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REVIEW

# Antiplatelet Therapy in ST-Segment Elevation Myocardial Infarction: A Contemporary Review

**Silviu-Ionel Dumitrescu[1,2], Ioana-Cristina Doicin[1*], Francesco Perone[3], Stefan Timofte[4], Luana Iorescu[1], Irina Prisacariu[1], Bogdan Vilceleanu[1], Ruxandra Ionescu[1], Andreea Ciuperca[1], Alice Elena Munteanu[1,2]**

[1] Carol Davila Central Military Emergency Hospital, Bucharest, Romania
[2] Titu Maiorescu University – Faculty of Medicine, Bucharest, Romania
[3] Cardiac Rehabilitation Unit, Rehabilitation Clinic "Villa delle Magnolie", 81020 Castel Morrone, Italy
[4] Department of Cardiology, "Prof. Th. Burghele" Clinical Hospital, 050653 Bucharest, Romania

*Correspondence: I.C.D. cristinadoicin98@gmail.com

**Abstract:** Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ inhibitor is the pharmacological cornerstone of ST-segment elevation myocardial infarction (STEMI) management. The evolution from clopidogrel to the more potent agents prasugrel and ticagrelor has improved ischaemic outcomes in PCI-treated patients, while cangrelor provides an intravenous alternative when oral absorption is compromised. Direct comparative data from ISAR-REACT 5 have established a hierarchy favoring prasugrel in patients proceeding to invasive management, although methodological limitations temper definitive conclusions. Beyond agent selection, contemporary practice increasingly emphasizes calibrated therapy: DAPT duration, de-escalation strategies — guided by CYP2C19 genotyping, platelet function testing, or applied empirically — and $P2Y_{12}$ monotherapy after short DAPT have been validated as approaches that preserve ischaemic protection while reducing bleeding. Special populations, including elderly patients, those with renal impairment, concomitant oral anticoagulation, or cardiogenic shock, require individualized decision-making that exceeds what any single trial provides. Emerging approaches, including subcutaneous selatogrel for patient self-administration and anti-GPVI strategies, signal a shift toward precision antiplatelet therapy. This review synthesizes current evidence across agent pharmacology, duration modulation, special population management, and future directions, framing the transition from potency to precision as the defining trajectory of the field.

**Keywords:** Keywords STEMI; dual antiplatelet therapy; $P2Y_{12}$ inhibitors; prasugrel; ticagrelor; de-escalation; bleeding risk

## INTRODUCTION

ST-segment elevation myocardial infarction (STEMI) remains the most acute manifestation of coronary atherothrombosis. Globally, more than three million individuals present with STEMI each year, and despite advances in reperfusion systems, the condition continues to exact a disproportionate toll in cardiovascular mortality — particularly in low- and middle-income countries [1–5]. In the United States, age-adjusted STEMI hospitalization rates fell by approximately 50% between 2004 and 2020, driven by improved primary prevention, widespread statin use, and declining smoking prevalence [6,7]. Yet this progress has not been uniform across socioeconomic strata, and even in contemporary primary PCI–treated cohorts, the first 90 days after infarction carry substantial excess mortality relative to the age- and sex-matched general population, with risk converging only thereafter [8]. The Fourth Universal Definition of Myocardial Infarction has further refined diagnostic precision, but the fundamental therapeutic imperative remains unchanged: every modifiable determinant of early outcome — antiplatelet therapy foremost among them — retains direct prognostic relevance [4].

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The biological rationale for antiplatelet therapy is rooted in the convergent platelet activation pathways that sustain coronary thrombosis after plaque disruption. Exposure of subendothelial collagen, tissue factor, and von Willebrand factor triggers platelet adhesion and recruitment through thromboxane $A_2$–dependent amplification, ADP-driven $P2Y_{12}$ signaling, and thrombin-mediated protease-activated receptor stimulation [9–11]. Optical coherence tomography studies have revealed that plaque erosion — rather than rupture — accounts for approximately

one-quarter to one-third of STEMI cases, with distinct platelet-rich thrombus composition that may respond differently to antiplatelet strategies, though this has not yet been translated into differentiated treatment algorithms. Because mechanical reperfusion does not extinguish this thrombotic milieu — platelet activation persists during and after stenting, driving distal embolization, microvascular obstruction, and acute stent thrombosis — pharmacological suppression of platelet function is indispensable [1,3,12]. Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ receptor inhibitor constitutes the pharmacological backbone of STEMI care.

The field has evolved through three overlapping phases. The first established DAPT itself, with aspirin and clopidogrel reducing ischaemic events in the landmark CLARITY and COMMIT trials [13,14]. The second introduced potent $P2Y_{12}$ inhibitors — prasugrel and ticagrelor — which were driven by the recognition that CYP2C19-dependent response variability left a substantial minority of clopidogrel-treated patients inadequately protected [15–17]. The third, and current, phase focuses not on maximal platelet inhibition but on calibrated therapy: matching agent, dose, and duration to each patient's composite ischaemic and bleeding risk profile [1,2].

The question of when to initiate $P2Y_{12}$ inhibitor loading — before or after coronary anatomy is defined — remains actively debated. The ATLANTIC trial found no benefit of prehospital ticagrelor administration, though with a median time advantage of only 30 minutes [56]. Registry data from the Bern-PCI and CREA-ARIAM cohorts have provided conflicting signals, and current ESC guidelines assign only a class IIb recommendation to routine pretreatment [1,57,58]. Adding complexity, opioid analgesics — administered to the majority of STEMI patients for chest pain — delay gastric absorption and attenuate the antiplatelet effect of oral $P2Y_{12}$ inhibitors by up to several hours, as demonstrated in the IMPRESSION trial [59]. Strategies to mitigate this interaction include crushed or chewed tablets and cangrelor bridging [60,61]. This review synthesizes the evidence across the full clinical arc, from pharmacological mechanisms through comparative efficacy, duration modulation, special populations, and emerging precision approaches.

## ANTIPLATELET AGENTS: MECHANISMS, EVIDENCE, AND COMPARATIVE HIERARCHY

### Aspirin

Aspirin's role in STEMI rests on irreversible acetylation of platelet cyclooxygenase-1, suppressing thromboxane $A_2$ synthesis. Its efficacy was established definitively by ISIS-2, which demonstrated a 23% reduction in five-week vascular mortality [18]. The Antithrombotic Trialists' Collaboration subsequently confirmed a 25% reduction in serious vascular events across high-risk populations [61]. Current guidelines recommend aspirin loading (150–300 mg oral or 250–500 mg intravenous) as early as possible, followed by indefinite low-dose maintenance (75–100 mg daily) [1,2]. Notably, accumulating evidence for $P2Y_{12}$ monotherapy after short DAPT has begun to raise the question of whether aspirin remains necessary beyond the acute periprocedural phase — a conceptual shift that would have seemed heretical a decade ago.

### Clopidogrel

Clopidogrel is a second-generation thienopyridine prodrug requiring hepatic CYP2C19-mediated bioactivation. Its onset is delayed (2–6 hours), and inter-individual variability is substantial: approximately 25–30% of Europeans and a higher proportion of East Asian populations carry loss-of-function CYP2C19 alleles that impair conversion to the active metabolite, translating into higher rates of stent thrombosis and recurrent ischaemic events [15,19]. The CLARITY trial demonstrated that adding clopidogrel to aspirin and fibrinolytic therapy in STEMI significantly improved infarct-artery patency [13], and COMMIT confirmed a modest but significant reduction in death, reinfarction, and stroke with clopidogrel in over 45,000 patients with acute MI [14]. Despite being superseded by potent agents for most STEMI indications, clopidogrel remains the preferred $P2Y_{12}$ inhibitor in patients requiring concomitant oral anticoagulation, and retains a central role in genotype-guided de-escalation strategies after the acute phase [1,2].

### Prasugrel

Prasugrel is a third-generation thienopyridine with more efficient hepatic conversion, achieving faster and more consistent platelet inhibition than clopidogrel. The pivotal TRITON-TIMI 38 trial demonstrated a significant reduction in the composite of cardiovascular death, myocardial infarction, and stroke compared with clopidogrel in ACS patients undergoing PCI (HR 0.81, 95% CI 0.73–0.90), with particular benefit in the STEMI subgroup, where prasugrel additionally reduced myocardial reinfarction (HR 0.70) and cardiovascular death (HR 0.61) at 30 days [16,20]. However, this came at the cost of increased major bleeding (HR 1.32), and three subgroups — patients with prior stroke or transient ischaemic attack (net harm), age ≥75 years, and body weight <60 kg — had attenuated or absent net benefit. A meta-analysis of 12 randomized trials including over 14,000 STEMI patients confirmed the superiority of prasugrel over clopidogrel in mortality (RR 0.56) and MACE (RR 0.71) without a significant excess in TIMI major bleeding [62]. Prasugrel is contraindicated in prior cerebrovascular disease and requires dose reduction (5 mg maintenance) in elderly and low-weight patients [1,2].

### Ticagrelor

Ticagrelor is a direct-acting, reversible $P2Y_{12}$ inhibitor belonging to the cyclopentyltriazolopyrimidine class that does not require hepatic bioactivation. The PLATO trial, enrolling over 18,000 ACS patients, showed superiority over clopidogrel for the composite of vascular death, myocardial infarction, or stroke (HR 0.84, 95% CI 0.77–0.92), with a notable and statistically significant all-cause

mortality reduction (HR 0.78) — a finding not replicated by any other oral $P2Y_{12}$ inhibitor [17]. However, in the STEMI subgroup (n=7,544) the primary composite endpoint did not reach statistical significance (HR 0.87, p=0.07), though stent thrombosis and total mortality were individually reduced. Ticagrelor additionally increases adenosine plasma levels through inhibition of cellular uptake, a pleiotropic effect that may contribute to its mortality signal but also explains the dyspnoea observed in 15–20% of patients [22].

**Table 1:** Major antiplatelet agents

| Property | Aspirin | Clopidogrel | Prasugrel | Ticagrelor | Cangrelor | Selatogrel* |
|---|---|---|---|---|---|---|
| **Chemical class** | Salicylate | Thienopyridine (2nd gen) | Thienopyridine (3rd gen) | Cyclopentyltriazolopyrimidine | ATP analogue | 2-phenylpyrimidine-4-carboxamide |
| **Target** | COX-1 ($TXA_2$ synthesis) | $P2Y_{12}$ receptor | $P2Y_{12}$ receptor | $P2Y_{12}$ receptor | $P2Y_{12}$ receptor | $P2Y_{12}$ receptor |
| **Route** | Oral / IV | Oral | Oral | Oral | Intravenous | Subcutaneous |
| **Prodrug** | No | Yes (CYP2C19-dependent) | Yes (CYP-dependent) | No (active parent + metabolite) | No (active compound) | No (active compound) |
| **Binding** | Irreversible | Irreversible | Irreversible | Reversible | Reversible | Reversible |
| **Onset of action** | 20 min oral, 5 min IV | 2–6 hours | 30 min – 4 hours | 30 min – 2 hours | ~2 minutes | 15–30 minutes |
| **Offset of action** | 7–10 days | 5–10 days | 7–10 days | 3–5 days | 30–60 minutes | ~24 hours |
| **Loading dose** | 150–300 mg oral or 250–500 mg IV | 600 mg | 60 mg | 180 mg | 30 µg/kg bolus | 16 mg SC |
| **Maintenance dose** | 75–100 mg daily | 75 mg daily | 10 mg daily (5 mg if ≥75 y or <60 kg) | 90 mg twice daily | 4 µg/kg/min infusion | Single dose (no maint.) |
| **CYP dependency** | No | Major (LOF → resistance) | Minor | None | None | None |
| **Renal dose adjustment** | No | No | No | No | No | No (fecal >92%) |
| **Key contraindications** | Active peptic ulcer, aspirin-exacerbated resp. disease | CYP2C19 poor metabolisers (relative) | Prior stroke/TIA (absolute), ≥75 y, <60 kg (caution) | Prior ICH, active pathological bleeding | Active bleeding | Under investigation |
| **Pivotal STEMI evidence** | ISIS-2 (1988) | CLARITY, COMMIT | TRITON TIMI 38 (STEMI subgroup) | PLATO (STEMI subgroup) | CHAMPION PHOENIX | Phase II (Sinnaeve 2020), SOS-AMI (phase III ongoing) |
| **Head-to-head data** | Universal foundation | Inferior to prasugrel & ticagrelor | Superior to ticagrelor (ISAR-REACT 5) | Superior to clopidogrel (PLATO) | Superior to clopidogrel (CHAMPION) | |
| **Pre-CABG discontinuation** | Not required | ≥5 days | ≥7 days | ≥3 days | ~1 hour | NA (SC bypasses GI) |
| **Opioid interaction** | Minimal | Yes (delayed absorption) | Yes (delayed absorption) | Yes (delayed absorption) | None | No (SC bypasses GI) |

| Property | Aspirin | Clopidogrel | Prasugrel | Ticagrelor | Cangrelor | Selatogrel* |
|---|---|---|---|---|---|---|
| **Unique advantage** | Universal, lowest cost, anti-inflammatory | Low cost, preferred with OAC; genotype-guided reversal | Most potent oral; lowest stent thrombosis | Reversible mortality signal; no prodrug | Instant onset; titratable; surgical bridging | Self-injectable; pre-hospital; no IV needed |

* Selatogrel is investigational (point estimates from phase II / ongoing SOS-AMI phase III trial).

SC = subcutaneous; IV = intravenous; LOF = loss-of-function; OAC = oral anticoagulation; $TXA_2$ = thromboxane $A_2$; CYP = cytochrome P450; ICH = intracranial haemorrhage; PFT = platelet function test.

Premature discontinuation due to side effects has been associated with adverse outcomes in registry data, highlighting adherence as a clinically relevant limitation of ticagrelor therapy [21].

**Cangrelor**

Cangrelor is the only available intravenous $P2Y_{12}$ inhibitor, providing near-complete platelet inhibition within two minutes of bolus administration and offset within 30–60 minutes after discontinuation. The CHAMPION PHOENIX trial, enrolling over 11,000 patients undergoing urgent or elective PCI, demonstrated a significant reduction in periprocedural ischaemic events compared with clopidogrel (OR 0.78, 95% CI 0.66–0.93), driven primarily by a reduction in periprocedural MI and stent thrombosis [23]. A pooled analysis of all three CHAMPION trials confirmed these findings with consistent benefit across stable and ACS presentations. Its clinical niche is patients unable to absorb oral agents — those presenting with cardiogenic shock, mechanical ventilation, active vomiting, or severe opioid-induced gastroparesis. The ongoing DAPT-SHOCK-AMI trial is prospectively comparing cangrelor with crushed ticagrelor as the initial $P2Y_{12}$ strategy specifically in cardiogenic shock complicating acute MI [51]. An important pharmacological consideration is that cangrelor competitively inhibits the binding of thienopyridine active metabolites to the $P2Y_{12}$ receptor; therefore, clopidogrel or prasugrel loading should be deferred until the end of cangrelor infusion, whereas ticagrelor — which binds a distinct site on the receptor — can be administered at any time during or after infusion without pharmacodynamic interaction [24,25].

**Comparative hierarchy**

The ISAR-REACT 5 trial directly compared ticagrelor with prasugrel in 4,018 ACS patients, of whom approximately 41% presented with STEMI. At one year, the composite of death, myocardial infarction, and stroke was significantly lower with prasugrel (6.9% vs 9.3%, HR 1.36, favoring prasugrel), an effect primarily driven by reduced nonfatal MI, with a numerically lower all-cause mortality. Importantly, major bleeding (BARC ≥3) was similar between groups [26]. The STEMI subgroup showed a consistent numerical advantage for prasugrel (7.9% vs 10.1%), though this did not reach statistical significance (p=0.10). These results have influenced guideline positioning, with ESC 2023 guidelines now favoring prasugrel as the preferred $P2Y_{12}$ inhibitor in PCI-bound ACS patients [1]. However, ISAR-REACT 5 carries several methodological limitations that temper definitive interpretation: it was open-label; drug administration timing differed between arms (prasugrel at angiography, ticagrelor at diagnosis); approximately 20% of patients crossed over between treatments; and adherence in the ticagrelor arm was lower than expected [27,28]. A network meta-analysis of over 52,000 patients from 12 randomized trials confirmed superior ischaemic protection with both potent agents over clopidogrel, with prasugrel showing the most favorable net clinical benefit [29]. Nationwide registry data from Denmark, Sweden, and other countries following the shift from ticagrelor to prasugrel have provided broadly supportive real-world evidence, though residual confounding remains a concern in these observational analyses. The most relevant data on antiplatelet agents are represented in Table 1.

## DURATION OF DAPT, DE-ESCALATION, AND $P2Y_{12}$ MONOTHERAPY

For much of the past two decades, DAPT after STEMI was conceptualized as a uniform 12-month course. Contemporary evidence has revealed that ischaemic and bleeding risks follow divergent temporal trajectories: thrombotic events — particularly stent thrombosis and spontaneous MI — concentrate in the first weeks after PCI, whereas bleeding accumulates steadily over time and independently worsens prognosis [1,2]. This temporal mismatch provides the biological rationale for three modulation strategies: de-escalation, shortened DAPT with monotherapy, and extended DAPT. The choice among these strategies depends on each patient's composite risk profile, which can be estimated using validated tools including the ARC-HBR criteria for bleeding risk [41], the PRECISE-DAPT score [44], and the DAPT score for ischaemic benefit of prolonged therapy [43].

**De-escalation**

De-escalation involves switching from a potent $P2Y_{12}$ inhibitor to clopidogrel after the acute high-risk window. This can be guided by platelet function testing, as in TROPICAL-ACS, where PFT-guided de-escalation from prasugrel to clopidogrel at 14 days was non-

inferior for ischaemic events with a trend toward reduced bleeding [30]; or by CYP2C19 genotyping, as in POPular Genetics, which demonstrated that genotype-guided therapy in primary PCI patients was non-inferior for ischaemic outcomes while significantly reducing bleeding (HR 0.78) [31]. Unguided de-escalation has also been validated in multiple trials: TOPIC demonstrated benefit of empiric switching from any potent $P2Y_{12}$ inhibitor to clopidogrel at one month [32]; HOST-REDUCE-POLYTECH-ACS showed that halving the prasugrel dose at one month reduced bleeding without ischaemic harm [33]; and TALOS-AMI confirmed safety and bleeding reduction with ticagrelor-to-clopidogrel switching at one month, including in patients with high ischaemic risk features [34,36]. A network meta-analysis of these strategies confirmed consistent benefit across guided and unguided approaches [35]. Critically, no evidence supports de-escalation within the first 30 days — a boundary that should be considered non-negotiable given the high early thrombotic risk [1].

**$P2Y_{12}$ monotherapy**

Shortened DAPT (1–3 months) followed by $P2Y_{12}$ inhibitor monotherapy represents an alternative strategy for patients at high bleeding risk. The concept rests on pharmacodynamic evidence that in the presence of potent $P2Y_{12}$ blockade, aspirin adds relatively little incremental antithrombotic effect while contributing substantially to gastrointestinal bleeding. The double-blind TWILIGHT trial demonstrated that ticagrelor monotherapy after 3 months of DAPT significantly reduced BARC 2, 3, or 5 bleeding (HR 0.56) without increasing ischaemic events in high-risk PCI patients [37]. TICO confirmed similar findings in a Korean ACS population with ticagrelor monotherapy after 3 months [39], while STOPDAPT-2 tested an even shorter 1-month DAPT followed by clopidogrel monotherapy [38]. MASTER DAPT further validated abbreviated DAPT in ARC-HBR patients. An individual patient data meta-analysis of over 24,000 patients from these and other trials confirmed that $P2Y_{12}$ monotherapy reduces bleeding by approximately 40% with preserved ischaemic protection, solidifying this approach as a viable alternative to standard 12-month DAPT [40]. The ARC-HBR criteria provide a structured framework for identifying candidates: patients meeting at least one major or two minor criteria (including age ≥75 years, oral anticoagulant use, severe CKD, anemia, thrombocytopenia, active malignancy, and recent major surgery) qualify as high bleeding risk and may benefit from shortened DAPT [41].

**Extended DAPT and risk stratification**

Conversely, patients with high residual ischaemic risk and acceptable bleeding profiles may benefit from extended DAPT beyond 12 months. The PEGASUS-TIMI 54 trial enrolled over 21,000 patients with prior MI (1–3 years earlier) and demonstrated that ticagrelor 60 mg twice daily added to aspirin significantly reduced the composite of cardiovascular death, MI, and stroke (HR 0.84, 95% CI 0.74–0.95), at the cost of increased TIMI major bleeding (HR 2.32) without an increase in fatal or intracranial haemorrhage [42]. Identifying which patients derive net benefit from prolonged therapy requires formal risk stratification. The DAPT score integrates clinical and procedural variables to predict benefit from extended therapy, with scores ≥2 identifying patients in whom ischaemic risk reduction outweighs bleeding hazard [43]. The PRECISE-DAPT score complements this by identifying patients at high bleeding risk (score ≥25) who should be considered for shortened rather than prolonged DAPT [44]. Together, these tools operationalize the principle that DAPT duration should be a personalized decision rather than a default prescription.

## SPECIAL POPULATIONS

The “average STEMI patient” enrolled in landmark trials does not capture the complexity of everyday practice. Several subpopulations require individualized management that extends beyond guideline defaults.

**Advanced age, low body weight, and prior cerebrovascular disease**

These characteristics share a common consequence: they narrow the therapeutic window of potent $P2Y_{12}$ inhibitors. The TRITON-TIMI 38 trial identified prior stroke or transient ischaemic attack as a setting of net harm with prasugrel, age ≥75 years as a modifier of attenuated benefit, and body weight <60 kg as a risk factor for excess bleeding [16]. A reduced maintenance dose of prasugrel (5 mg daily) is recommended in elderly and low-weight patients by both ESC and ACC/AHA guidelines, although the evidence for this adjustment derives from pharmacokinetic modeling rather than a dedicated outcome trial [1,2]. Ticagrelor does not carry the same label contraindication in prior cerebrovascular disease, making it the preferred potent $P2Y_{12}$ inhibitor in this subgroup. However, its twice-daily dosing and dyspnoea profile may be particularly burdensome in elderly patients with comorbid pulmonary disease, and adherence tends to decline with age. In frail elderly patients with high bleeding risk and limited anticipated ischaemic benefit from potent therapy, clopidogrel — ideally informed by CYP2C19 genotyping to confirm adequate metabolizer status — remains an entirely reasonable choice [1,2,41].

**Renal dysfunction**

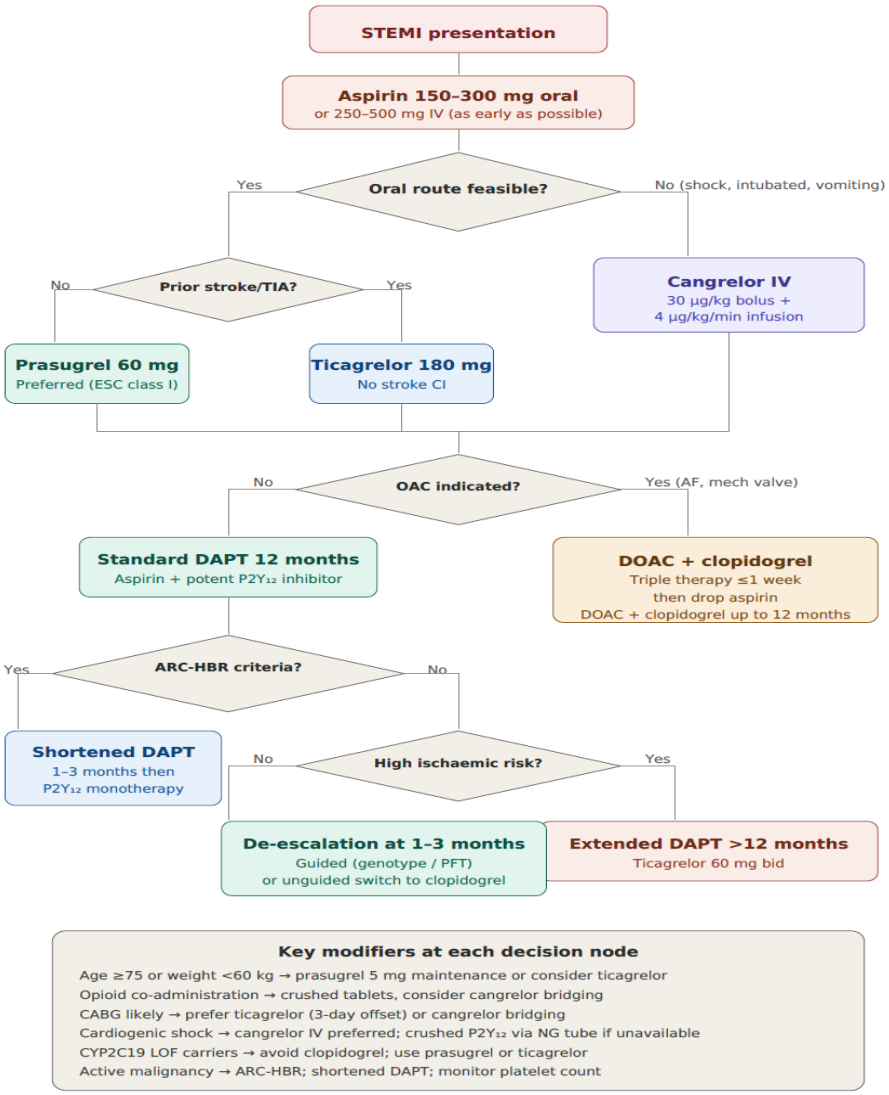
Chronic kidney disease intensifies both ischaemic and bleeding risk through overlapping mechanisms: uraemic platelet dysfunction, accelerated vascular calcification, more diffuse coronary disease, concomitant anemia, and systemic inflammation. Patients with eGFR below 30 mL/min/1.73 $m^2$ or on dialysis were excluded from, or minimally represented in, the trials that established the superiority of prasugrel and ticagrelor over clopidogrel [16,17]. No dedicated outcome trial has been completed exclusively in this population. In practice, clinicians face a dual uncertainty: potent antiplatelet therapy may amplify an already elevated bleeding risk, yet CKD patients

derive greater absolute benefit from ischaemic event prevention given their higher baseline event rates. Ticagrelor does not require renal dose modification; prasugrel pharmacokinetics are not altered by CKD per se, but the combination of renal impairment with advanced age or low weight magnifies risk. CKD should be viewed as a marker of prognostic complexity requiring careful individualization rather than a simple contraindication to potent therapy [1,2].

**Concomitant oral anticoagulation**

Patients with STEMI and atrial fibrillation represent one of the most challenging antithrombotic scenarios, as they require simultaneous prevention of AF-related stroke and stent thrombosis. Triple therapy (OAC plus DAPT) carries bleeding rates approaching 10% per year, which are unacceptable beyond a very brief periprocedural window. The evidence has evolved through a series of landmark trials: WOEST first demonstrated the feasibility of dropping aspirin [45]; PIONEER AF-PCI, RE-DUAL PCI, and ENTRUST-AF PCI tested DOAC-based dual therapy [46–48]; and AUGUSTUS, the only trial with a 2×2 factorial design (4,614 patients), definitively showed that apixaban plus a $P2Y_{12}$ inhibitor without aspirin resulted in significantly less bleeding and fewer hospitalisations without an increase in ischaemic events [49]. A four-way analysis confirmed that DOAC-based dual therapy yields the best composite outcome [50]. Current guidelines recommend limiting triple therapy to ≤1 week peri-procedurally, then continuing DOAC plus clopidogrel for up to 12 months [1,2]. Clopidogrel is the preferred $P2Y_{12}$ inhibitor in this context because the combination of a DOAC with prasugrel or ticagrelor has not been prospectively validated.

**Figure 1:** Decision algorithm



**Need for coronary artery bypass grafting**

In 5–10% of STEMI patients, coronary anatomy necessitates CABG rather than PCI. In this scenario, the differences in offset times among $P2Y_{12}$ inhibitors become clinically decisive: current recommendations advise discontinuation of ticagrelor at least 3 days, clopidogrel at least 5 days, and prasugrel at least 7 days before elective surgery [1,2]. Cangrelor provides an alternative bridging strategy with a 60-minute offset, and the BRIDGE trial demonstrated that cangrelor bridging maintained platelet suppression without increasing CABG-related bleeding [25]. When there is uncertainty about coronary anatomy, the choice of $P2Y_{12}$ inhibitor should incorporate the patient's potential surgical trajectory — a practical argument against indiscriminate pretreatment.

**Cardiogenic shock**

Cardiogenic shock complicates 5–8% of STEMI presentations and fundamentally alters the pharmacokinetic landscape for oral medications. Splanchnic hypoperfusion, vasopressor-driven vasoconstriction, mechanical ventilation, and opioid use collectively impair gastric absorption to a degree that renders oral $P2Y_{12}$ inhibitor loading unreliable — even with crushed tablets. This is the clinical scenario in which intravenous cangrelor has the strongest theoretical and practical justification: it bypasses the gastrointestinal tract entirely and provides immediate, titratable, and rapidly reversible $P2Y_{12}$ blockade. The ongoing DAPT-SHOCK-AMI trial is prospectively comparing cangrelor with crushed ticagrelor as the initial $P2Y_{12}$ strategy in this population [51]. Until these results are available, clinical practice is guided by expert consensus favoring cangrelor where available, or crushed potent $P2Y_{12}$ inhibitors via nasogastric tube, with the recognition that platelet inhibition may be delayed and unpredictable regardless of the oral agent chosen [1].

The management of antiplatelet therapy in these special populations cannot be reduced to a simple algorithm. What unites these subgroups is that they demand a level of clinical judgment, risk balancing, and therapeutic flexibility that exceeds what any single trial or guideline recommendation can provide. Sex-specific considerations further complicate ACS management, as women present later, receive less aggressive antiplatelet regimens, and are underrepresented in clinical trials [63]. These disparities underscore the need for personalized secondary prevention strategies that consider sex-related differences and associated comorbidities, an approach increasingly recognized across cardiovascular and other medical disciplines, including the management of patients with preexisting conditions that may predispose them to adverse outcomes [63,64]. The consistent theme is one of deliberate individualization: choosing the agent, dose, and duration that best fit the patient's composite risk profile rather than reflexively applying the strategy optimized for the average trial participant.

## CONCLUSIONS AND FUTURE PERSPECTIVES

Antiplatelet therapy remains one of the most decisive modifiable determinants of outcome in STEMI, while delays from symptom onset to reperfusion continue to critically influence myocardial salvage and overall clinical prognosis [65]. Aspirin continues to serve as the universal acute-phase foundation, while the evolution from clopidogrel to prasugrel and ticagrelor has materially improved ischaemic protection. Cangrelor fills a critical niche when the oral route fails, and the pharmacokinetic challenges imposed by opioids and hemodynamic compromise are now well recognized and increasingly addressable.

Yet the most important evolution has been conceptual rather than pharmacological. Contemporary management is no longer defined by maximal platelet inhibition alone, but by calibrated inhibition — potent and timely when ischaemic risk is greatest; modified, shortened, or de-escalated when bleeding risk predominates; and tailored to age, frailty, genotype, renal function, surgical trajectory, and anticoagulation needs. The 2023 ESC and 2025 ACC/AHA guidelines embody this shift toward individualized decision-making, providing frameworks that accommodate standard-duration DAPT, shortened DAPT with $P2Y_{12}$ monotherapy, guided or unguided de-escalation, and extended therapy as parallel options calibrated to individual risk [1,2].

Several emerging paradigms may further reshape the field. Selatogrel, a subcutaneous $P2Y_{12}$ inhibitor achieving near-complete platelet inhibition within 15–30 minutes, is being evaluated in the phase III SOS-AMI trial (~14,000 patients) for patient self-administration at suspected MI recurrence — a concept that represents the logical endpoint of the pretreatment debate [52,53]. Strategies targeting the platelet collagen receptor GPVI and neutrophil extracellular traps offer the theoretical possibility of antithrombotic efficacy without hemostatic impairment [54,55]. Genotype-guided therapy, already validated in dedicated STEMI populations, is likely to transition from niche to standard practice as point-of-care platforms become faster and cheaper [31].

In practical terms, the contemporary clinician managing a patient with STEMI must navigate a decision matrix that begins with aspirin loading and agent selection (prasugrel preferred unless contraindicated; ticagrelor as alternative; cangrelor when oral absorption is compromised), continues through the acute phase with attention to opioid interactions and potential CABG, and extends into the maintenance phase with individualised duration decisions informed by bleeding and ischaemic risk scores. The management of concomitant oral anticoagulation, advanced age, renal impairment, and cardiogenic shock each introduces additional layers of complexity that no single algorithmic pathway can fully capture. The central challenge for the next era is not simply to develop stronger antiplatelet drugs but to deploy existing and emerging agents with greater biological and clinical precision. The transition from potency to precision is the defining trajectory of the field.

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***Authors' contribution***

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

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ARTICLE

# Beyond grief: Developing a transdiagnostic conceptual model of bereavement-related psychopathology

**Octavian Vasiliu[1,2], Bogdan Mircea Petrescu[1,2], Andrei Gabriel Mangalagiu[1,2*], Mihaela Pătrașcu[2], Andreea Filareta Făinărea[2], Irina Amanolesei[2], Ciprian Muja[2], Silviu-Ionel Dumitrescu[3,4*], Cristian Năstase[5], Florentina Cristina Pleșa[1,6]**

[1] Clinical Neurosciences Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Psychiatry Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
[3] Titu Maiorescu University – Faculty of Medicine, Bucharest, Romania
[4] Cardiology Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
[5] Neurosurgery Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
[6] Neurology Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania

*Correspondence: andrei-gabriel.mangalagiu@umfcd.ro (A.G.M.); Silviu-Ionel Dumitrescu, silviudumius@yahoo.com (S.I.D.)

**Abstract:** Bereavement constitutes a shared environmental exposure that may result in distinct psychopathological outcomes, including prolonged grief disorder (PGD), posttraumatic stress disorder (PTSD), and major depressive disorder (MDD). The objective of this research was to construct a transdiagnostic conceptual model for interpreting bereavement-related pathologies that integrates core psychopathological data. A structured conceptual synthesis of the literature was conducted, focusing on diagnostic, epidemiological, pathophysiological, clinical, and therapeutic findings related to PGD and associated disorders. Shared and disorder-specific mechanisms were identified and mapped across diagnostic categories in order to construct an integrative framework. The proposed model suggests that bereavement-related psychopathology can be conceptualized as a spectrum of partially overlapping syndromes organized around three principal domains: attachment dysregulation and separation distress (PGD), trauma- and threat-processing abnormalities (PTSD, acute stress disorder, adjustment disorder), and depressive-cognitive vulnerability (MDD). This framework provides a clinically relevant perspective on the relationship between grief-, trauma-, and depression-related disorders following loss. More research is needed to empirically validate this model, but at a theoretical level, it can serve as an orienting aid for mapping bereavement-related diagnoses and treatments.

**Keywords:** bereavement; prolonged grief disorder; major depressive disorder; posttraumatic stress disorder; adjustment disorder; acute stress disorder; new conceptual model; vulnerability to stress; attachment styles

## INTRODUCTION

Grief is a normal emotional reaction to interpersonal loss, usually transient, as the individual's coping mechanisms are activated and adjustment to the new life situation occurs. On the contrary, pathological grief (PG) is a phenomenon that is considered excessive, reported to deviate from the person's cultural and social norms in terms of amplitude, duration, discomfort, and/or functional impairment.

PG has been approached in the literature from multiple perspectives across major psychological and psychiatric traditions, with historically distinct aspects of this clinical phenomenon emphasized, ranging from unconscious impulses to neurobiological dysfunctions, and from attachment styles to existential crisis (Fig. 1).

The psychoanalytic perspective, starting with Sigmund Freud, evaluates PG as the result of difficulties in detaching libido from the loved one, and distinguishes between normal mourning and melancholia [1]. As described in Freud's seminal work, "Mourning and melancholia" (1917), mourning is terminated when the subject severs its emotional attachment to the lost one and reinvests the freed libido in a new object [2]. Later, the founder of psychoanalysis revisited his theory on grief and stated that identification associated with melancholia is an integral component of

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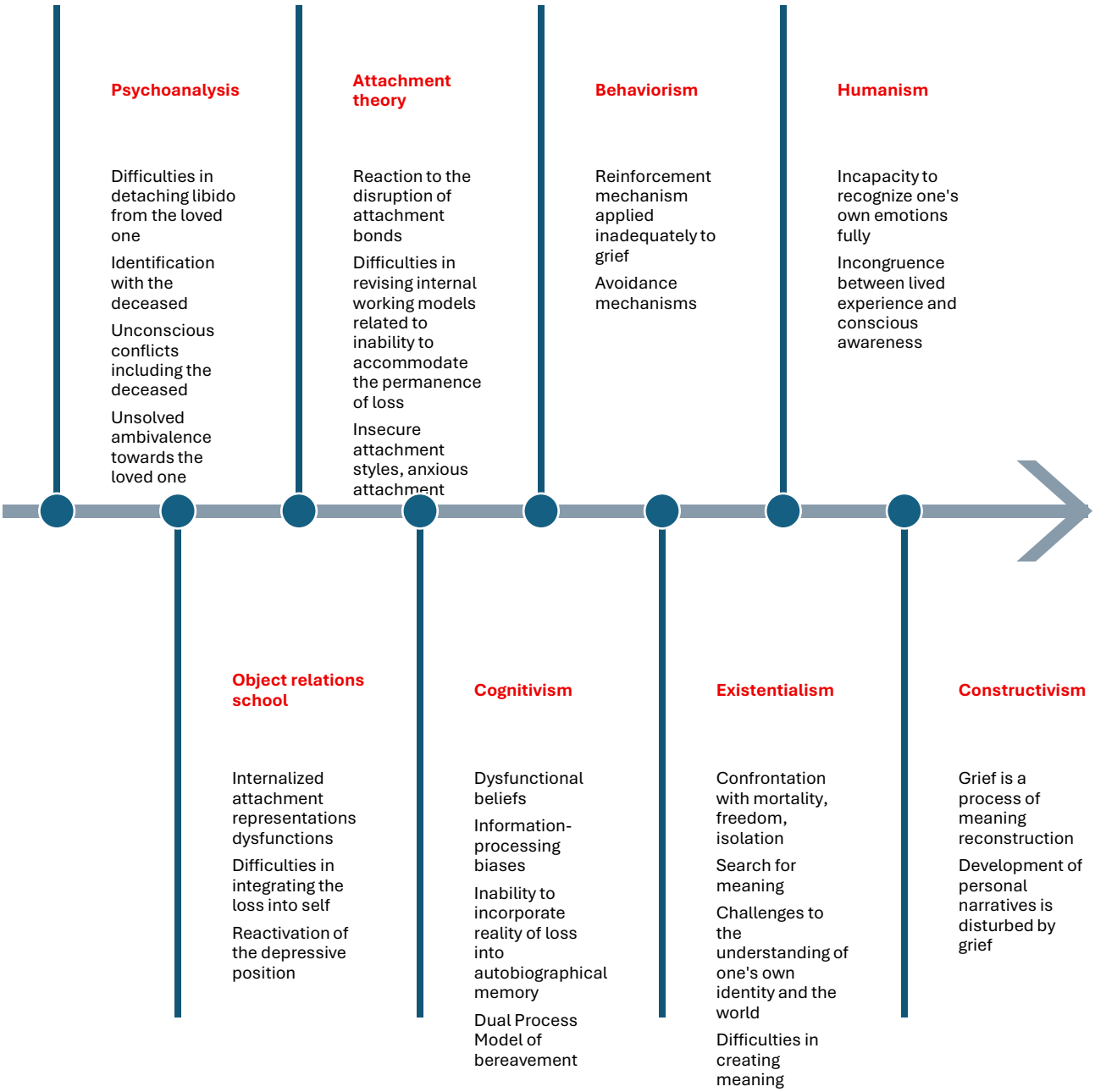
mourning [2]. Identification, at the unconscious level, with the deceased may lead to self-reproach, guilt, and persistent suffering, and unconscious conflicts and unresolved ambivalence toward the same individual are other contributors to the onset and persistence of PG, in the psychoanalytic framework [1,3,4]. According to psychodynamic theory, pathological mourning may be manifested as defensive processes such as splitting (one part of the subject is aware of the loss, while the other wishes that the loved one were still alive), repression, and dissociation [5].

**Figure 1:** Historical perspectives on pathological grief



Modern research is looking to connect psychoanalytic core concepts with neurobiological findings, starting from the observation that pathological grief and depression involve persistent activation of brain systems related to attachment, reward, and self-referential processing of stimuli [6]. These data suggest that difficulties disengaging from the lost object may be associated with identifiable neural mechanisms, providing a neurobiological basis for psychoanalytic accounts of melancholia [6]. According to this framework, activation of the subgenual cingulate may represent a neurobiological correlate of repression, whereas the default mode network is presented as a potential neural substrate for ego functions [6].

Later object-relations theorists have explored the disruptions in internalized attachment representations and difficulties integrating the loss into the self [7,8]. Building on earlier psychoanalytic formulations, Melanie Klein proposed that mourning reactivates the depressive position, a developmental state characterized by anxiety over the loss or destruction of loved internal objects [4,9]. Successful mourning involves the restoration and reintegration of these internal representations, whereas PG arises when this process is disrupted, resulting in persistent guilt, despair, or an inability to relinquish the lost object [7]. Within this paradigm, therapy for PG focuses on helping the individual to consciously re-experience repressed destructive impulses, overcome the unconscious guilt, and integrate the "good" and "bad" aspects of the lost object [10]. Working through the depressive position (by encouraging the patient to overcome denial and omnipotent control, and to tolerate the ambivalence of loving and hating the lost one), interpreting projective identification, addressing pathological organizations (or "psychic retreats" in the face of guilt over destructive impulses), and encouraging reparation (by verbalizing and mourning their destructive unconscious fantasies) are processes of healing in patients with PG [10,11].

Building on these ideas, attachment theory, developed by John Bowlby, conceptualized grief as a response to the disruption of attachment bonds and suggested that chronic mourning reflects difficulties in revising internal working models to accommodate the permanence of the loss [12]. The vulnerability to PG is considered enhanced by early attachment insecurities, especially anxious attachment, which can prevent individuals from developing stable internal models and engaging in "hyperactivating" strategies [12-15]. Due to these insecurities, individuals are prone to prolonged yearning, separation anxiety, and severe dependency that make the process of mourning especially difficult [13-15]. Also, avoidant attachment may lead to deactivating strategies that suppress painful emotions; therefore, an inhibited mourning is negatively interfering with the recovery from the loss of the loved one [13-15].

Cognitive perspectives on PG emphasize maladaptive beliefs and information-processing biases, as well as difficulties in integrating the reality of loss into autobiographical memory [16]. Examples of dysfunctional beliefs related to PG are "Life is meaningless without the deceased", "I should have prevented the death", or "Moving on would be a betrayal". Continuous processing of these cognitions (i.e., in a ruminative manner) and catastrophic interpretations maintain a high level of distress and discomfort [16-18]. A prominent contemporary model is the cognitive-behavioral formulation of prolonged grief developed by Stroebe and Schut (1999) through the Dual Process Model (DPM) of bereavement, and later expanded by Boelen and colleagues (2005), who emphasized maladaptive cognitions, avoidance behaviors, and difficulties integrating the reality of the loss [16-18]. DPM is based on the presence of loss- and restoration-oriented stressors and on the dynamic regulatory process of oscillation between confrontation and avoidance across different tasks of grieving [16]. According to DPM, adaptive coping comprises confrontation of reality and avoidance of loss and restoration stressors [16]. This model is important because it normalizes relief by validating experiences of distraction, productivity, or happiness as healthy mechanisms, rather than simply forgetting the deceased, and it also explains individual differences in grieving at different paces [16-18].

From a behavioral perspective, PG is maintained through reinforcement mechanisms and avoidance behaviors that interfere with adaptation to loss [18-21]. Individuals experiencing prolonged grief may avoid reminders of the deceased, including places, objects, memories, or situations associated with the loss, thereby preventing adequate emotional processing and integration of the bereavement experience [18-21]. This avoidance is negatively reinforced because it temporarily reduces distress; however, it simultaneously impedes confrontation with the reality of the loss and prolongs grief-related symptoms. In addition, bereaved individuals often withdraw from social, occupational, and recreational activities, reducing opportunities for positive reinforcement and meaningful engagement with life. Such behavioral patterns contribute to persistent sadness, isolation, and functional impairment, creating a self-perpetuating cycle in which avoidance and disengagement maintain emotional suffering over time [18-21]. As a consequence of these theories, behavioral therapists should encourage exposure to the feared situations by the use of imagination (e.g., patients recount the story of loss while working with the therapist), or in vivo (e.g., the patients are gradually guided to visit places where they were together with the deceased, and which they avoided during the bereavement period), but also behavioral activation (e.g., scheduling pleasurable, meaningful, or previously enjoyed activities for the patient) [22,23].

From an existential perspective, grief is understood as a profound confrontation with the fundamental realities of human existence, including mortality, freedom, isolation, and the search for meaning [24]. The death of a significant other is able to disrupt established assumptions about the self and the world, often challenging an individual's sense of identity, purpose, and continuity, and that is why normal grief can facilitate personal growth and existential reflection, while pathological grief may make the bereaved person unable to reconstruct meaning following the loss or integrate the experience into a coherent life narrative [25]. In the last situation, bereavement may precipitate an existential crisis characterized by persistent questioning of one's identity, life purpose, and capacity to live in the face of mortality [24]. Existential theorists argue that successful adaptation to loss requires engaging with, rather than

avoiding, these existential concerns and developing a renewed sense of meaning and self-understanding [24,25]. Consequently, pathological grief is viewed not only as emotional suffering but also as a disruption in the individual's capacity to create meaning, confront existential anxiety, and reconstruct identity after loss [24,25].

From a humanistic perspective, grief is viewed as a natural and meaningful response to loss that reflects the individual's capacity for attachment, love, and personal growth [26-28]. Humanistic theorists emphasize the importance of experiencing and expressing grief authentically rather than suppressing or avoiding painful emotions [26-28]. PG may develop when individuals are unable to fully acknowledge their emotional experiences, either due to internal defenses or to external pressures that discourage the open expression of sorrow [26-28]. Such incongruence between lived emotional experience and conscious awareness can impede adaptation to loss and hinder psychological growth [26-28]. Recovery is therefore understood not as the elimination of grief but as a process of acceptance, emotional integration, and the reconstruction of a meaningful life in the absence of the deceased [26-28]. Through self-exploration, emotional congruence, and renewed engagement with personal values and relationships, bereaved individuals can achieve greater self-understanding and continue their developmental journey despite loss [26-28]. Within this framework, grief is regarded as a potentially transformative process that may foster resilience, authenticity, and self-actualization when navigated successfully [26-28].

From a constructivist perspective, grief is understood as a process of meaning reorganization, following the disruption of an individual's created perspective on the world [25,29,30]. People develop personal narratives that provide coherence, purpose, and continuity to their lives; however, bereavement can profoundly challenge these narratives and destabilize previously held beliefs about the self, relationships, and the world [25,29,30]. PG may arise when the loss cannot be successfully integrated into the individual's existing meaning system, resulting in persistent distress, identity disruption, and difficulty adapting to life without the deceased [25,29,30]. Constructivist theorists emphasize that successful mourning involves reconstructing meaning, revising one's life narrative, and developing a renewed sense of identity that accommodates the reality of the loss [25,29,30].

As can easily be observed, virtually all the relevant schools of thought in clinical psychology and psychotherapy have approached the phenomenon of bereavement. However, there are multiple differences in conceptualizing the dimensions of normal and pathological bereavement, stages of these processes, and the most efficient way to approach individuals presenting problems in processing grief. Therefore, the objective of this article is to develop a conceptual framework that explains how bereavement may lead to distinct yet overlapping psychiatric outcomes, such as different clinical diagnoses, and to improve case management in these cases. At the origin of this research is the unmet need to formulate a transdiagnostic perspective with clinical relevance, due to the overlap of PTSD (posttraumatic stress disorder), MDD (major depressive disorder), and PGD symptoms, but also due to the diagnostic controversies related to PG.

## MATERIALS AND METHODS

The proposed model was developed through a structured conceptual synthesis of the literature. Relevant constructs associated with bereavement-related psychopathology were identified and organized into three domains: diagnostic classification, shared transdiagnostic mechanisms, and clinical outcomes. Mechanisms repeatedly described across disorders were mapped by their presence and relative centrality, yielding a transdiagnostic conceptual model. This analysis was based on data from the literature, structured according to three main directions: (1) current psychiatric perspective on diagnosing PG and related phenomena; (2) epidemiological, pathophysiological, and therapeutic data supporting a continuum model of PG; (3) clinical data on bereavement-related psychopathology. The integration of relevant data into a conceptual model of PG is expected to highlight the current state of research in the field and the main directions for future research, with the ultimate objective of improving the quality of medical assistance for patients with PG-related functional impairments.

## RESULTS

The retrieved corpus of literature was organized according to the formulated research objectives, resulting in a synthesis of evidence supporting a comprehensive theoretical model of PG.

### 1. Psychiatric perspective on prolonged grief

In the DSM-5 (2013), pathological grief was not recognized as an official mental disorder. Instead, it was included in Section III under the designation Persistent Complex Bereavement Disorder (PCBD), a category reserved for conditions requiring further research [31]. PCBD was characterized by persistent yearning for the deceased, intense emotional pain, difficulty accepting the loss, identity disruption, and significant impairment in social or occupational functioning [31]. Although the condition acknowledged that some bereaved individuals experience severe and enduring grief reactions, its placement in Section III reflected ongoing uncertainty regarding its diagnostic boundaries and distinction from other psychiatric disorders, particularly MDD and PTSD [31].

Accumulating empirical evidence over the following decade led to a substantial revision in the DSM-5-TR (2022), which formally recognized Prolonged Grief Disorder (PGD) as a distinct mental disorder [32]. The diagnosis applies to individuals who continue to

experience persistent longing or preoccupation with the deceased beyond culturally expected periods of mourning, accompanied by symptoms such as identity disruption, disbelief, avoidance of reminders of the loss, emotional numbness, intense loneliness, difficulty reintegrating into everyday life, or a sense that life has lost its meaning [32]. For adults, symptoms must persist for at least 12 months following the death and cause clinically significant distress or functional impairment [32].

The transition from PCBD to PGD reflects a broader shift toward recognizing pathological grief as a distinct clinical entity rather than merely a variant of depression or trauma-related disorders. Compared with the DSM-5 criteria, the DSM-5-TR diagnostic framework is more streamlined and clinically applicable, while retaining an emphasis on functional impairment and symptom persistence. Furthermore, the DSM-5-TR places greater emphasis on evaluating grief reactions within their cultural, religious, and social context to avoid pathologizing normative mourning experiences.

The revised diagnosis of PGD also aligns more closely with the ICD-11 classification of Prolonged Grief Disorder, reflecting growing international consensus regarding the nature and diagnosis of persistent maladaptive grief reactions [33]. According to the World Health Organization (WHO) classification, uncomplicated bereavement (code QE62) corresponds to normal grief, while PGD is a disturbance characterized by persistent and pervasive grief, with longing for the deceased or persistent preoccupation with the deceased, accompanied by intense emotional pain [33]. ICD-11 acknowledges that the duration of core PGD symptoms should be at least 6 months, and mentions that symptoms are in excess of cultural norms [33]. Grief reactions that are persistent, but within the normative period of grieving, taken into consideration the individual's cultural and religious context, are considered normal bereavement [33]. The functional impairment criterion is also mentioned by ICD-11 for PGD [33].

In conclusion, the conceptual evolution regarding pathological grief in the DSM-5 and 5-TR reflects significant advances in the understanding of this phenomenon, starting from clinical and epidemiological data. The formal recognition of PGD in the most recent nosographic systems acknowledges that, for some individuals, grief can become persistent, debilitating, and clinically significant beyond culturally expected periods of mourning. At the same time, both DSM-5-TR and ICD-11 emphasize the importance of considering normative religious and social contexts when evaluating grief responses, thereby distinguishing PG from normal bereavement, thus avoiding overpathologization. The increasing convergence between DSM-5-TR and ICD-11 criteria further supports an ongoing international consensus regarding the identification, diagnosis, and treatment of prolonged and maladaptive grief reactions, facilitating more accurate assessment and improved access to appropriate clinical care.

**Table 1:** Shared and disorder-specific mechanisms across bereavement-related psychopathology

| Mechanism | PGD | PTSD | MDD | ASD | AjD | Key references |
|---|---|---|---|---|---|---|
| **Attachment dysregulation** | ✓ | ± | ± | – | ± | [12-15,35,36] |
| **Persistent yearning/separation distress** | ✓ | – | – | – | ± | [32-35,48] |
| **Intrusive cognitions/memories** | ✓ | ✓ | ± | ✓ | ± | [35,36,50] |
| **Avoidance behaviors** | ✓ | ✓ | ± | ✓ | ± | [16-21,36] |
| **Emotional dysregulation** | ✓ | ✓ | ✓ | ✓ | ✓ | [35,36] |
| **Identity disruption** | ✓ | ± | ± | – | ± | [11,32,36,37] |
| **Meaning-making difficulties** | ✓ | ± | ± | – | ± | [23-25,29,30] |
| **Altered reward processing** | ✓ | ± | ✓ | – | – | [6,35] |
| **Fear/threat processing** | ± | ✓ | – | ✓ | ± | [48-53] |
| **Low mood/anhedonia** | ± | ± | ✓ | – | ± | [34,37,52,53] |
| **Functional impairment** | ✓ | ✓ | ✓ | ✓ | ✓ | [32,33,51] |

AjD= adjustment disorder, ASD= acute stress disorder, PGD= prolonged grief disorder, PTSD= posttraumatic stress disorder, MDD= major depressive disorder
Note: ✓ = core feature; ± = frequently present but not defining; – = not typically characteristic.

## 2. Epidemiological, pathophysiological, and therapeutic data

The existing data in the literature support a continuum model of PG, in which PGD, PTSD, acute stress disorder, MDD, and adjustment disorder represent partially overlapping but clinically distinguishable responses to loss-related stress, while preserving a certain degree of individual specificity. Epidemiological studies demonstrate substantial comorbidity between PGD, PTSD, and MDD, while also showing that PGD constitutes a distinct diagnostic construct characterized primarily by persistent separation distress, yearning, and preoccupation with the deceased rather than the fear-based symptoms of PTSD or the pervasive anhedonia and low mood typical of MDD [34,35].

Regarding the pathophysiology of these disorders, they were observed as sharing several mechanisms, including emotional dysregulation, intrusive cognitions, avoidance behaviors, alterations in reward processing, and difficulties integrating stressful experiences into autobiographical memory, suggesting common vulnerability pathways following severe stress or attachment loss [36,37]. However, PGD seems to involve grief-specific processes, especially persistent activation of attachment mechanisms, difficulty of accepting the irrefutability of the loss, and disturbance of identity and meaning-making following bereavement [36,37].

Therapeutic evidence is also supportive of such a continuum perspective. Although symptoms of depression, anxiety, and trauma may improve with general, non-specific psychotherapeutic and counseling interventions, grief-focused cognitive behavioral therapies consistently demonstrate superior efficacy in reducing PG manifestations and improving functional outcomes, thus showing that PG requires targeted treatment approaches beyond those developed for depression or trauma alone [23,38-40]. Treatments for MDD are continually expanding, but PGD-targeted pharmacological interventions are still lacking, maybe due also to the novelty of the diagnosis [41,42].

Consequently, PG may be understood as a distinct yet closely related condition within the broader spectrum of stress- and loss-related psychopathology, placed in an intermediate position between normative bereavement and other trauma- and stressor-related disorders. To further illustrate this diagnosis overlap and specificity of the principal mechanisms involved in PG-related psychopathology, the major transdiagnostic dimensions identified in the literature are summarized in Table 1.

## 3. Clinical evidence on bereavement-related psychopathology

Clinical studies support the idea that responses to bereavement arise along a spectrum from normal grief to psychiatric disorders, supporting a continuum model of bereavement-related psychopathology [43-46]. Longitudinal cohort studies indicate that the majority of individuals with bereavement experience a gradual decline in grief severity in time, while a significant minority develop persistent and impairing clinical manifestations [43-46]. Data in the literature suggest that almost 7–10% of bereaved adults meet criteria for PGD at 6–12 months after the loss of a dear one [35,47]. Comorbidity percentage across the spectrum varies, with between 20 and 30% of individuals with PGD meeting criteria for MDD, and up to 30–40% meeting criteria for PTSD; also, a substantial percentage of individuals with PTSD or MDD report a death loss as the precipitating event in the recent personal history [34,48,49]. Acute stress disorder is a nosologic entity occurring in the immediate aftermath of a loss and stands as a strong predictor of later PTSD and PGD onset [50].

**Table 2:** Clinical comparison between psychiatric disorders related to pathological grief

| Characteristics | Normal bereavement | Adjustment disorder | Acute stress disorder | Major depressive disorder | Posttraumatic stress disorder | Prolonged grief disorder |
|---|---|---|---|---|---|---|
| **Position on continuum** | Normative/adaptive | Mild psychopathology | Acute trauma response | Depressive syndrome | Trauma-related syndrome | Persistent grief syndrome |
| **Typical onset** | Immediate–weeks | Within weeks | Days to 1 month | Weeks–months | Weeks–months | ≥6 months (ICD-11); ≥12 months (DSM-5-TR) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Core symptoms** | Sadness, yearning, transient distress | Distress disproportionate to stressor; impaired functioning | Intrusions, avoidance, dissociation, arousal | Low mood, anhedonia, guilt, worthlessness | Re-experiencing, avoidance, hyperarousal | Persistent yearning, preoccupation, identity disruption |
| **Approximate prevalence among bereaved** | 80–85% | 10–20% | 10–30% (acute phase) | 10–20% | 10–20% | 7–10% |
| **Risk of persistence** | Low | Low–moderate | Moderate | Moderate–high | Moderate–high | High |
| **Key predictors** | — | Prior mental illness, low support | Intense acute distress, dissociation | Prior depression, functional impairment | Traumatic loss, prior trauma | Attachment insecurity, loss centrality, ongoing stressors |

Adjustment disorder, characterized by distress that is disproportionate to the stressor and associated functional impairment, is frequently observed following bereavement and typically represents a milder and more time-limited clinical response [51]. Factor-analytic and latent class studies consistently demonstrate that PGD, PTSD, and MDD are related but distinguishable syndromes with partially overlapping symptom clusters and disorder-specific core features: yearning and preoccupation with the deceased in PGD, fear-based re-experiencing and hyperarousal in PTSD, and pervasive low mood and anhedonia in MDD [37,52,53]. Analyzed together, these data are in favor of a dimensional stress-response continuum in which individual vulnerability, characteristics of the loss, and ongoing psychosocial stressors determine whether bereaved persons experience transient distress or progress toward persistent psychopathology (Table 2).

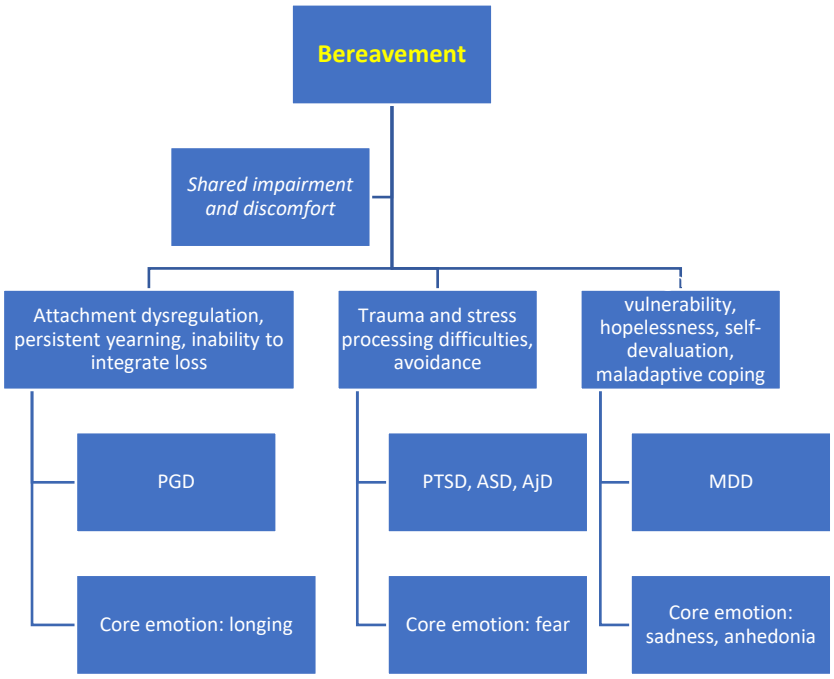
## DISCUSSION

Based on the presented data, the continuum hypothesis of grief-related phenomena, from a normal reaction to stress, to psychiatric disorders, can be framed into a conceptual model. Common vulnerability factors that may increase risk for MDD (reactive type), PTSD, acute stress disorders, adjustment disorder, and PGD are prior psychiatric history, insecure attachment, childhood adversity, social isolation, low resilience, female sex, sudden death, and violent death [34-40].

### A proposed model of shared vulnerability to stress and trauma-related disorders

This suggested conceptual model is based on the dominant mechanisms and main emotions identified for each disorder cluster during the literature search (Fig. 2). The three domains shown in Figure 2 were derived by clustering the mechanisms summarized in Table 1, thereby reuniting the most relevant features of each psychiatric disorder within this spectrum. Starting with the PGD, the dominant mechanisms include attachment dysregulation, separation distress, persistent yearning, and inability to integrate loss, while the core emotion placed at the core of the clinical manifestations may be considered longing.

**Figure 2:** Model of shared vulnerability and clinical features of pathological grief




AjD= adjustment disorder, ASD= acute stress disorder, PGD= prolonged grief disorder, PTSD= posttraumatic stress disorder, MDD= major depressive disorder

Regarding the vital stress and trauma processing cluster, the dominant mechanisms are considered traumatic exposure, threat processing, intrusive memories, and avoidance. This cluster of disorders shares the core emotion of fear (i.e., of death or destruction).

The third cluster, represented by disorders centered on depressive manifestations, which have as representative the MDD, has as dominant mechanisms hopelessness, self-devaluation, anhedonia, and negative cognitive schemas. The core emotions in these disorders are sadness and anhedonia.

Therefore, although the current nosographic systems treat PGD, PTSD, acute stress disorder, adjustment disorder, and MDD as separate entities, comorbidity is common, symptoms overlap, risk factors overlap, and treatment targets are also similar. In this context, bereavement-related psychopathology may be better conceptualized as a spectrum of partially overlapping syndromes rather than completely distinct disorders. Therapeutic management of MDD and PTSD benefits from the same lines of interventions [54-57]; extrapolation of these to PGD seems more than logical, at a clinical level.

The development of transdiagnostic frameworks is not unique to psychiatry, since similar integrative approaches have been proposed in other medical specialties where heterogeneous clinical manifestations arise from common underlying biological mechanisms. For example, systemic amyloidosis may present with diverse gastrointestinal, neurological, and cardiovascular manifestations despite sharing a common pathogenic substrate [58,59]. Such observations support the broader principle that apparently distinct clinical syndromes may be better understood through shared mechanistic pathways rather than exclusively categorical classifications. This perspective reinforces the rationale for conceptualizing bereavement-related psychopathology as a spectrum of partially overlapping disorders rather than completely independent entities [58,59]. The proposed framework adopts a dimensional perspective while preserving categorical distinctions. PGD remains a distinct diagnosis, but one that exists within a broader continuum of bereavement-related psychopathology. Shared mechanisms justify partial transfer of therapeutic principles across disorders, but available evidence indicates that grief-focused interventions remain preferable when PGD is the primary diagnosis.

Limitations of this research are related to the limited availability of studies dedicated to PGD, since it was only recently recognized in the DSM-5 TR (2022), and to the non-systematic strategy for collecting data from the literature. Also, this model requires empirical validation, which can only be achieved through prospective studies that monitor and treat PGD and related disorders.

Future directions of research include conducting large-scale trials focused on early detection markers for vulnerability to PG-related disorders, but also on new treatments, both pharmacological and psychotherapeutic, that can be used in approaching patients with complicated bereavement.

## CONCLUSION

The recognition of PGD has represented an important advance in bereavement psychiatry, reflecting the growing clinical relevance of this phenomenon. Nevertheless, the substantial overlap between PGD, PTSD, acute stress disorder, adjustment disorder, and MDD suggests that these conditions may represent related manifestations of a broader bereavement-response spectrum. A transdiagnostic framework may improve diagnostic precision, facilitate personalized interventions, and guide future research into the mechanisms that determine divergent trajectories following loss.

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***Authors' contribution***

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***Ethics approval and consent to participate***

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ARTICLE

# Cardiac Autonomic Response to Musical Rhythm: Sex-Specific Heart Rate Variability Modulations by Diverse Musical Genres

**Ilinca Savulescu-Fiedler[1,2], Sandica Bucurica[3,4*], Ionela Maniu[5,6], Constantin Pistol[7], Ioana Toader[2]**

[1] Department of Internal Medicine, "Carol Davila" University of Medicine and Pharmacy, 050474, Bucharest, Romania; ilinca.savulescu@umfcd.ro
[2] Department of Internal Medicine, Coltea Clinical Hospital, 030167, Bucharest, Romania; i.toader12@yahoo.com
[3] Department of Gastroenterology, "Carol Davila" University of Medicine and Pharmacy, 050474, Bucharest, Romania; sandica.bucurica@umfcd.ro
[4] Department of Gastroenterology, University Emergency Central Military Hospital "Dr. Carol Davila", 010825, Bucharest, Romania
[5] Department of Mathematics and Informatics, Faculty of Sciences, Lucian Blaga University Sibiu, 550012 Sibiu, Romania; ionela.maniu@ulbsibiu.ro
[6] Research Team, Pediatric Clinical Hospital Sibiu, 550166 Sibiu, Romania
[7] Physics Department, University of Bucharest, 405 Atomistilor Street, Bucharest, Romania; costi.pistol@fizica.unibuc.ro

*Correspondence: sandica.bucurica@umfcd.ro (S.B)

**Abstract:** Introduction: Heart rate variability (HRV) reflects autonomic nervous system (ANS) activity and emotional responsiveness. Music influences autonomic regulation through the heart–brain axis, yet sex-specific autonomic responses to different musical genres remain insufficiently characterized. Materials and methods: HRV parameters were recorded in 24 healthy young adults (17 females, 7 males; mean age 27.04 ± 1.97 years) at rest and during exposure to six musical genres: Baroque, Jazz, Heavy Metal, Romantic, Traditional Romanian, and Ambient. Time-domain and frequency-domain HRV parameters, including low frequency (LF), high frequency (HF), normalized LF (nLF), normalized HF(nHF), LF/HF ratio, and normalized coherence (NCoh), were analyzed. Anxiety was assessed using the Hospital Anxiety and Depression Scale (HADS).Results: Heart rate did not differ between rest and musical sessions. In contrast, significant changes were observed in HRV parameters, particularly in the frequency domain. Compared with rest, nLF and LF/HF increased, and nHF decreased during all musical sessions, with more pronounced effects in males. Differences between musical genres were limited, whereas sex-related differences were consistent across sessions. rMSSD and HF were strongly correlated in all conditions except Jazz in males. LF/HF correlated directly with nLF and NCoh and inversely with nHF. Conclusion: Music modulates cardiac autonomic balance without altering heart rate. Autonomic responses are predominantly sex-dependent rather than genre-dependent, underscoring the importance of sex-specific analysis in studies investigating music-induced autonomic modulation.

**Keywords:** autonomic nervous system; heart rate; HRV; rMSSD; normalized coherence

## 1.INTRODUCTION

"Life is about rhythm. We vibrate, our hearts are pumping blood, we are a rhythm machine, that's what we are" (Mickey Hart)

Heart rate exhibits continuous physiological variability driven by the dynamic interplay between the sympathetic and parasympathetic branches of the autonomic nervous system (ANS)(1,2). Heart rate variability (HRV), defined as the temporal fluctuations between consecutive cardiac cycles, is a validated marker of autonomic regulation and an indirect indicator of emotional and cognitive processing (2–5).

According to the neurovisceral integration model proposed by Thayer and Lane, HRV reflects the functional integrity of a network linking cortical and subcortical brain structures to autonomic output, supporting context-appropriate emotional regulation (6). Reduced HRV has been reported in stress-related and affective conditions, including anxiety and depressive symptomatology (5,7).

Music is a complex auditory stimulus that can elicit emotional responses and modulate autonomic function. Music engages central structures involved in autonomic control, including cortico-subcortical circuits, thereby influencing the heart–brain axis and downstream cardiovascular and respiratory rhythms (8–12).

**Citation**: Savulescu-Fiedler I, Bucurica S, Maniu I, Pistol C, Toader I. *Cardiac Autonomic Response to Musical Rhythm: Sex-Specific Heart Rate Variability Modulations by Diverse Musical Genres*. R. J. Mil. Med. 2026, CXXIX(4): 362-378 https://doi.org/10.55453/rjmm.2026.129.4.3

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Prior work suggests that musical tempo and arousal can shift the sympathetic-vagal balance, with more stimulating music generally associated with greater sympathetic activation (11,13–17). However, the available evidence remains heterogeneous across study designs and analytical choices (12,18,19).

Biological sex is a major determinant of baseline HRV and autonomic reactivity, including differences in sympathetic and parasympathetic markers (20). Despite this, sex-specific responses to musical exposure—particularly across multiple genres—remain underexplored (14). In addition, anxiety and depressive symptoms may influence baseline autonomic regulation and reactivity, potentially modifying music-induced HRV responses (5,7).

The present study aimed to investigate cardiac autonomic responses to musical rhythm across multiple musical genres using HRV analysis. Secondary objectives were to examine sex-specific differences in HRV modulation and explore the influence of anxiety symptoms on autonomic responses to music.

## 2. MATERIALS AND METHODS

### 2.1 Study objectives

The primary objective of this study was to assess ANS activity, as reflected in HRV, during exposure to musical stimuli across different genres and tempos. Secondary objectives included evaluating sex-specific differences in HRV responses and exploring the influence of anxiety on music-induced autonomic modulation.

### 2.2 Participants

A prospective study was conducted involving resident physicians of similar age, academic level, and apparent health status. All participants were informed about the study objectives and protocol and provided written informed consent before enrollment. Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS). The Hospital Anxiety and Depression Scale (HADS) is a well-established screening tool extensively used in both clinical and research settings and demonstrates robust psychometric performance (21). Prior validation studies have reported good reliability and internal consistency, with Cronbach's alpha coefficients generally ranging between 0.80 and 0.93 for the anxiety subscale. In this study, the Romanian validated version of the HADS was employed, which has been standardized for use in clinical and research populations (22). All participants completed the questionnaire in a self-administered format (22).

Exclusion criteria included known cardiovascular, neurological, or psychiatric disorders; current medication affecting autonomic function; and failure to comply with pre-recording instructions.

The sample size was determined by the limited duration of the study and the number of eligible participants available within that timeframe. The investigation was carried out during a predefined study period, and all resident physicians who met the inclusion criteria and provided informed consent were consecutively enrolled. Consequently, the final sample comprises the entire accessible population of eligible residents during the study interval. This recruitment strategy reduced the risk of selection bias while ensuring a relatively homogeneous cohort with respect to age, health status, and professional workload.

The study was conducted between May 28, 2024, and October 1, 2024, with approval by the Ethics Committee of Colțea Clinical Hospital (approval No. 6006/10.04.2024) and in accordance with the Declaration of Helsinki.

### 2.3 Experimental setting

Recordings were performed in a quiet, closed room maintained at a temperature of 20–24 °C. Only the participant and the investigator conducting the recordings were present. All electronic devices were removed during recording sessions to minimize interference.

Participants were instructed to avoid alcohol, caffeine, nicotine, chocolate, and energy drinks for at least one hour before testing, refrain from smoking for 30 minutes before recordings, and avoid intense physical activity for 12 hours beforehand, in line with standardized HRV recommendations (21). All recordings were conducted in the morning.

### 2.4 Recording protocol

HRV data were recorded using the emWave Pro system- Inner Balance app (HeartMath Inc., USA) with the Inner Balance application. After a 10-minute seated relaxation period, recordings were obtained under the following conditions:

**Resting session (session A):** seated position, no auditory stimulus, spontaneous breathing, 5-minute recording.

**Musical sessions:** six musical excerpts, each lasting 2 minutes, separated by 30-second silent (rest) intervals, presented in a fixed order:

- Session B: Baroque (J.S. Bach, Double Violin Concerto)
- Session C: Jazz (Dave Brubeck, Take Five)

- Session D: Heavy Metal (AC/DC, Big Gun)
- Session E: Romantic (W.A. Mozart, Piano Concerto No. 21)
- Session F: Traditional Romanian music (Clejani orchestra)
- Session G: Ambient music (432 Hz)

The auditory stimuli consisted of pre-existing musical excerpts selected to represent distinct stylistic genres. All tracks were obtained from publicly available recordings and were selected by the investigators to clearly reflect the defining musical characteristics of each genre (e.g., tempo, harmonic structure, rhythmic profile). To ensure methodological consistency, excerpts were standardized with respect to duration, sound intensity (volume level), and playback conditions, and were presented in a controlled environment under identical acoustic settings for all participants. Although a validated or psychometrically standardized musical stimulus database was not employed, all participants were exposed to the same predefined excerpts, thereby ensuring internal consistency of the experimental protocol. The absence of a validated musical stimulus set is acknowledged as a methodological limitation.

**2.5 HRV analysis**

HRV parameters were analyzed in both time and frequency domains (2,3) . Data were processed using MATLAB 2017b (MathWorks, Natick, MA, USA) and R v4.0.5.

Short-term and ultra-short recordings were analyzed using frequency-domain metrics, which are sensitive to time-window selection (3,22).

**Time-domain parameters, as follows:**

- SDNN: Standard deviation of all normal-to-normal (NN) intervals (2).
- rMSSD: root mean square of successive differences between NN intervals; surrogate of parasympathetic activity (23,24).

**Spectral domain parameters (absolute values), as follows:**

- TP (total power): representing all frequencies ≤ 0.4 Hz
- HF (high frequency ms2/Hz): frequency regime in the range 0.15-0.4 Hz; marker of parasympathetic activity.
- LF (low frequency ms2/Hz): frequency regime in the range 0.04-0.15 Hz; reflects both sympathetic and parasympathetic modulation, predominantly sympathetic.
- VLF (very low frequency ms2/Hz): recording frequencies between 0.0033-0.04 Hz; often associated with sympathetic activity.
- ULF (ultra- low frequency ms2/Hz): frequency regime ≤ 0.0033 Hz; its physiological significance remains uncertain

**Frequency-domain parameters (relative values):**

- nHF/nhf (the relative value of HF): represents the relative portion of the HF band in the TP band. It is calculated according the following formulas: nHF = HF / (TP – VLF) (24,25)
- nLF/nlf (the relative value of LF): represents the relative portion of the LF band in the TP band. It is calculated according to the following formulas: nLF = LF / (TP – VLF) (24,25)
- LF/HF ratio

The analyzed parameters are synthetically presented in Table 1.

**2.6 Statistical analysis**

Data analysis was performed using MATLAB (MathWorks, Natick, MA, USA) and R software (version 4.0.5). HR and HRV parameters were compared between rest and musical sessions and between musical genres. Linear regression models explored relationships between HRV parameters and age, sex, and anxiety score. Group comparisons were conducted using ANOVA for multiple comparisons or t-tests if appropriate. Correlations between HRV parameters were assessed using Spearman's rank correlation coefficient, and correlograms were used to display the (upper triangular) correlation matrix. Statistical significance was set at $p < 0.05$.

**Table 1:** The synthetic presentation of heart rate variability parameters analyzed

| Parameter | Significance | ANS- sympathetic and parasympathetic contributions |
|---|---|---|
| **Time domain** | | |
| SDNN (ms) | The standard deviation of the NN intervals | Sympathetic and parasympathetic contribution |
| rMSSD (ms) | The root mean square of the mean squared differences of successive NN intervals | A marker of parasympathetic nervous system activity |
| **Frequency domain** | | |
| TP (total power) ($ms^2$) | all frequencies ≤ 0.4 Hz | Sympathetic and parasympathetic contribution |
| HF (high frequency power) ($ms^2$) | frequencies between 0.15-0.4 Hz | A marker of parasympathetic nervous system activity |
| LF (low frequency power) ($ms^2$) | frequencies ranging from 0.04-0.15 Hz | Changes in sympathetic and parasympathetic activities |
| VLF (very-low frequency power) ($ms^2$) | frequencies between 0.0033-0.04 Hz | Increased sympathetic nervous system activity |
| nLF (nu)* | The relative portion of LF band in the TP of the power spectral domain | |
| nHF (nu)* | The relative portion of the HF band in the TP of the power spectral domain | |
| LF/HF | | The global sympathetic/parasympathetic balance |
| NCoh (normalized coherence) (%) | The amount of cardio-respiratory coherence(35). | |

*nu- normalized units; nLF= LF/TP-VLF (23), or nLF= LF/HF+LF (23); nHF= HF/TP-VLF , nHF= HF/HF+LF (2,23–26)

## 3. RESULTS

A total of 31 participants were initially enrolled in the study. Following visual inspection of the recordings, 24 subjects (17 females and 7 males) were retained for analysis. This quality control step was undertaken to prevent distortion of both time- and frequency-domain HRV parameters. Recordings were excluded in cases of excessive noise, missed or spurious beats, or when frequency values clearly exceeded the accepted physiological range described in the literature.

The final study cohort (general lot, GL), therefore, comprised 24 participants. The average age was 27.04 years (SD = 1.97). The mean anxiety score was 7.04 (SD = 3.97).

Based on HADS classification, participants were stratified into subgroups. According to anxiety scores, two subgroups were defined: A0–7 (score ≤ 7; n = 15; 7 males and 8 females) and A8–21 (score ≥ 8; n = 9; all females).

HRV parameters were analyzed in relation to age, anxiety, and depression scores, and comparative changes across experimental sessions were evaluated for each parameter. Across all musical exposures, heart rate remained stable and did not significantly differ from resting values. In contrast, several HRV parameters—particularly frequency-domain indices—demonstrated consistent modulation during music listening.

### 3.1 HRV Parameters Behavior Within Individual Musical Sessions

*Jazz Music*

Jazz exposure was characterized by higher LF/HF values in males overall (p=0.0321).

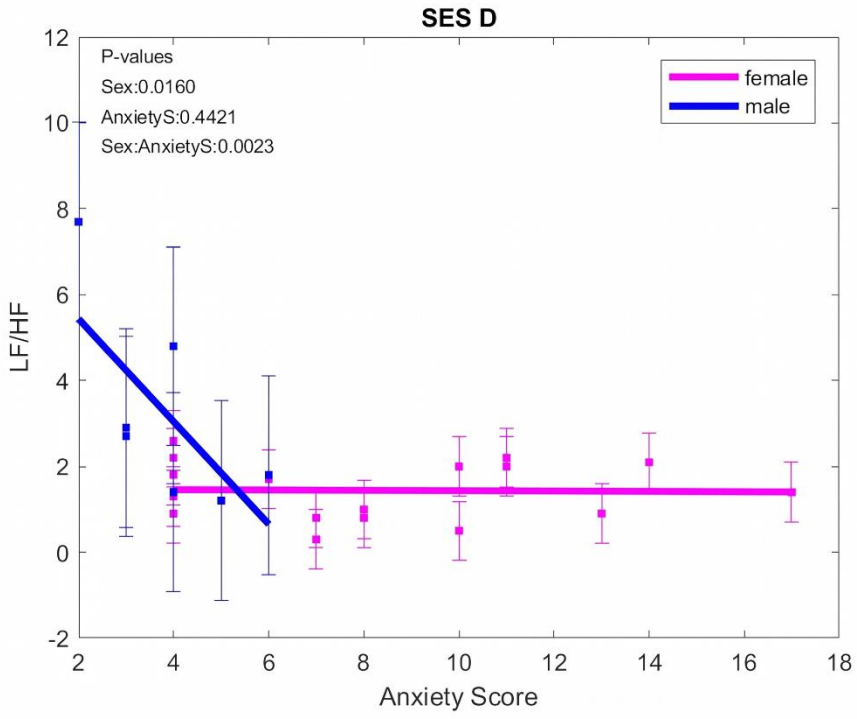
*Heavy Metal Music*

Heavy metal music elicited a pattern similar to Jazz in several respects. nLF and LF/HF were significantly higher in males (p=0.0317, respectively, p=0.0115). nHF remained higher in females (p=0.0317). LF/HF showed differences between sexes, and anxiety, decreasing in males with anxiety (p=0.0023) (Figure 1).

*Baroque Music*

During Baroque music exposure, nLF and LF/HF ratio were significantly higher in males compared with females (p= 0.0002, respectively, p= 0.0000), while nHF showed reciprocal elevation in females (p= 0.0002). These statistically significant sex-related differences were maintained when stratifying by anxiety scores, with divergent patterns, without a relationship with anxiety score. With increasing anxiety, nLF and LF/HF decreased in females but increased in males (p=0.0019 and p=0.0000, respectively), indicating divergent autonomic patterns. rMSSD declined with age in both sexes (p= 0.0486), without significant sex differences (Figure 2).

**Figure 1:** LF/HF variation by sex and anxiety scores in Heavy Metal music session (SES D- Heavy Metal music)




*Romantic Music*

Romantic music exposure was associated with increased nLF and LF/HF in males (p=0.0103 and p=0.0011, respectively) and higher nHF values in females (p=0.0103). These statistically significant sex-related differences were maintained when stratifying by anxiety scores, with divergent patterns, without a relationship with anxiety score. With increasing anxiety, nLF and LF/HF decreased in females but increased in males (p=0.0338 and p=0.0038, respectively), indicating divergent autonomic patterns. NCoh was higher in males (p=0.0303) and showed a tendency to decrease with age, though not statistically significant.

*Traditional Music*

Traditional music was associated with an increase in VLF power with increasing anxiety scores in both sexes (p=0.0058). rMSSD values were higher in females (p=0.0313) (Figure 3).

*Ambient Music*

During Ambient music, rMSSD was higher in females than in males, at rest (p=0.0206) and in anxiety (p=0.0254).

**3.2 HRV Parameters comparisons between Resting condition (session A) and Musical Sessions (session B to G)**

Comparative analysis between the basal (resting) condition and the various musical sessions revealed that the most consistent and pronounced modifications were observed in nLF, nHF, and the LF/HF ratio across the general study cohort, as well as in participants stratified by anxiety (A0–7) scores, as shown in Table 2.

**nLF Dynamics**

Across the general cohort, all musical genres were associated with a significant increase in nLF compared with the basal condition, with a more marked elevation in male participants.

In participants without anxiety (A0–7), nLF remained significantly elevated compared with rest during Baroque, Metal, Romantic, and Ambient sessions, again predominantly in males.

Overall, nLF increases were substantially greater in females than in males on GL and A0-7.

**nHF Modifications**

nHF displayed a reciprocal pattern to nLF. In the general cohort, nHF values decreased during all musical sessions compared with basal, with a more pronounced reduction in males.

Among participants without anxiety, Baroque, Metal, Romantic, and Ambient music were associated with significant decreases in nHF relative to rest, again more evident in males. Traditional music did not differ from basal in either sex.

**Figure 2:** VLF/TP variation by sex and anxiety scores in SES B- Baroque music

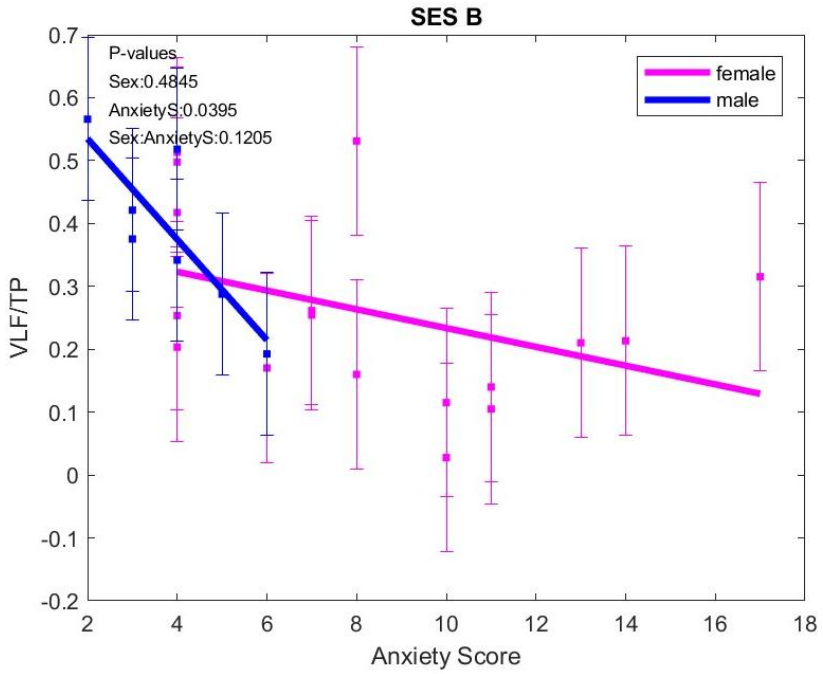



**Figure 3:** VLF variation by sex and anxiety scores in SES F – Traditional music session

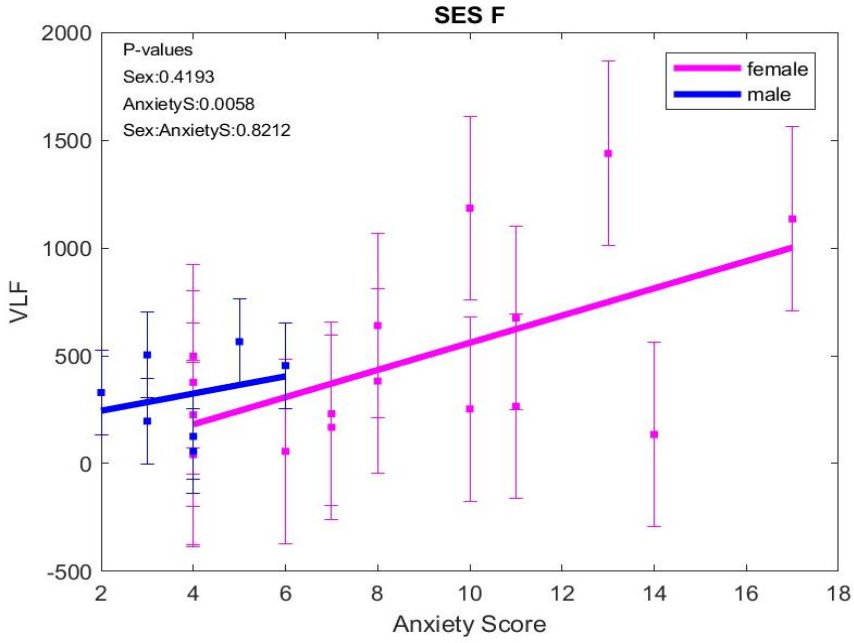


**Table 2:** The most significant differences between rest and musical sessions

| **For GL (24p)** | Baroque vs. Basal<br><br>F M<br>(p-value) | Jazz vs. Basal<br><br>F M<br>(p-value) | Metal vs. Basal<br><br>F M<br>(p-value) | Romantic vs. Basal<br><br>F M<br>(p-value) | Traditional vs. Basal<br><br>F M<br>(p-value) | Ambient vs. Basal<br><br>F M<br>(p-value) |
|---|---|---|---|---|---|---|
| nLF | + ++<br>0.0002[#]<br>0.0002* | + ++<br>0.0250[#]<br>0.0030* | + ++<br>0.0119[#]<br>0.0006* | + ++<br>0.0040[#]<br>0.0032* | + ++<br>0.0523[#]<br>0.0012* | + ++<br>0.0010[#]<br>0.0002* |
| nHF | - - -<br>0.0004[#] | - - -<br>0.0337[#] | - - -<br>0.0175[#] | - - -<br>0.0060[#] | - - -<br>0.0638[#] | - - -<br>0.0018[#] |
| LF/HF | + ++<br>0.0000[#]<br>0.0005* | + ++<br>0.0044[#]<br>0.0091* | + ++<br>0.0020[#]<br>0.0156* | + ++<br>0.0003[#]<br>0.0130* | + +<br>0.0085* | + ++<br>0.0010[#]<br>0.0020* |
| NCoh | + +<br>0.0518* | | | - +<br>0.0575[#] | | |
| **For A0-7 (15p)** | | | | | | |
| HF | - -<br>0.0070[#]<br>0.0070* | - -<br>0.0003* | - -<br>0.0056* | | | |
| nLF | + ++<br>0.0028[#]<br>0.0010* | + ++<br>0.0516[#] | + ++<br>0.0365[#]<br>0.0583* | + + +<br>0.0278#<br>0.0413* | | + ++<br>0.0043[#]<br>0.0227* |
| nHF | - - -<br>0.0028[#]<br>0.0010* | - - -<br>0.0516[#] | - - -<br>0.0365[#]<br>0.0583* | - - -<br>0.0279[#]<br>0.0413* | | - - -<br>0.0043[#]<br>0.0227* |
| LF/HF | + ++<br>0.0007[#]<br>0.0003* | + ++<br>0.0258[#]<br>0.0323* | + ++<br>0.0291[#]<br>0.0442* | + + +<br>0.0118[#]<br>0.0183* | + +<br>0.0332* | |
| NCoh | + +<br>0.0104* | | - +<br>0.0198[#]<br>0.0442[*] | | | |

*significant p-value for differences between sessions; # significant p-value for differences between sexes; + indicates a higher value, - indicates a lower value, ++indicates a greater increase, - - indicates a greater decrease; F-female participant, M-male participant; the basal session represented the reference; nLF= LF/HF+LF, nHF= HF/HF+LF, HF- high frequency, LF- low frequency, NCoh- normalized coherence
A0-7= participants without anxiety

**LF/HF Ratio**

The LF/HF ratio demonstrated the most homogeneous and robust pattern across all analyzed groups. In the general population and in anxiety stratified subgroups, LF/HF increased significantly during all musical sessions compared with basal conditions, with greater increments in males.

An exception was observed in Ambient music among participants without anxiety, where the increase was less consistent. For Traditional music, differences in the general cohort and in participants without depression were detected primarily between sessions rather than between sexes.

These findings reinforce the interpretation that musical exposure, irrespective of genre, shifts autonomic balance toward relative sympathetic predominance, particularly in male participants.

**NCoh and Secondary Parameters**

Normalized coherence (NCoh) exhibited a more restricted and genre-specific modulation pattern. During Baroque exposure, NCoh increased compared with basal in the general cohort and in participants without anxiety, without sex differences. In contrast, Romantic music induced divergent responses: NCoh increased in males but decreased in females in both the general cohort and the A0–7 subgroup.

Additional parameters showed only minor or isolated changes. rMSSD decreased in the A0–7 subgroup during Jazz sessions.

**Overall Interpretation**

When compared with the resting condition, musical exposure consistently modulated frequency-domain HRV parameters, with nLF and LF/HF increasing and nHF decreasing across most genres. These effects were amplified in males and were more consistent than changes observed in secondary parameters. Genre-specific variations were present but generally secondary to the overarching autonomic shift toward sympathetic predominance.

The normalized low-frequency component (nLF) increased during all musical sessions compared with baseline, particularly in males. This pattern was observed in the general cohort and in participants without anxiety.

Among subjects without anxiety, no significant differences were found between Traditional music and other sessions. During Jazz music listening, differences were observed only between sexes rather than between sessions and baseline.

For nHF, significant differences were primarily sex-related, with a more pronounced decrease in males across sessions.

The LF/HF ratio demonstrated the most consistent pattern across groups. Increases were observed in all musical sessions compared with baseline, again more pronounced in males. The only exception occurred during Ambient music listening in participants without anxiety.

NCoh demonstrated a more limited pattern of variation. It increased during Baroque music listening compared with rest, regardless of sex, in both the general cohort and participants without anxiety. NCoh increased in males but decreased in females during Metal music compared with baseline in participants without anxiety.

Taken together, these results may confirm that musical stimulation exerts measurable and reproducible effects on cardiac autonomic balance, with sex representing a principal determinant of magnitude and direction of response.

**3.3 HRV Parameters Comparative Analysis Across Musical Sessions in the General Lot and Subgroups (A0-7)**

A comparative evaluation of HRV parameters across musical sessions demonstrated that inter-session variability was predominantly sex-dependent rather than magnitude-dependent. In other words, statistically significant differences between musical genres were largely attributable to divergent male–female autonomic responses, while absolute parameter amplitudes remained broadly comparable across sessions (Table 3)

**Table 3:** The most significant differences between musical sessions regarding the parameters that had the most variation in the general study cohort, with significant differences by sex, in the general study population

| **GL** (24p) | rMSSD<br>F M<br>(p-value) | HF<br>F M<br>(p-value) | nLF<br>F M<br>(p-value) | nHF<br>F M<br>(p-value) | LF/HF<br>F M<br>(p-value) | NCoh<br>F M<br>(p-value) | VLF/TP<br>F M<br>(p-value) |
|---|---|---|---|---|---|---|---|
| Jazz vs. Baroque | + -<br>0.0504 | | 0 -<br>0.0000 | - +<br>0.0000 | + -<br>0.0000 | - - -<br>0.0225 | ++ +<br>0.0363 |
| Metal vs. Baroque | | | + -<br>0.0000 | - +<br>0.0000 | + -<br>0.0000 | - - -<br>0.0323 | |
| Romantic vs.Baroque | | | 0 -<br>0.0000 | + ++<br>0.0000 | - +<br>0.0000 | - - -<br>0.0120 | |
| Traditional vs. Baroque | + -<br>0.0155 | ++ +<br>0.0376 | + -<br>0.0001 | - +<br>0.0001 | + -<br>0.0003 | | ++ +<br>0.0548 |
| Ambient vs. Baroque | + -<br>0.0145 | + ++<br>0.0417 | + -<br>0.0000 | - +<br>0.0000 | + -<br>0.0000 | | |
| Metal vs. Jazz | + ++<br>0.0102 | | 0 +<br>0.0024 | - -<br>0.0024 | - +<br>0.0004 | + -<br>0.0097 | |
| Romantic vs. Jazz | + ++<br>0.0151 | | - +<br>0.0008 | + -<br>0.0008 | - +<br>0.0001 | - - -<br>0.0002 | |
| Traditional vs. Jazz | + -<br>0.0027 | + -<br>0.0382 | ++ +<br>0.0117 | - - -<br>0.0109 | ++ +<br>0.0410 | | |
| Ambient vs. Jazz | ++ +<br>0.0015 | ++ +<br>0.0365 | 0 +<br>0.0002 | 0 -<br>0.0002 | + ++<br>0.0002 | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Romantic vs. Metal | + 0.0224 | ++ | | - 0.0003 | + | + - 0.0003 | | - + 0.0000 | | - 0.0066 | 0 | |
| Traditional vs. Metal | + 0.0039 | - | ++ 0.0324 | + | + 0.0061 | - | - + 0.0056 | | | | | |
| Ambient vs. Metal | + 0.0025 | - | - 0.0455 | + | 0 0.0001 | + | 0 - 0.0001 | | + ++ 0.0001 | | | |
| Traditional vs. Romantic | + 0.0054 | - | + 0.0518 | - | + 0.0020 | - | - + 0.0019 | | + - 0.0013 | | + - 0.0562 | |
| Ambient vs. Romantic | + 0.0038 | - | - 0.0065 | - - | + 0.0000 | ++ | - - 0.0000 | | + - 0.0000 | | + - 0.0430 | |
| Ambient vs. Traditional | - 0.0007 | + | - 0.0029 | + | - 0.0006 | + | + - 0.0005 | | - + 0.0046 | | | |

GL- general study cohort; significant p-value for differences between sexes; + indicates a higher value, - indicates a lower value, ++indicates a greater increase, - - indicates a greater decrease; 0- no change; F- female subject, M- male subject; rMSSD- root mean square of the mean squared differences of successive NN intervals, HF- high frequency, nLF= LF/HF+LF, nHF= HF/HF+LF, NCoh- normalized coherence VLF- very low frequency, TP- total power; statistical significance P<0.05

**General Study Cohort**

In the general population, frequency-domain indices (nLF, nHF, LF/HF) and selected time-domain parameters (rMSSD) exhibited the most consistent inter-session contrasts.

For nLF, differences between musical genres reflected inverse modulation patterns in females and males. Comparisons such as Jazz vs. Baroque and Romantic vs. Baroque showed either stability or increases in females, accompanied by decreases in males. Conversely, transitions involving Traditional or Ambient music frequently induced increases in females and reductions in males, reinforcing the presence of sex-opposed autonomic adjustments rather than genre-specific amplitude effects.nHF demonstrated a reciprocal pattern relative to nLF. In multiple comparisons (e.g., Jazz vs. Baroque, Metal vs. Baroque), females tended to show increases while males exhibited decreases, or vice versa, indicating alternating parasympathetic predominance depending on sex.

The LF/HF ratio showed coherent shifts mirroring nLF dynamics. Across numerous pairwise comparisons, males demonstrated larger increases or smaller decreases than females, emphasizing a stronger sympathetic reactivity to changes in musical structure. Notably, comparisons involving Ambient or Traditional music frequently accentuated these divergences.

rMSSD exhibited variable but interpretable trends. In comparisons such as Metal vs. Jazz or Romantic vs. Metal, both sexes showed increases, although the magnitude was often greater in males. These findings suggest that transitions toward more stimulating genres were accompanied by augmented short-term vagal modulation.

NCoh displayed selective modifications, particularly in comparisons involving Baroque and Romantic sessions. In several instances, females demonstrated greater decreases, whereas males exhibited relative stability or increases, suggesting sex-differentiated central–autonomic coupling mechanisms.

The comparative evolution of HRV parameters that showed significant differences between musical sessions, and the relationship between music genre and the sympathetic and parasympathetic balance in males and females, are presented in Table 5.

**Table 4:** The synoptic presentation of the parameter dynamics and comparative sessions on the general study cohort by sex parameter variability without session differences

| | **nLF** | | **RMSSD** | | **HF** | | **nHF** | | **LF/HF** | | **NCoh** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compared sessions | F | M | F | M | F | M | F | M | F | M | F | M |
| Jazz vs. Baroque | ↔ | ↓ | ↑ | ↓ | | | ↓ | ↑ | ↑ | ↓ | ↓↓ | ↓ |
| Metal vs. Baroque | ↑ | ↓ | | | | | ↓ | ↑ | ↑ | ↓ | ↓↓ | ↓ |
| Romantic vs. Baroque | ↔ | ↓ | | | | | ↑ | ↑↑ | ↓ | ↑ | ↓↓ | ↓ |
| Traditional vs. Baroque | ↑ | ↓ | ↑ | ↓ | ↑↑ | ↑ | ↓ | ↑ | ↑ | ↓ | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ambient vs. Baroque | ↑ | ↓ | ↑ | ↓ | ↑ | ↑↑ | ↓ | ↑ | ↑ | ↓ | | |
| Metal vs. Jazz | ↔ | ↑ | ↑ | ↑↑ | | | ↓ | ↓↓ | ↓ | ↑ | ↑ | ↓ |
| Romantic vs. Jazz | ↓ | ↑ | ↑ | ↑↑ | | | ↑ | ↓ | ↓ | ↑ | ↓ | ↑ |
| Traditional vs. Jazz | ↑↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↓↓ | ↓ | ↑↑ | ↑ | | |
| Ambient vs. Jazz | ↔ | ↑ | ↑↑ | ↑ | ↑↑ | ↑ | ↔ | ↓ | ↑ | ↑↑ | | |
| Romantic vs. Metal | ↓ | ↑ | ↑ | ↑↑ | | | ↑ | ↓ | ↓ | ↑ | ↓ | ↔ |
| Traditional vs. Metal | ↑ | ↓ | ↑ | ↓ | ↑↑ | ↑ | ↓ | ↑ | | | | |
| Ambient vs. Metal | ↔ | ↑ | ↑ | ↓ | ↓ | ↑ | ↔ | ↓ | ↑ | ↑↑ | | |
| Traditional vs. Romantic | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↑ | ↑ | ↓ | ↑ | ↓ |
| Ambient vs. Romantic | ↑ | ↑↑ | ↑ | ↓ | ↓ | ↓↓ | ↓ | ↓↓ | ↑ | ↓ | ↑ | ↓ |
| Ambient vs. Traditional | ↓ | ↑ | ↓ | ↑ | ↓ | ↑ | ↑ | ↓ | ↓ | ↑ | | |

F- female subject, M- male subject; HF- high frequency, ↓- decrease; ↑- increase; ↔- unchanged values,↑↑-indicates a greater increase,↓↓-indicates a greater decrease; rMSSD- root mean square of the mean squared differences of successive NN intervals, HF- high frequency, LF- low frequency, NCoh- normalized coherence

*Anxiety-Free Group (A0–7)*

In participants without anxiety, inter-session comparisons revealed predominantly sex-related differences without consistent session-specific amplitude effects.

VLF/TP was lower in females during Traditional compared with Jazz, Romantic, and Metal sessions, without significant male variation. Conversely, Metal vs. Jazz comparisons showed higher VLF/TP in females.

rMSSD increased during Metal, Romantic, and Ambient sessions compared with Jazz, suggesting that less rhythmically complex or more immersive genres may enhance parasympathetic modulation in anxiety-free individuals. However, these effects remained sex-modulated.

*Overall Interpretation*

The integrated comparative analysis confirms that transitions between musical genres do not produce large, uniform shifts in HRV magnitude. Instead, they generate sex-specific directional adjustments in autonomic balance.

Across the general and anxiety-free groups, the dominant pattern was characterized by:

- Reciprocal modulation of nLF and nHF between sexes.
- Consistent LF/HF shifts reflecting stronger sympathetic dominance in males.
- Selective modulation of coherence (NCoh), suggesting differentiated central–autonomic integration.

Collectively, these findings indicate that the autonomic response to different musical genres is governed primarily by sex-related regulatory mechanisms rather than by intrinsic genre intensity alone. The stability of overall HRV magnitude, coupled with directional sex-specific shifts, supports the concept that musical processing engages adaptive autonomic tuning rather than indiscriminate sympathetic activation.

**3.4 HRV Parameters Comparative Analysis Across Musical Sessions in Subgroups with Anxiety (A8–21)**

In the anxiety subgroup (A8–21), autonomic modulation was primarily characterized by redistribution within the total spectral power rather than marked global HRV shifts.

Among females with anxiety, VLF/TP increased during Jazz, Romantic, and Traditional sessions compared with basal, demonstrating a pattern opposite to that observed in females without anxiety.

Direct comparisons between females with and without anxiety revealed minimal differences across musical genres. The only significant divergence emerged during the Traditional session, where VLF values were higher in anxious females, while lower values were recorded in non-anxious participants.

Overall, in participants with clinically relevant anxiety symptoms, musical exposure predominantly induced shifts in spectral distribution (VLF/TP) and selective modulation of vagal indices (rMSSD, HF), rather than uniform changes in global autonomic balance. These findings support the hypothesis that psychological burden modifies the qualitative pattern of autonomic adaptation to musical stimuli.

**3. 5. Overall results- HRV Parameter Variations Between Musical Sessions in GL and Subgroups.**

Among the HRV parameters analyzed, HF, rMSSD, nLF, nHF, LF/HF, and normalized coherence (NCoh) showed the most notable variations across sessions and between sexes.

HF values decreased during comparisons such as Ambient vs. Romantic music in both sexes, while increases were observed in comparisons including Traditional and Ambient vs. Baroque, Ambient vs. Jazz, and Traditional vs. Metal. Some comparisons showed opposite trends between sexes.

rMSSD showed substantial variability. Increases were observed in both sexes during Metal, Romantic, and Ambient sessions compared with Jazz. Conversely, decreases were observed in females and increases in males during Ambient vs. Traditional sessions. Additional opposite patterns between sexes were also observed across several session comparisons.

nLF values increased in both sexes during comparisons such as Traditional vs. Jazz and Ambient vs. Romantic. However, divergent sex-related patterns were evident in several other comparisons, emphasizing the influence of gender on autonomic responses.

The nHF parameter demonstrated a largely mirrored pattern compared with nLF. Decreases were observed in both sexes in comparisons such as Metal and Traditional vs. Jazz and Ambient vs. Romantic, whereas increases were noted in Romantic vs. Baroque sessions.

The LF/HF ratio exhibited consistent increases across several comparisons, particularly during Traditional and Ambient sessions relative to Jazz and Ambient vs. Metal. Nevertheless, opposite patterns between males and females were frequently observed.

NCoh values were generally lower during Jazz, Metal, and Romantic sessions compared with Baroque sessions. Some sex-specific patterns were also identified, including decreases in females and increases in males during Romantic vs. Jazz comparisons.

**3.6 Correlations between HRV parameters**

Across all sessions, LF/HF was strongly and directly correlated with nLF and inversely correlated with nHF ($p < 0.001$), confirming the expected relationship between normalized spectral components and sympathovagal balance. Similarly, HF and rMSSD showed strong positive correlations in all groups and sessions, except during Jazz music exposure in males.

NCoh demonstrated a more variable pattern, with correlations differing according to sex and anxiety status. In contrast to the stable relationships observed among conventional HRV indices, coherence-related associations appeared to be influenced by both musical genre and participant characteristics.

Overall, the principal autonomic response to music was reflected by consistent changes in nLF, nHF, and LF/HF, whereas coherence-related measures showed more selective and context-dependent modulation (Figure 4).

**3.7 HRV Evolution in Relation to Age, Sex, and Anxiety**

For Baroque music, nLF and LF/HF values were generally higher in males, whereas females demonstrated reciprocal trends, independent of anxiety scores. The parameters nLF, nHF, and LF/HF showed variable relationships with age and psychological scores. (Table 5)

During Metal music exposure, nLF increased with age in females and remained relatively stable in males, although absolute values were higher in males. In contrast to Baroque music, the evolution of nLF and LF/HF with anxiety or depression in males showed an opposite trend.

In the Jazz session, LF/HF showed minimal variation with age in males but tended to increase with age in females. Additionally, LF/HF demonstrated a quasi-linear relationship with anxiety scores in females, whereas in males it showed an inverse relationship with these scores.

In the Romantic music session, nLF displayed divergent age-related patterns between sexes without reaching statistical significance, although values remained higher in males. nLF increased in males and decreased in females with increasing anxiety scores.

During Ambient music listening, nLF increased with age in both sexes, again with higher values in males.

The VLF component (or VLF/TP ratio) demonstrated distinct patterns depending on musical style:

- increasing with anxiety score during Traditional music,
- decreasing with anxiety score during Baroque music

The rMSSD parameter decreased with age during Baroque music listening in both sexes. Sex-related differences were observed only during Traditional and Ambient music sessions, where rMSSD values were higher in females. During Ambient music listening, rMSSD followed the same age-related decreasing trend but remained higher in females.

During the Jazz session, NCoh decreased with age in females but increased with age in males. NCoh also decreased with increasing anxiety scores in both sexes. During the Romantic session, NCoh decreased with age in both sexes, although values remained higher in males, and decreased with anxiety scores in females.

**Figure 4:** Figure 4 A, B, C, D: Correlations between HRV parameters in female and male individuals, and in subjects with and without anxiety. rMSSD-root mean square of the mean squared differences of successive NN intervals; LF- low frequency; HF- high frequency; LF- low frequency; NCoh- normalized coherence

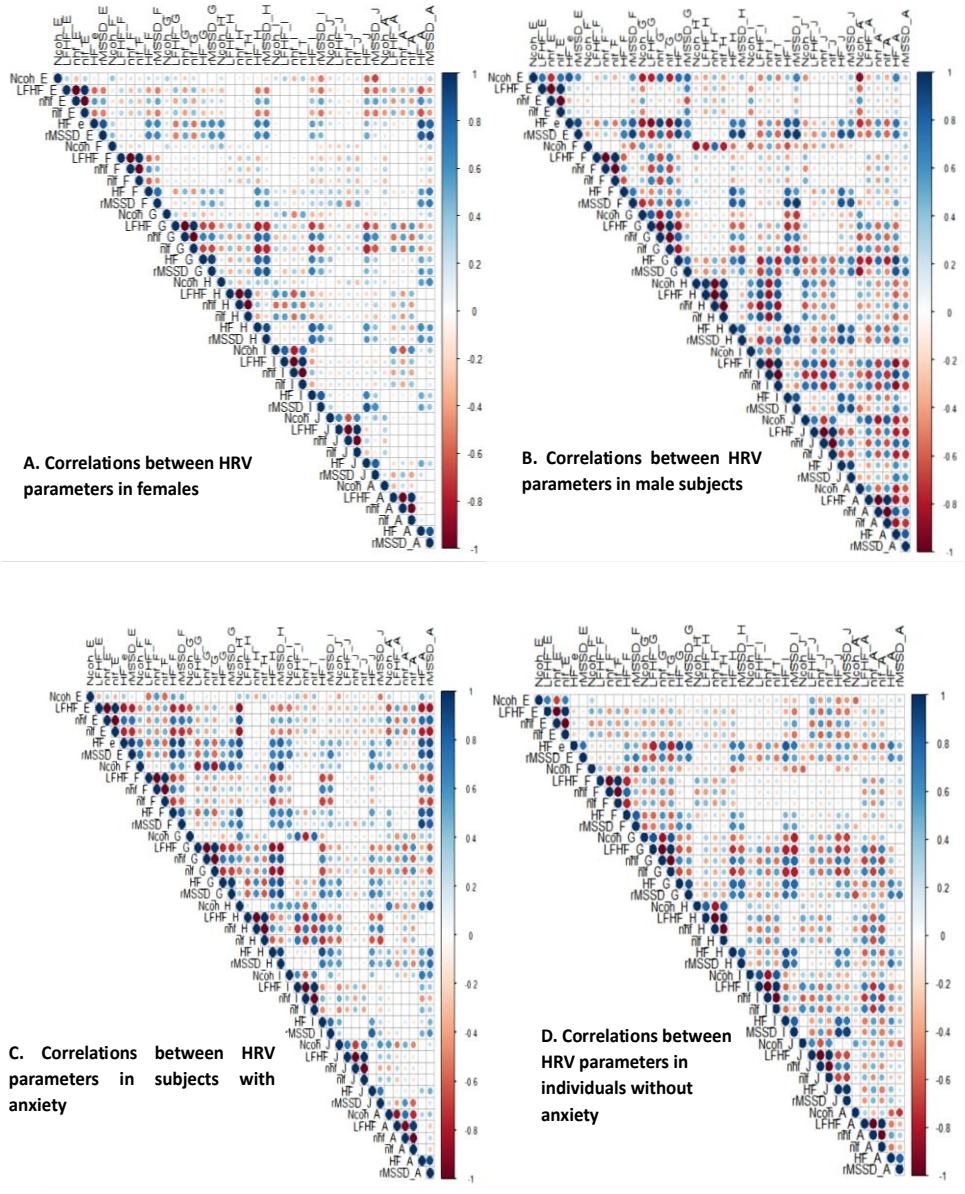


**Table 5:** HF, nHF, LF/HF, and NCoh variation in the compared sessions by sex in the general study cohort

| Evolution of HF by sex | Comparison between sessions |
|---|---|
| ↓ in both sexes | Ambient vs. Romantic |
| ↑ in both sexes | Traditional and Ambient vs. Baroque; Ambient vs. Jazz; Traditional vs. Metal |
| ↓ in females and ↑ males | Ambient vs. Metal and Traditional |
| ↑ in females and ↓ males | Traditional vs. Jazz and Romantic |
| **Evolution of rMSSD by sex** | |
| ↑ for both sexes | Metal, Romantic, and Ambient vs. Jazz; Romantic vs. Metal |
| ↓ in females and ↑ males | Ambient vs. Traditional |
| ↑ in females and ↓ males | Jazz, Traditional, Ambient vs. Baroque; Traditional vs. Jazz, Metal, and Romantic; Ambient vs and Romantic |
| **Evolution of nLF by sex** | |
| ↑ in both sexes | Traditional vs. Jazz; Ambient vs. Romantic |
| ↓ in females and ↑ males | Romantic vs. Jazz and Metal; Ambient vs. Traditional |
| ↑ in females and ↓ males | Metal, Traditional, Ambient vs. Baroque; Traditional vs. Metal and Romantic |
| ↔ | In females: Jazz vs Baroque; Romantic vs. Baroque; Metal vs. Jazz; Ambient vs. Metal and Jazz |
| **Evolution of nHF by sex** | |
| ↓ in both sexes | Metal and Traditional vs. Jazz; Ambient vs. Romantic |
| ↑ in both sexes | Romantic vs. Baroque |
| ↓ in females and ↑ males | Jazz, Metal, Traditional, and Ambient vs. Baroque; Traditional vs. Metal and Romantic |
| ↑ in females and ↓ males | Romantic vs. Jazz and Metal; Ambient vs. Traditional |
| ↔ | In females: Ambient vs. Jazz and Metal |
| **Evolution of LF/HF by sex** | |
| ↑ in both sexes | Traditional and Ambient vs. Jazz; Ambient vs. Metal |
| ↓ in females and ↑ males | Romantic vs. Baroque and Jazz; Metal vs. Jazz; Ambient vs. Traditional |
| ↑ in females and ↓ males | Jazz, Metal, Traditional, and Ambient vs. Baroque; Ambient vs. Romantic |
| **Evolution of NCoh by sex** | |
| ↓ in both sexes | Jazz, Metal, and Romantic vs. Baroque |
| ↓ in females and ↑ males | Romantic vs. Jazz |
| ↑ in females and ↓ males | Metal vs. Jazz; Traditional and Ambient vs. Romantic |
| ↔ | In males: Romantic vs. Metal |

F- female subject, M- male subject; HF- high frequency, ↓- decrease; ↑- increase; ↔- unchanged values; rMSSD- root mean square of the mean squared differences of successive NN intervals, HF- high frequency, LF- low frequency, NCoh- normalized coherence.

## 4. DISCUSSION

Our study found no significant changes in heart rate (HR) when comparing musical exposure with the resting condition or when comparing different musical sessions with each other. Some studies have reported that music with positive emotional valence is associated with higher HR values (28,29).

Our findings suggest that different musical styles influence time-domain and spectral heart rate variability (HRV) parameters in distinct ways. Significant variations were observed in several HRV parameters between rest and musical listening sessions, primarily expressed as differences between sessions rather than absolute value changes. Importantly, most variations were sex-related rather than magnitude-related, indicating that gender plays a significant role in autonomic responses to music.

A PubMed-based review of 26 studies conducted between 1980 and 2020 examining musical interventions in healthy and diseased populations reported heterogeneous results. Some authors concluded that the highest HRV values occur during mantra singing, whereas others observed no significant difference between mantra singing and conventional music listening with respect to HRV parameters (19,30).

Research evaluating the effects of musical style and rhythm on HRV has produced inconsistent results (10). Slow-tempo music, such as classical or meditative compositions, has been associated with reduced HR and increased relaxation, although contradictory findings have also been reported. For example, one study involving 11 healthy males exposed to 20 minutes of heavy metal music (Gamma Ray) and 20 minutes of baroque music (Pachelbel) compared the effects of these genres on HRV parameters, including LF, HF, nLF, nHF, LF/HF, rMSSD, pNN50, and SDNN (31). No significant differences between the two musical sessions were identified.

### HRV Differences Between Musical Styles

Among time-domain HRV parameters, rMSSD showed the most extensive variation. In participants without anxiety, rMSSD (and HF) decreased only during Jazz sessions compared with other musical styles, without sex differences. This observation aligns with findings

from other authors suggesting that baroque music does not significantly affect parasympathetic time-domain parameters such as rMSSD and pNN50 (13).

No significant variation in SDNN was observed across musical sessions compared with rest. However, some authors have reported lower SDNN values during stimulating music and higher values during calming music (13,15,20,32).

rMSSD increased in both sexes during Metal, Romantic, and Ambient music compared with Jazz, and was higher during Romantic than Metal sessions. TP, LF, and VLF did not show significant changes across sessions.

HF decreased during Ambient music compared with Romantic music. HF was also reduced during Baroque, Jazz, and Metal sessions in individuals without anxiety. HF values were higher during Romantic sessions than Ambient sessions, and higher during Metal, Traditional, and Baroque sessions than in other genres.

Sex differences were also observed. HF values were lower in females during Jazz, Romantic, Ambient, and Traditional sessions compared with other genres, but higher during Metal and Traditional sessions compared with Jazz.

Consistent with previous studies, our results support the observation that both LF and HF components are lower during music listening than during silence and are further reduced during stimulating music compared with relaxing music. Other authors reported that relaxant music reduces the LF/HF ratio and LF index and increases the HF index in both sexes (13). The HF component was higher in silence and while listening to relaxing music (13).

**Comparison With Previous Studies**

Previous studies reported that heavy metal music decreases SDNN, LF, nLF, HF, and nHF, whereas Baroque music reduces LF power and the LF/HF ratio (28).

Another study involving 21 healthy female students comparing heavy metal music (Gamma Ray) with Romantic music (Schumann or Chopin) concluded that neither musical fragment significantly altered HR, although heavy metal exposure showed a trend toward decreased vagal modulation (31,33).

In another investigation comparing musical works by Mozart and Strauss with ABBA compositions, only Mozart and Strauss significantly reduced blood pressure and HR. Mozart's music has been widely recommended as relaxation-inducing (34).

Heavy metal music has been associated with greater decreases in SDNN compared with Baroque music (35). The same authors reported that Baroque music primarily reduces LF activity, reflecting decreased sympathetic modulation, whereas heavy metal music reduces both LF and HF amplitudes, indicating reductions in both sympathetic and parasympathetic tone (14,31,33,34).

Other authors have suggested that relaxing music reduces the LF/HF ratio and LF index while increasing HF index in both sexes .(14) HF values were higher during silence and while listening to relaxing music. (14)

This study has several limitations. The groups with and without depression were unequal in size, and the anxiety and depression groups consisted exclusively of female participants. Additionally, inclusion criteria were based on academic and professional homogeneity rather than anxiety or depression scores.

A major strength of the study is the inclusion of a wide range of musical styles and rhythms. We compared HRV parameters during musical exposure and resting conditions and examined variations across musical genres while accounting for sex and anxiety.

One of the most consistent findings of the present study was the presence of sex-related differences in autonomic responses across musical genres. Although all musical stimuli induced modifications in frequency-domain HRV parameters, the direction and magnitude of these changes differed substantially between females and males, suggesting that biological sex may be a stronger determinant of autonomic responsiveness than musical genre itself.

During Baroque music exposure, females exhibited higher nHF values, whereas males showed higher nLF and LF/HF ratios. Because nHF is generally considered a marker of parasympathetic modulation, while higher nLF and LF/HF values indicate relative sympathetic predominance, these findings suggest a greater vagal contribution in females and a stronger sympathetic response in males during exposure to Baroque music. Furthermore, the decrease in VLF/TP with increasing anxiety scores may indicate reduced sympathetic activation among individuals with higher anxiety levels while listening to Baroque music, although this interpretation should be approached cautiously given the ongoing debate regarding the physiological significance of the VLF component.

A similar sex-specific pattern was observed during Romantic music exposure. Females demonstrated higher nHF values, whereas males exhibited higher nLF, LF/HF, and NCoh values. These results suggest that Romantic music may preferentially enhance parasympathetic modulation in females, while promoting greater sympathetic predominance and cardiorespiratory coherence in males. Interestingly, increasing anxiety scores were associated with reductions in nLF and LF/HF among females, potentially indicating attenuation of sympathetic predominance during exposure to Romantic music in anxious women.

Heavy Metal music also produced marked sex-dependent autonomic responses. Higher nLF and LF/HF values in males together with higher nHF values in females suggest that males experienced greater sympathetic predominance during listening. However, among males, LF/HF decreased with increasing anxiety scores, which may indicate a reduced sympathetic response to Heavy Metal music in participants with higher anxiety levels. This observation is intriguing because highly stimulating music is generally expected to increase sympathetic activation, suggesting that familiarity, emotional appraisal, or adaptive autonomic regulation may modulate the physiological response.

Traditional Romanian music displayed a distinct autonomic profile. The positive association between VLF power and anxiety scores in both sexes suggests that anxiety may amplify autonomic arousal during exposure to this genre. Although VLF cannot be considered an exclusive marker of sympathetic activity, the observed pattern is compatible with enhanced sympathetic involvement among more anxious participants. In contrast, females maintained higher rMSSD values, indicating preserved vagal modulation despite this apparent increase in autonomic arousal.

Ambient music elicited one of the clearest sex-specific parasympathetic responses. Females demonstrated significantly higher rMSSD values both at baseline and across anxiety levels, suggesting enhanced vagal activity during exposure to this genre. These findings support previous observations that slow, repetitive, and relaxing auditory stimuli may facilitate parasympathetic regulation and emotional relaxation. The persistence of this effect irrespective of anxiety status suggests that Ambient music may exert a relatively robust autonomic calming influence in females.

Taken together, these findings indicate that autonomic responses to music are not uniform across individuals. Rather than musical genre alone determining the physiological outcome, sex appears to substantially influence the balance between sympathetic and parasympathetic activation. The recurrent observation of higher nHF and rMSSD values in females, together with higher nLF and LF/HF values in males, suggests that women may exhibit a relatively greater parasympathetic response to several musical genres, whereas men tend to display stronger sympathetic predominance. These differences may reflect sex-related variations in autonomic regulation, emotional processing, and neurovisceral integration and should be considered in future investigations of music-induced autonomic modulation.

## 5. CONCLUSIONS

Exposure to musical passages did not produce measurable changes in heart rate but was associated with multiple variations in HRV parameters.

Comparisons between musical listening and rest revealed sporadic variations in time-domain parameters and absolute spectral values. The most prominent variations involved nLF, nHF, and LF/HF, reflecting both session-dependent effects and sex-related differences.

When comparing different musical genres directly, variations were more strongly associated with sex differences than with musical style alone. Significant changes were also observed in rMSSD and parameters related to the VLF band.

The VLF band, which is less frequently discussed in the literature, appeared particularly relevant in subjects with anxiety and may represent a marker of sympathetic activity.

A strong concordance was observed between parasympathetic indices (rMSSD and HF) across all sessions except Jazz in males. Similarly, LF/HF was strongly correlated with nLF and inversely correlated with nHF across all sessions.

Although all musical genres modulated autonomic function, the observed responses were predominantly sex-dependent. Females generally demonstrated greater parasympathetic modulation during Baroque, Romantic, and Ambient music, whereas males exhibited higher sympathetic predominance across several genres. Anxiety further modified these responses in a genre-specific manner.

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***Authors' contribution***

*I.S-F. – conceptualization, study design, and writing, prepared the tables, data interpretation, I.T.- data collection, data interpretation, writing and reviewing, I.M. and C.P.- statistical analysis and figures, data interpretation, S.B.- writing and reviewing, and prepared the tables, data interpretation. All authors reviewed the manuscript.*

***Data availability statement***

*The data supporting this study's findings are available upon reasonable request. Contact person: Ioana Toader at e-mail: i.toader12@yahoo.com*

***Ethics approval and consent to participate***

*The study was conducted between May 28, 2024, and October 1, 2024, with approval by the Ethics Committee of Colțea Clinical Hospital (approval No. 6006/10.04.2024) and conducted in accordance with the Declaration of Helsinki.*

***Patient consent for publication***

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

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ARTICLE

# Clinicopathological Correlations and Predictors of Surgical Margins and Reconstruction in Head and Neck Non-Melanoma Skin Cancers: A retrospective study

**Maria Moga[1], Adrian Costache[2*], Romica Cergan[3*], Mihai Dumitru[4], Daniela Vrinceanu[4], Crenguta Serboiu[5], Gabriela Musat[6], Adina Zamfir Chiru Anton[7], Daniel Octavian Costache[1]**

1 Dermatology Department, Carol Davila Military University Emergency Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
2 Pathology Department, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
3 Anatomy Department, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
4 ENT Department, Bucharest Emergency University Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
5 Molecular Biology and Histology Department, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
6 ENT Department, Saint Mary Clinical Hospital, Bucharest, Romania
7 7ENT Department, Grigore Alexandrescu Children's Hospital, Bucharest, Romania

*Correspondence: adriancostacheeco@yahoo.com (A.C.) and r.cergan@gmail.com (R.C.)

**Abstract:** Background: Head and neck keratinocyte carcinomas are frequently managed surgically; anatomic constraints may increase the risk of incomplete excision and influence reconstructive choices. Objectives: To describe the clinicopathological characteristics of head and neck non-melanoma skin cancers (NMSC) treated in a tertiary care dermatology department and to explore factors associated with resection status (R0 vs R1) and reconstructive approach after conventional excision. Methods: We performed a retrospective observational study of 117 patients undergoing excision of 134 primary head and neck NMSC between September 2025 and January 2026. Demographic, clinical, histopathological, and procedure-related variables were extracted from medical records and pathology reports. Margin status was assessed for conventionally excised lesions. Results: Most lesions were basal cell carcinomas (113/134, 84.3%) and were located on the cheek (31.3%) or nose (29.8%). Conventional excision was performed in 126/134 lesions (94%). Among conventionally excised lesions, 92% had complete resection (R0) and 8% had positive margins (R1), most commonly involving the lateral margin. Conclusions: In this single-center cohort, head and neck NMSC clustered in high-risk anatomic regions, and a small proportion of conventionally excised tumors showed positive margins. Clear reporting of margin involvement and reconstruction patterns may support quality improvement and guide future prospective follow-up.

**Keywords:** non-melanoma skin cancer; basal cell carcinoma; squamous cell carcinoma; head and neck; surgical margins; reconstruction

## INTRODUCTION

Non-melanoma skin cancers (NMSC) are among the most frequently diagnosed neoplasms worldwide [1]. According to the International Agency for Research on Cancer, NMSC ranks 5th in terms of global cancer incidence, with Europe reporting the second-highest mortality rates [2]. While non-melanoma skin cancers include a broad spectrum of cutaneous malignancies, the keratinocyte-derived neoplasms of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) account for approximately 99% of all NMSC [3].

Cutaneous BCC is the most common malignancy in the human population, with a steadily rising incidence that is projected to exceed the incidence of all other cancers combined [4] It constitutes almost 80% of NMSC cases, whereas SCC accounts for around 20%, with a reported BCC:SCC ratio of up to 10:1. While both BCC and SCC generally have a favourable prognosis, basal cell carcinoma is typically less aggressive and contributes minimally to NMSC-related mortality. In contrast, SCC accounts for

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a substantial proportion of deaths due to NMSC, with a reported metastatic rate of up to 9.9% [5].

The pathogenesis of these tumors is multifactorial, with risk factors including fair skin phenotype, UV radiation, genetic susceptibility, personal history of skin cancer, immunosuppression, and HPV infection. Furthermore, epidemiological data show that advanced age and male sex are independently associated with an increased incidence of NMSC [6].

BCC originates from basal cells of the epidermis and typically occurs in chronically sun-exposed areas. Accordingly, approximately 80% of these tumors are found on the skin of the head and neck [7]. Basal cell carcinoma is usually slow-growing and locally invasive, with limited metastatic potential. Although rare, metastatic BCC can involve lymph nodes, bone, or lungs [8]. According to the National Comprehensive Cancer Network, basal cell carcinomas located in the head and neck region are considered high-risk, regardless of size [9].

In contrast, SCC often arises from precursor lesions such as actinic keratosis or Bowen's disease and may progress to an invasive carcinoma. Tumor progression may ultimately lead to metastatic disease in a minority of cases. Similar to BCC, SCC is strongly influenced by UV radiation and sun exposure, which shape its anatomic distribution. However, compared with BCC, a smaller proportion of SCC occurs in the head and neck region, with many tumors involving other sites such as the forearms, hands, and legs [10]. Several factors are considered when assessing recurrence and metastatic risk, including tumor size, depth of invasion, perineural involvement, and degree of histological differentiation; in addition, head and neck locations such as the lip and ear are generally considered at least high-risk sites [11].

For most NMSC, surgical removal is the first-line and often curative treatment. However, due to anatomic and functional constraints in the head and neck region, surgery can be challenging for both patient and clinician. The cephalic region is an area where function and aesthetics are of considerable concern, and the surgical plan often represents a compromise between cosmetic expectations and oncologic safety. As a result, narrower excision may be performed in some cases, potentially leading to positive surgical margins and a higher risk of local persistence/recurrence. In a 2025 retrospective study including 377 basal cell carcinomas, up to 37.2% of cases exhibited involved margins, largely due to constraints imposed by complex head and neck anatomy [12].

This study retrospectively analyzes a cohort of 117 patients with histopathologically confirmed head and neck NMSC treated surgically in a tertiary care center. The lesions included 113 basal cell carcinomas and 21 squamous cell carcinomas, including precursor lesions. Although classification remains controversial, some authors consider keratoacanthoma a premalignant lesion, whereas others regard it as a self-limited variant of SCC; therefore, histopathologically confirmed keratoacanthomas were included in the analysis. We describe the clinical and histopathological features of the tumors (including subtype and anatomic distribution) and summarize surgical management (excision technique, reconstruction, and margin status). We further explore factors associated with incomplete excision (R1) and with reconstruction choice following conventional excision.

**MATERIALS AND METHODS**

We conducted a retrospective observational study, analyzing all primary head and neck NMSC treated from September 2025 to January 2026 in the Department of Dermatovenerology at the "Dr. Carol Davila" Central Military Emergency University Hospital of Bucharest, Romania. The study cohort included 117 patients who underwent resection of 134 head and neck NMSC; twelve patients underwent excision of more than one lesion. All cases were diagnosed based on histopathological evaluation of a single excision under local anesthetic (1% lidocaine). Excisional methods included conventional surgical excision and shave excision. In cases treated by shave excision, electrodesiccation of the lesion base was subsequently performed, preventing accurate histopathological assessment of maximum tumor depth; this limitation was encountered in eight cases. No incisional biopsies were performed, as current evidence supports primary excision as the preferred initial management for many NMSC [13]. Specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for microscopic evaluation.

Inclusion criteria comprised patients with histologically confirmed head and neck NMSC who underwent surgical excision between September 2025 and January 2026, inclusive. The following variables were recorded for each patient: demographic data, clinical characteristics, histopathological parameters, and treatment-related data, as detailed in Table 1.

BCCs were classified histologically into six specified subtypes: superficial, nodular, micronodular, infiltrative, metatypical (basosquamous), and pigmented. No morpheaform or sclerosing BCC was identified during the study period. Some lesions (n=18) were reported as basal cell carcinoma, not otherwise specified (NOS), while others were classified as mixed, with coexisting histological patterns within the same lesion (e.g., nodular with adenoid and/or pigmented differentiation). The study also included squamous cell carcinomas and precursor lesions, including keratoacanthoma, actinic keratosis, and Bowen's disease. While recent NCCN data suggest classifying SCC into two differentiation categories (well-to-moderately differentiated and poorly differentiated), in this study, squamous cell carcinomas were classified using the classic grading system: well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) [14]. No anaplastic (undifferentiated, G4) cases were identified.

Due to the retrospective design, tumor dimensions were reconstructed from available clinical and histopathological records to provide a three-dimensional assessment. Tumor length and width were derived from clinical measurements, while maximum thickness was determined from histopathology reports.

The exclusion criteria included histologically unconfirmed NMSC, NMSC located outside the head and neck region, cutaneous melanomas, skin cancers treated by methods other than surgical excision and NMSC excised during periods other than the one specified above.

*Outcomes*

The primary outcome was resection status for conventionally excised lesions, categorized as complete (R0) or incomplete (R1), with R1 cases further classified by margin involvement (lateral, deep, or both). Secondary outcomes included the reconstruction method after conventional excision (direct closure, local flap, skin graft, or secondary intention) and flap subtype among flap reconstructions. Because prospective follow-up was not available within this study period, post-excision recurrence could not be assessed; a history of prior skin cancer excision within the previous 24 months was recorded as a separate baseline variable.

*Statistical analysis*

Categorical variables were summarized as counts and percentages, and continuous variables as means and ranges, as reported in the Results section and Tables. Associations between selected predictors and outcomes were explored using appropriate hypothesis tests for categorical data (e.g., chi-square), with multiple-testing control where indicated by false discovery rate (FDR) reporting.

**Table 1:** Variables included in the study

| Category | Variable | Type | Definition |
|---|---|---|---|
| **Demographic** | Age | Continous | Age (years) |
| | Sex | Categorical | Male/ Female |
| | Area of residence | Categorical | Urban/ Rural |
| **Clinical** | Tumor location | Categorical | The specific anatomical site of the head and neck (e.g nasal, cheek, auricular) |
| | Evolution time | Continous | Duration of lesion prior to excision (months) |
| | Tumor size | Continous | Tumor length, width and maximum depth |
| **Temporal** | Month of excision | Categorical | Month in which the lesion was surgically excised (September to January) |
| **Histopathological** | Tumor type | Categorical | BCC/ SCC/ Bowen/ AK/ Keratoachantoma |
| | Histological subtype | Categorical | Superficial, nodular, infiltrative etc. (for BCC)/ G1, G2, G3 (for SCC) |
| | pT stage | Ordinal | Pathological tumor stageing according to AJCC 8$^{th}$ edition (just for BCCs) |
| | Depth of invasion | Categorical | Deepest layer involved: dermis (papillary/ reticular), hypodermis, muscle or lamina propria (for mucosal, lip lesions) |
| | Perineural invasion | Binary | Present/ Absent |
| | Perivascular invasion | Binary | Present/ Absent |
| | Ulceration | Binary | Present/ Absent |
| | Desmoplastic reaction | Categorical | Absent/ Minimal/ Moderate/ Important |
| | Necrosis | Binary | Present/ Absent |
| | Solar elastosis | Categorical | Absent/ Grade 1/ Grade 2/ Grade 3 |
| **Surgical** | Surgical procedure | Categorical | Conventional excision/ Excision-shave |
| | Reconstruction method (for conventional excisions) | Categorical | Direct closure/ Local flap/ Skin graft/ Secondary intention |
| | Flap subtype technique (for flap cases only) | Categorical | Advancement, rotation, transposition |
| | Surgical margins | Continous | Lateral and deep margins (mm) |
| | Resection status | Binary | Complete (R0)/ Incomplete (R1) |
| | Margin involvement (for R1 cases only) | Categorical | Lateral/ Deep/ Both |
| | Reintervention in the past | Binary | Yes/ No |

BCC = basal cell carcinoma; SCC = squamous cell carcinoma; AK = actinic keratosis; G1=well-differentiated SCC; G2= moderately differentiated SCC; G3= poorly differentiated SCC; AJCC = American Joint Committee of Cancer.

In the performed study, the diagnostic and staging criteria of the American Joint Committee of Cancer (AJCC) were available only for a subset of cases, predominantly BCCs [15]. Given the retrospective design, staging data were limited to the pathological tumor component (pT), as nodal and metastatic status require clinical and imaging correlation that were not consistently documented for all cases.

The surgical procedures were performed by 7 doctors. The case cohort collected was divided into two classes: clean (R0) and positive (R1) margins, the latter being subdivided in turn according to the margin touched by the cancer (lateral, deep, or both).

The reconstruction methods were also recorded, being classified as direct closure, local flap, skin graft, or healing by secondary intention. For cases managed using local flaps, the flap's subtype (such as advancement, rotation, or transposition) was documented, as well as the specific design modifications (M-plasty, A-T plasty, S-plasty, or V-Y plasty). During the research time period, only one case required reconstruction using a full-thickness skin graft. Thus, the defect involving the nose and adjacent cheek was covered by a right laterocervical skin patch. In the same way, a single case in the cohort was managed by secondary intention healing. After the excision of an auricular SCC with suspected cartilage involvement, the defect was intentionally left open, followed by the application of topical 5-fluorouracil.

**RESULTS**

The study included 117 Caucasian patients who underwent resection of 134 primary NMSC located in the head and neck region. Of these, 75 (64.1%) were male and 42 (35.9%) were female, with a slight predominance of urban residents (n=65, 55.6%) compared with rural residents (n=52, 44.4%); this difference was not statistically significant. Patient age ranged from 39 to 92 years, with a mean age of 70.24 years. The most represented age group was 70–79 years (n=48, 41%), followed by 60–69 years (n=24, 20.5%). The remaining groups were 80–89 years (n=18, 15.4%), 50–59 years (n=15, 12.8%), 40–49 years (n=7, 6%), and 90–92 years (n=4, 3.4%); one patient (0.9%) was younger than 40 years. The month with the highest number of operated NMSC cases was December (n= 35/117), while November had the fewest (n=17/117) (Figure 1).

During the study period, 104 patients (88.9%) underwent surgical removal of a single lesion, whereas 13 patients (11.1%) underwent excision of two or more tumors (31 lesions in total). Two lesions were excised in 10/117 patients (8.5%); four lesions were excised in 2/117 patients (1.7%); and three lesions were excised in 1/117 patients (0.9%).

Out of the 134 lesions, 113 (84.3%) were basal cell carcinomas and 21 (15.7%) were squamous cell carcinomas, including precursor lesions. Among BCCs, 36.3% (n=41/113) exhibited mixed histological patterns, whereas 63.7% (n=72/113) showed a single morphological pattern; 18 lesions (16.0%) were reported as BCC, NOS. The most frequent specified subtype was nodular (n=32/113, 28.3%), followed by superficial (n=8/113, 7.1%), infiltrative (n=5/113, 4.4%), metatypical (n=4/113, 3.5%), micronodular (n=3/113, 2.7%), and pigmented (n=2/113, 1.8%). Pathological staging according to AJCC was available for the BCCs included in this cohort and showed a predominance of early-stage tumors: pT1 (n=104, 92%), pT2 (n=8, 7%), and pT3 (n=1, 1%); no pT4 lesion was recorded.

Regarding the histological distribution of SCC-related lesions, 8/21 (38.1%) were invasive squamous cell carcinomas, while the remaining 13 lesions (61.9%) were categorized as precursor or related lesions, including actinic keratosis (n=6/21, 28.6%), SCC in situ (Bowen's disease, n=4/21, 19.0%), and keratoacanthoma (n=3/21, 14.3%). Among invasive SCCs (n=8), most were well differentiated (G1, n=5/8, 62.5%), followed by moderately differentiated (G2, n=2/8, 25.0%), with one poorly differentiated case (G3, n=1/8, 12.5%). The distribution of the primary histopathological diagnoses identified in the study cohort is illustrated in Figure 2.

When stratified by age group, statistics demonstrates the predominance of basal cell carcinoma across all age categories, particulary in patients aged 70-79 years. SCC were less frequent, but were identified across most age categories (except <50 years) (Figure 3).

The most frequent anatomical site involved was the cheek area (n=42, 31.3%), followed by the nose (n=40, 29.8%). Within the nasal region, the nasal pyramid (n=31/40) was more commonly affected than the nasal alae (n=9/40). The ear and periauricular area were affected in 12 cases (9.0%), the temporal region in 10 cases (7.4%), and the lips in 8 cases (6.0%), with the upper lip (n=5/8) more frequently involved than the lower lip (n=3/8). The scalp, forehead, and periocular region showed equal involvement (6 cases each, 4.5% each), while the neck was least frequently affected (n=4, 3.0%). Regarding laterality, 42 lesions were located on the left side of the face (31.3%) and 41 on the right side (30.6%); 31 lesions involved the midline (23.1%), and laterality was not specified for 20 lesions (15.0%). The anatomical distribution of lesions across facial regions is highlighted by the "heatmap" representation shown in Figure 4, where warmer colors (red–orange) indicate areas with higher case density (nose and cheeks), while cooler colors (green–blue) represent regions with lower frequency, such as the scalp and neck.

The mean maximum tumor diameter was 10.0 mm (range: 1–23 mm), and the mean maximum depth was 1.9 mm (range: 0.2–10 mm).

Lesion evolution time before surgery varied considerably: most lesions (n=112/134, 83.6%) had been present for more than one year, while 22/134 (16.4%) had an onset within one year. Additional histological characteristics, including depth of invasion, perineural and perivascular involvement, ulceration, desmoplastic reaction, necrosis, and solar elastosis, are presented in Table 2.

Regarding the surgical approach, most lesions underwent conventional excision (n=126/134, 94.0%), while 8 lesions (6.0%) were treated by shave excision. Wound management after conventional excision was implemented primarily by direct closure in 94 lesions (74.6%), followed by local flaps in 30 cases (23.8%). More complex techniques were required in a small subset of cases: a full-thickness skin graft (harvested from the right laterocervical area) was used in one patient (0.8%) for reconstruction of a defect involving the nasal pyramid and part of the right cheek; secondary intention healing was also used in one patient (0.8%) for an auricular defect. Among flap reconstructions, 66.7% (n=20/30) were transposition flaps, 20.0% (n=6/30) were advancement flaps, and 13.3% (n=4/30) were mixed rotation-advancement flaps.

As illustrated in Figure 5, direct closure represented the predominant reconstructive approach across most anatomical regions, while local flaps were used more frequently in regions requiring greater reconstructive complexity, particularly the lips (n=6/8) and nasal area (n=3/6). When analyzed according to histopathological diagnosis, direct closure remained the predominant reconstructive technique across most lesion types, particularly in basal cell carcinoma (n=82/113). In contrast, SCC cases more frequently required complex reconstructive procedures, including local flaps (n=4/8) and secondary intention healing (n=1/8). The only skin graft case was observed in a BCC lesion (Figure 6).

The average lateral surgical margin was 3.2 mm, while the average deep margin was 1.4 mm. Histological evaluation of conventionally excised specimens showed that 92% (n=116/126) had complete margins (R0) and 8% (n=10/126) had positive margins (R1). Among R1 cases, lateral margins were most commonly involved (n=6/10, 60%), followed by deep margins (n=3/10, 30%); in one case (n=1/10, 10%), both lateral and deep margins were involved. Positive margins were observed in the nose (n=2/10, 20%), scalp (n=2/10, 20%), auricular (n=2/10, 20%), and periocular region (n=2/10, 20%). The temporal and cheek areas presented one case each with positive margins (n=1/10, 10%). No positive margins were recorded on the forehead, lips, or neck.

**Table 2:** Demographic, anatomical, clinical and histological data

| Variable | Values |
|---|---|
| **Mean Age** | 70.24 |
| **Gender** | |
| **Male** | 75 (64.1%) |
| **Female** | 42 (35.9%) |
| **Residence** | |
| **Urban** | 65 (55.6%) |
| **Rural** | 52 (44.4%) |
| **Tumor location** | |
| **Cheek area** | 42 (31.3%) |
| **Nose** | 40 (29.8%) |
| **Ear and periauricular area** | 12 (9%) |
| **Temporal** | 10 (7.4%) |
| **Upper lip** | 5 (3.7%) |
| **Lower lip** | 3 (2.2%) |
| **Scalp** | 6 (4.5%) |
| **Forehead** | 6 (4.5%) |
| **Eye area** | 6 (4.5%) |
| **Neck** | 4 (3%) |
| **Affected side** | |
| **Left** | 42 (31.3%) |
| **Right** | 41 (30.6%) |
| **Midline** | 31 (23.1%) |
| **Not specified** | 20 (15%) |
| **Evolution time of the tumor before surgery** | |
| **≤12 months** | 22 (16.4%) |
| **>12 months** | 112 (83.6%) |
| **Tumor size** | |
| **Average length** | 10 mm |
| **Average depth** | 1.9 mm |
| **Tumor type** | |
| **BCC** | 113 (84.3%) |
| **SCC** | 8 (6%) |

| | |
|---|---|
| **AK** | 6 (4.5%) |
| **Bowen's disease** | 4 (3%) |
| **Keratoacanthoma** | 3 (2.2%) |
| **BCC Histological Subtypes** | |
| **Nodular** | 32 (28.3%) |
| **Superficial** | 8 (7%) |
| **Infiltrative** | 5 (4.4%) |
| **Metatypical** | 4 (3.5%) |
| **Micronodular** | 3 (2.7%) |
| **Pigmented** | 2 (1.8%) |
| **NOS** | 18 (16%) |
| **Mixed histology** | 41 (36.3%) |
| **Pt category (just for BCCs)** | |
| **Pt1** | 104 (92%) |
| **Pt2** | 8 (7%) |
| **Pt3** | 1 (1%) |
| **Pt4** | 0 |
| **SCC Differentiation Grade (G)** | |
| **G1** | 4 (50%) |
| **G2** | 2 (25%) |
| **G3** | 2 (25%) |
| **Depth of invasion** | |
| **Papillary dermis** | 13 (9.7%) |
| **Reticular dermis** | 76 (56.7%) |
| **Hypodermis** | 13 (9.7%) |
| **Muscle** | 5 (3.7%) |
| **Lamina propria (for mucosal lesions)** | 3 (2.2%) |
| **Not specified** | 24 (18%) |
| **Perineural invasion** | |
| **Yes** | 5 (3.7%) |
| **No** | 125 (93.3%) |
| **Not specified** | 4 (3%) |
| **Perivascular invasion** | 0 |
| **Ulceration** | |
| **Yes** | 78 (58.2%) |
| **No** | 56 (41.8%) |
| **Desmoplastic reaction** | |
| **Absent** | 82 (61.2%) |
| **Minimal** | 11 (8.2%) |
| **Moderate** | 32 (23.9%) |
| **Important** | 9 (6.7%) |
| **Necrosis** | |
| **Yes** | 7 (5.2%) |
| **No** | 127 (94.8%) |
| **Solar elastosis** | |
| **Absent** | 23 (17.2%) |
| **Grade 1** | 10 (7.5%) |
| **Grade 2** | 39 (29.1%) |
| **Grade 3** | 57 (42.5%) |
| **Not specified** | 5 (3.7 %) |
| **Surgical procedure** | |
| **Conventional excision** | 126 (94%) |
| **Shave-excision** | 8 (6%) |
| **Reconstruction method** | |
| **Direct closure** | 94 (74.6%) |
| **Local flap** | 30 (23.8 %) |
| **Skin graft** | 1 (0.8%) |
| **Secondary intention** | 1 (0.8%) |
| **Flap subtype technique** | |
| **Advancement** | 6 (20%) |
| **Rotation** | 0 |
| **Transposition** | 20 (66.7%) |

| | |
|---|---|
| **Mixed (rotation-advancement)** | 4 (13.3%) |
| **Surgical margins (mm)** | |
| **Average size of lateral surgical margins** | 3.2 mm |
| **Average size of deep surgical margins** | 1.4 mm |
| **Resection status** | |
| **Complete (R0)/Clean margins** | 116 (92%) |
| **Incomplete (R1)/Positive margins** | 10 (8%) |
| **Margin involvement (for R1)** | |
| **Lateral** | 6 (60%) |
| **Deep** | 3 (30%) |
| **Both** | 1 (10%) |
| **Reintervention in the past 2 years** | |
| **Yes** | 7 (6%) |
| **No** | 110 (94%) |

BCC= basal cell carcinoma; SCC= squamous cell carcinoma; AK= actinic keratosis; NOS= not otherwise specified; G1=well-differentiated SCC; G2= moderately differentiated SCC; G3= poorly differentiated SCC.

**Figure 1:** Monthly distribution of surgically treated head and neck non-melanoma skin cancer cases during the study period (September 2025–January 2026)

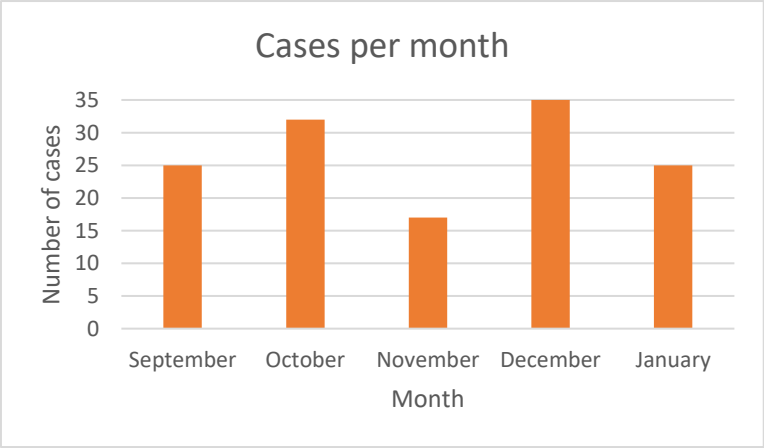



**Figure 2:** Distribution of primary histopathological diagnoses showing Basal Cell Carcinoma (BCC) as the predominant category, followed by Squamous Cell Carcinoma (SCC), Actinic Keratosis (AK), SCC in situ (Bowen's disease) and Keratoacanthoma

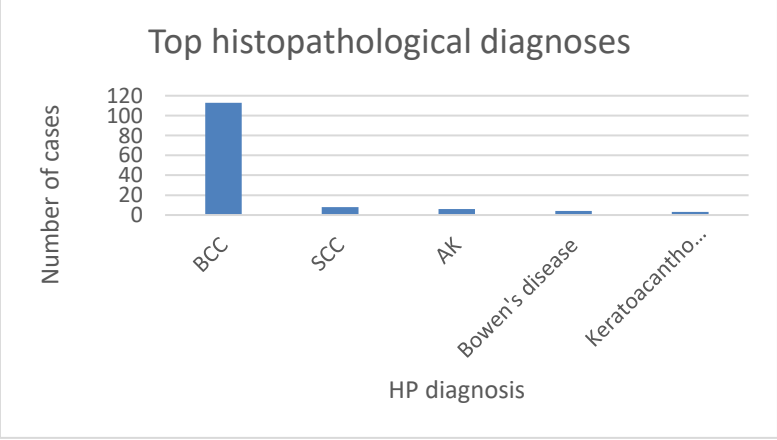


**Figure 3:** Distribution of histopathological diagnoses across age groups. Basal cell carcinoma (BCC) is the most frequent diagnosis in all age categories, with the highest incidence observed in patients aged 70–79 years. Squamous cell carcinoma (SCC) occurs less frequently but is represented across most of the age ranges (except <50 years). Other diagnoses, including keratoacanthoma, actinic keratosis and Bowen's disease, are present just in certain age groups.

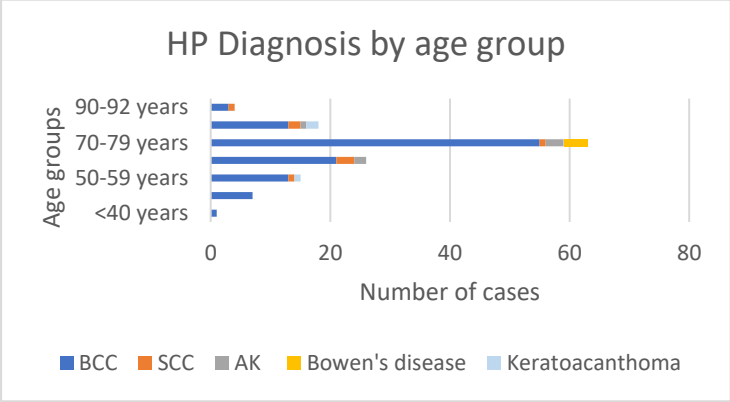



**Figure 4:** Heatmap illustrating the anatomical distribution of cases across facial regions. Warmer colors (red–orange) indicate areas with higher case density, predominantly on the nose and cheeks, while cooler colors (green–blue) represent regions with lower frequency, such as the scalp and neck

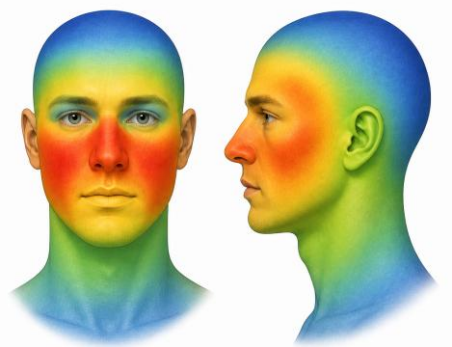


**Figure 5:** Distribution of reconstruction and auxiliary procedures by anatomical location. The highest number of reconstructions was observed in the cheek and nasal regions, predominantly managed by direct closure, followed by local flaps. Only one case required a skin graft (in the nasal area) and one case was managed by secondary intention healing (auricular defect). Notably, in the lip region, local flaps were used more frequently than direct closure. Other anatomical sites showed fewer cases, with direct closure remaining the most common approach.

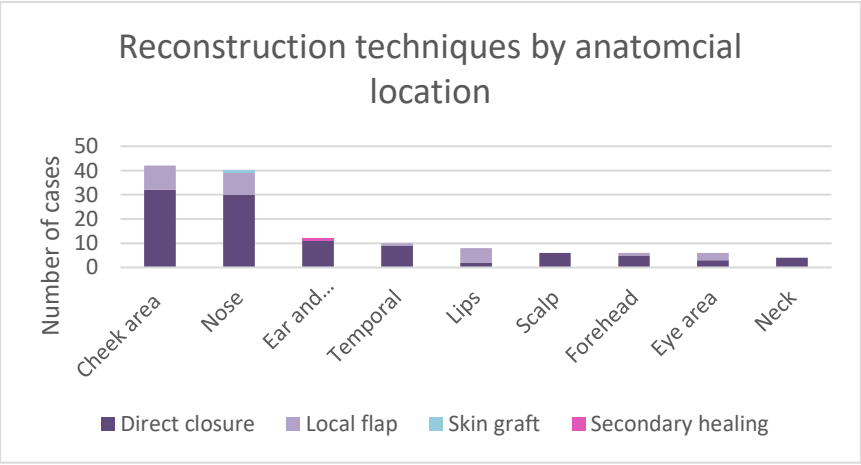


**Figure 6:** Distribution of reconstruction procedures according to histopathological diagnosis. Basal cell carcinoma (BCC) accounts for the highest number of cases, with direct closure and local flaps being the most frequently used reconstruction methods. Only one BCC lesion was treated by skin graft. Squamous cell carcinoma (SCC) shows a lower incidence, however requiring a complex range of reconstructive approaches: skin grafting (4 cases), direct closure (3 cases) and just one case of secondary healing. The other types of lesions, including Bowen's disease, actinic keratosis and keratoacanthoma showed minimal reconstructive requirements, being treated entirely by direct closure.

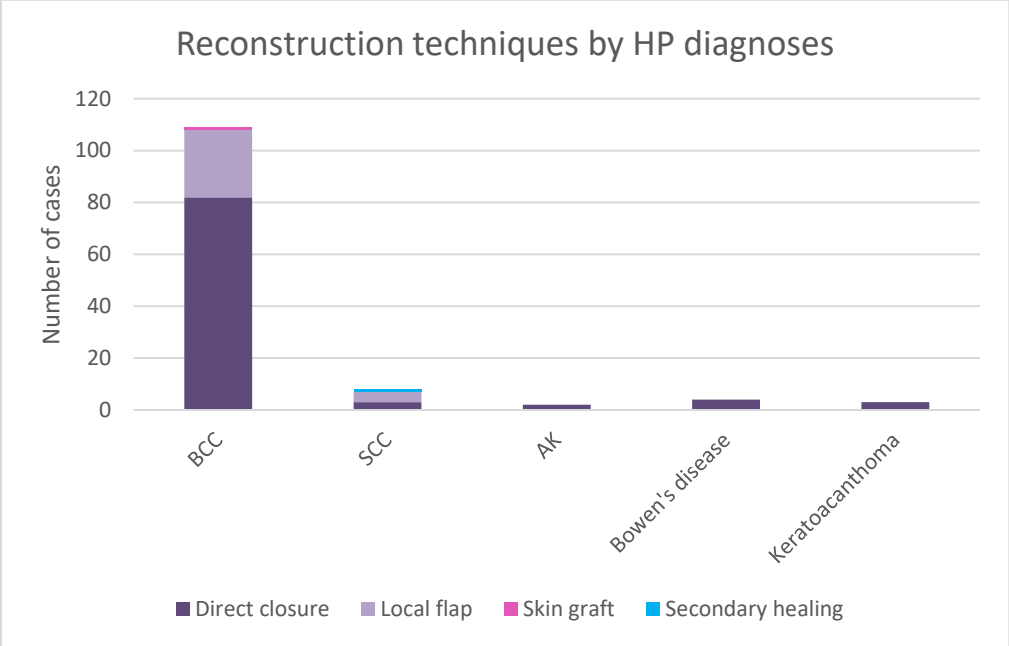
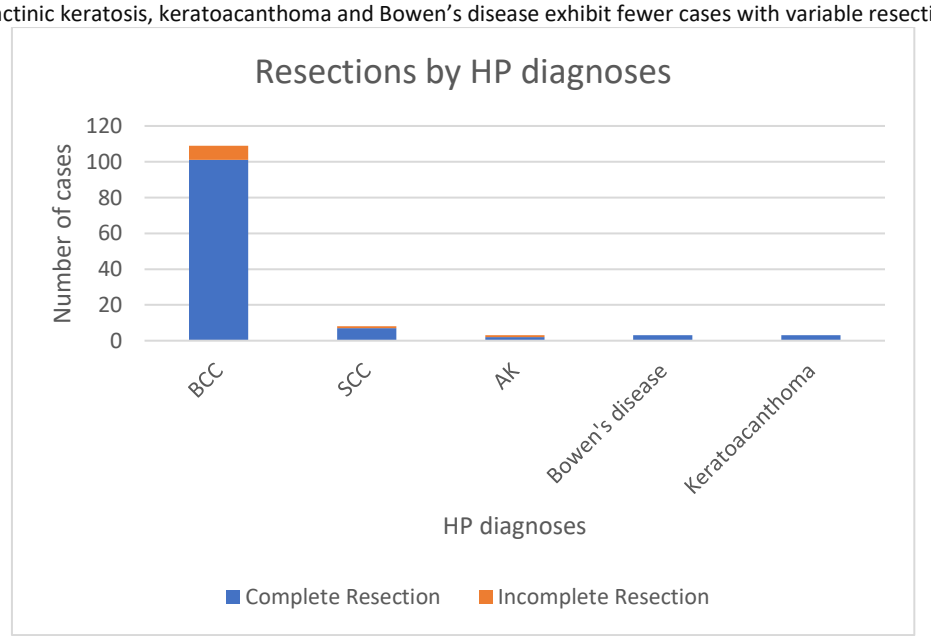



**Figure 7:** Distribution of complete and incomplete resections across histopathological diagnoses treated by conventional surgery. Basal cell carcinoma (BCC) shows the highest number of complete resections while other diagnoses such as squamous cell carcinoma (SCC), actinic keratosis, keratoacanthoma and Bowen's disease exhibit fewer cases with variable resection completeness.



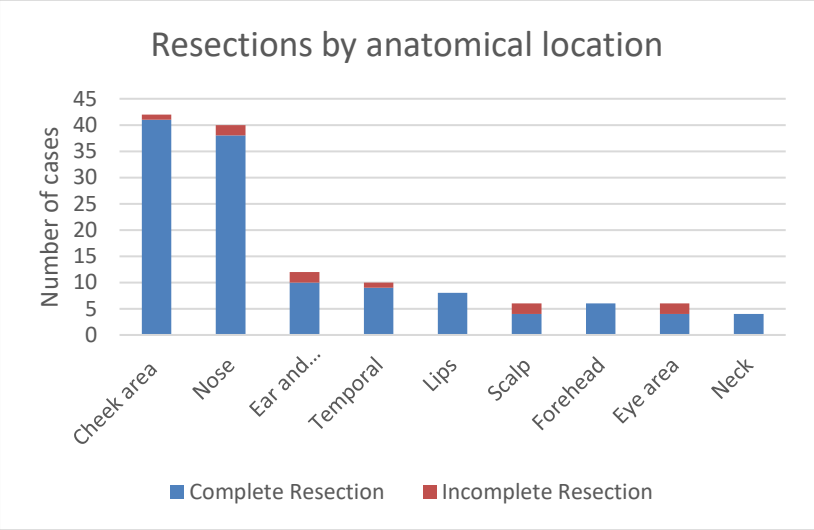

Regarding the relationship between resection status and histopathological diagnosis, basal cell carcinoma accounted for the majority of complete excisions (n=101/109). Although the remaining histopathological subtypes were represented by fewer cases, complete

excision remained the predominant outcome across all lesion types, including SCC (n=7/8), AK (n=2/3), Bowen's disease (n=3/3) and keratoacanthoma (n=3/3) (Figure 7).

When analyzed according to anatomical site, the cheek and nasal regions accounted for the highest number of complete resections (n=42 and n=38, respectively). Incomplete resections were observed more frequently in anatomically complex areas, particularly the nose, scalp, and periocular/ periauricular areas, all of which exhibited 2 cases each of positive edges (Figure 8).

Given the retrospective design and the short study period, prospective post-excision follow-up was not available to assess recurrence after the interventions included in this cohort. We therefore extracted baseline information from medical records regarding prior skin cancer surgery within the 24 months preceding the index procedure. Seven patients (6%) had undergone at least one previous surgical intervention for another skin cancer in the prior two years, while 110 patients (94%) had no such history documented.

**Figure 8:** Distribution of complete and incomplete resections by anatomical location. The cheek and nasal regions accounted for the highest number of cases, predominantly associated with complete resections. In contrast, the nose, scalp, auricular and periocular regions showed the highest frequency of incomplete resections with positive margins. Fewer resections were observed in the lips, forehead and neck, all of which were associated with negative margins following surgery.




The burden of adverse histopathological indicators varied considerably according to anatomical site, as shown in Table 3. While the cheek area showed the highest case burden with relatively lower-risk characteristics, the scalp, neck, and nasal regions exhibited higher frequencies of incomplete excision, deep invasion, ulceration, and perineural involvement, suggesting a more unfavorable histopathological profile. To further integrate these adverse histopathological features into a unified anatomical vulnerability model, a weighted composite risk index was calculated for each region (Table 4). The scalp region demonstrated the highest composite risk score (61.2), followed by the neck (44.0) and eye region (40.0), classifying these areas as high-risk anatomical sites. These findings are further illustrated in Figure 9, which provide a visual representation of the weighted anatomical risk distribution using a "traffic light" classification system.

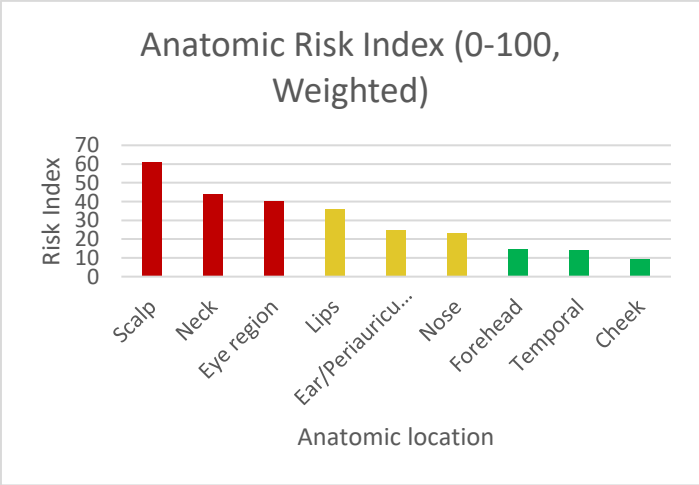
**Table 3:** Anatomic risk table (burden and key indicators) by location group

| Location Group | Cases | %Total | %Incomplete | %Mixed | %Deep Invasion | %Perineural Involvement | %Ulceration |
|---|---|---|---|---|---|---|---|
| Cheek area | 37 | 31.4 | 5.4 | 0.0 | 2.7 | 2.7 | 51.4 |
| Nose | 34 | 28.8 | 11.8 | 8.8 | 20.6 | 0.0 | 70.6 |
| Forehead | 16 | 13.6 | 12.5 | 0.0 | 6.2 | 6.2 | 43.8 |
| Lips | 8 | 6.8 | 0.0 | 0.0 | 62.5 | 0.0 | 62.5 |
| Temporal | 7 | 5.9 | 14.3 | 0.0 | 0.0 | 0.0 | 85.7 |
| Ear/Periauricular region | 6 | 5.1 | 0.0 | 33.3 | 16.7 | 0.0 | 100.0 |
| Scalp | 5 | 4.2 | 40.0 | 0.0 | 40.0 | 60.0 | 60.0 |
| Neck | 4 | 3.4 | 25.0 | 50.0 | 50.0 | 0.0 | 100.0 |
| Eye region | 1 | 0.8 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |

**Table 4:** Anatomic risk "traffic light" (weighted composite index) by location group

| Location | N | Margin% | Deep Invasion% | Perineural% | Ulceration% | Risk Index | Traffic Light |
|---|---|---|---|---|---|---|---|
| Scalp | 5 | 40.0 | 40.0 | 60.0 | 60.0 | 61.2 | RED |
| Neck | 4 | 25.0 | 50.0 | 0.0 | 100.0 | 44.0 | RED |
| Eye region | 1 | 100.0 | 0.0 | 0.0 | 0.0 | 40.0 | RED |
| Lips | 4 | 0.0 | 62.5 | 0.0 | 62.5 | 36.2 | AMBER |
| Ear/Periauricular region | 6 | 16.7 | 16.7 | 0.0 | 100.0 | 24.7 | AMBER |
| Nose | 34 | 16.2 | 20.6 | 0.0 | 70.6 | 23.4 | AMBER |
| Forehead | 16 | 12.5 | 6.2 | 0.0 | 43.8 | 14.4 | GREEN |
| Temporal | 7 | 14.3 | 0.0 | 0.0 | 85.7 | 14.3 | GREEN |
| Cheek | 37 | 5.4 | 2.7 | 0.0 | 51.4 | 9.5 | GREEN |

**Figure 9:** Weighted anatomic risk index across facial regions. The scalp and neck areas exhibit the highest risk scores, followed by the eye area and lips. Regions such as the forehead, temporal, and cheeks show lower risk levels, indicating reduced anatomical vulnerability




Univariate analyses identified several variables significantly associated with excision completeness. Using chi square tests with rare categories collapsed into an "OTHER" group, statistically significant associations were observed for surgical procedure, tumor stage, resection margin status (lateral and deep), solar elastosis, desmoplastic reaction, and depth of invasion. All associations remained significant after correction for multiple testing using the false discovery rate (FDR). The strongest association was observed for surgical procedure, followed by tumor stage and resection margin characteristics. Histopathological features reflecting tumor aggressiveness and tissue response—specifically solar elastosis, desmoplastic reaction, and deeper tissue invasion—were also significantly associated with excision completeness. These findings indicate that procedural factors, tumor extend, and pathological indicators of infiltrative growth are each related to the likelihood of achieving complete excision at a univariate level, Table 5.

**Table 5:** Univariate analysis of the study group

| Variable | Test | p-value | FDR q-value |
|---|---|---|---|
| **Procedure** | **Chi-square (rare → OTHER)** | **$1.79 \times 10^{-9}$** | **$4.12 \times 10^{-8}$** |
| **Tumor stage** | **Chi-square (rare → OTHER)** | **$2.07 \times 10^{-6}$** | **$1.62 \times 10^{-5}$** |
| **Resection margins (lateral and deep, mm)** | **Chi-square (rare → OTHER)** | **$2.11 \times 10^{-6}$** | **$1.62 \times 10^{-5}$** |
| **Solar elastosis** | **Chi-square (rare → OTHER)** | **$5.83 \times 10^{-4}$** | **$3.35 \times 10^{-3}$** |
| **Desmoplastic reaction** | **Chi-square (rare → OTHER)** | **$3.74 \times 10^{-3}$** | **$1.72 \times 10^{-2}$** |
| **Depth of invasion** | **Chi-square (rare → OTHER)** | **$4.77 \times 10^{-3}$** | **$1.83 \times 10^{-2}$** |

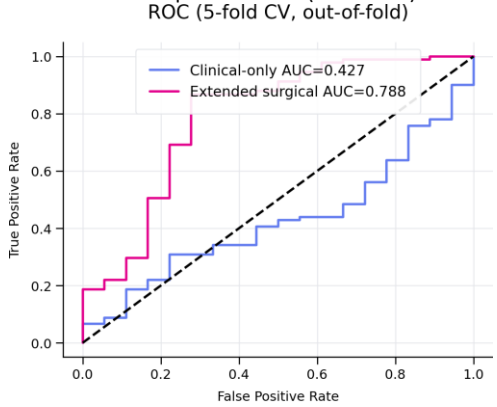
In multivariable logistic regression analysis evaluating independent predictors of complete excision, none of the examined clinical or histopathological variables demonstrated a statistically significant association after simultaneous adjustment. Patient age showed no

meaningful effect on excision completeness (odds ratio [OR] ≈1.0), while indicators of tumor aggressiveness—including deep invasion into the hypodermis or muscle and advanced pathologic stage (pT ≥ 2)—were associated with lower odds of complete excision, although these associations did not reach statistical significance. Similarly, histopathological features such as increasing solar elastosis grade and the presence of ulceration were not independently associated with completeness of excision. The absence of statistically significant predictors in the adjusted model contrasts with the univariate findings and likely reflects collinearity among tumor stage, depth of invasion, and related pathological characteristics, as well as limited power due to the relatively small number of incomplete excisions. Overall, these results suggest that while surgical and pathological factors are associated with excision completeness at a univariate level, no single variable independently predicts complete excision when considered within a multivariable framework (Table 6).

**Table 6:** Multivariate analysis of the study group

| Predictor | OR | 95% CI |
|---|---|---|
| **Age (per 1 year)** | 0.99 | 0.94 – 1.04 |
| **Deep invasion (hypodermis/muscle)** | 0.6 | 0.15 – 2.40 |
| **pT ≥ 2** | 0.44 | 0.09 – 2.18 |
| **Solar elastosis (per grade)** | 1.17 | 0.70 – 1.95 |
| **Ulceration (YES *vs* NO)** | 1.04 | 0.33 – 3.29 |

**Figure 10:** Receiver operating characteristic (ROC) curves comparing model performance under 5 fold cross validation. The clinical only model (blue) achieved an AUC of 0.427, indicating limited discriminative ability, while the extended surgical model (magenta) reached an AUC of 0.788, demonstrating substantially improved predictive accuracy. The dashed diagonal line represents random classifier performance (AUC = 0.5)




Receiver operating characteristic (ROC) analysis demonstrated superior predictive performance of the extended surgical model compared to the clinical-only model (Figure 10). The clinical-only model achieved an AUC of 0.427, indicating limited discriminatory ability, whereas the extended surgical model demonstrated substantially improved predictive accuracy, with an AUC of 0.788. Calibration analysis further demonstrated improved agreement between predicted and observed outcomes for the extended surgical model, particularly for complete and incomplete resection categories. As illustrated in Figure 11, the extended surgical model showed better calibration performance compared to the clinical-only model across most outcome categories. Prediction of mixed-pattern lesions remained limited in both models, likely reflecting the low number and heterogeneity of these cases. Overall, the inclusion of extended surgical and histopathological parameters improved both discrimination and calibration performance, supporting their added predictive value in resection outcome assessment.

**Figure 11:** Multiclass cross-validation performance summary (5-fold out-of-fold), comparing the clinical-only and extended surgical feature sets

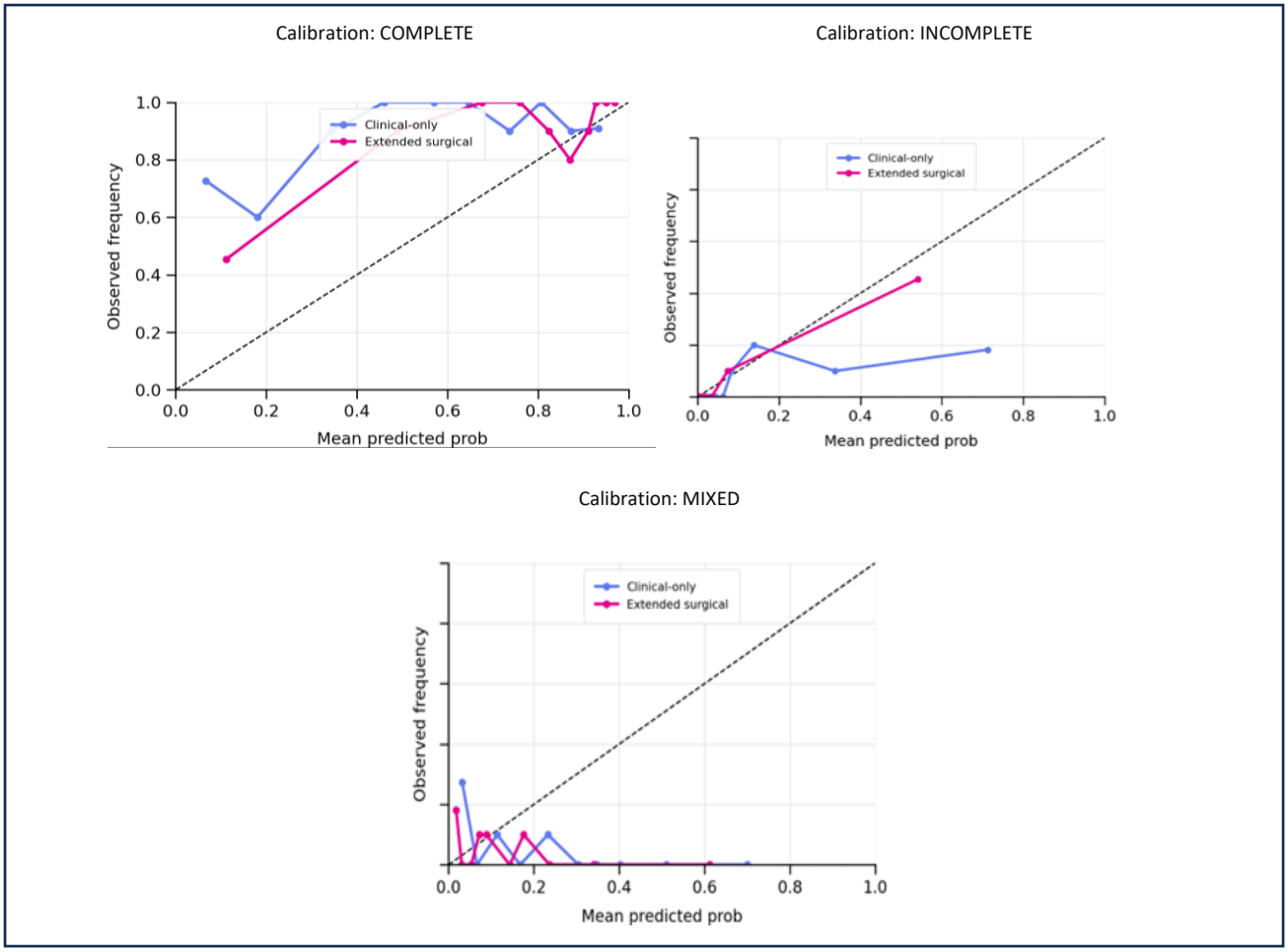



Table 7 summarizes 5 fold out of fold cross validation performance for a three class classification task (COMPLETE, INCOMPLETE, MIXED), comparing a clinical only model with an extended surgical feature set. The evaluation includes 102 cases, with marked class imbalance (91 COMPLETE, 6 INCOMPLETE, 5 MIXED), which limits the stability of estimates. Macro AUC (one vs rest) reflects average discrimination across classes and improves from 0.560 (clinical only) to 0.687 (extended). Log loss assesses the quality/calibration of predicted probabilities and improves substantially from 0.918 to 0.446, suggesting better probabilistic predictions with surgical features.

**Table 7:** Multiclass cross-validation metrics (5-fold out-of-fold), clinical-only vs extended surgical feature sets

| Metric | Clinical-only | Extended surgical |
|---|---|---|
| **N** | 102 | 102 |
| **Class counts** | {'COMPLETE': 91, 'INCOMPLETE': 6, 'MIXED': 5} | {'COMPLETE': 91, 'INCOMPLETE': 6, 'MIXED': 5} |
| **Macro AUC (OVR)** | 0.560 | 0.687 |
| **Log loss** | 0.918 | 0.446 |

Table 8 is a confusion matrix for the clinical-only model's 5 fold out of fold predictions in the three class task (COMPLETE, INCOMPLETE, MIXED). Rows are the true class and columns are the predicted class; values are the number of cases. Correct predictions are on the diagonal: 63 COMPLETE→COMPLETE, 2 INCOMPLETE→INCOMPLETE, and 0 MIXED→MIXED. The main errors are that many true COMPLETE cases are predicted as INCOMPLETE (11) or MIXED (17), and most true INCOMPLETE cases are predicted as MIXED (4). All true MIXED cases are misclassified (3 as COMPLETE, 2 as INCOMPLETE), indicating poor recognition of the MIXED class.

**Table 8:** Confusion matrix (clinical-only, out-of-fold) for the multiclass margin-status classification

| True Label | Predicted Complete | Predicted Incomplete | Predicted Mixed |
|---|---|---|---|
| **True Complete** | 63 | 11 | 17 |
| **True Incomplete** | 0 | 2 | 4 |
| **True Mixed** | 3 | 2 | 0 |

Table 9 is also a confusion matrix for the same three-class task (COMPLETE, INCOMPLETE, MIXED), but presented in a more compact "alternate" layout. Like Table 8, rows are the true labels and columns are the predicted labels, so the diagonal cells are correct classifications. In Table 9, the model correctly predicts 84/91 COMPLETE cases, 5/6 INCOMPLETE cases, and 0/5 MIXED cases. The main errors are misclassifying COMPLETE as MIXED (6) and a small number of COMPLETE as INCOMPLETE (1), plus misclassifying MIXED as COMPLETE (4) or INCOMPLETE (1). Overall, MIXED is not learned well.

**Table 9:** Confusion matrix (alternate display) for the multiclass margin-status classification

| True Label | Predicted Complete | | Predicted Incomplete | Predicted Mixed |
|---|---|---|---|---|
| **True Complete** | 84 | 1 | | 6 |
| **True Incomplete** | 0 | 5 | | 1 |
| **True Mixed** | 4 | 1 | | 0 |

**DISCUSSION**

In this single-center retrospective cohort of head and neck NMSC, basal cell carcinoma predominated and lesions clustered in chronically sun-exposed anatomic regions, particularly the cheek and nose. This distribution is consistent with the well-established role of cumulative UV exposure in keratinocyte carcinoma pathogenesis and with prior reports showing a high burden of disease in the facial "H-zone", where complex anatomy may constrain excision margins.

Among conventionally excised lesions, the proportion of positive margins (R1) was relatively low (8%). Lateral margin involvement was more frequent than deep margin involvement, which may reflect the practical tendency to preserve functionally and cosmetically sensitive tissue laterally in the head and neck. Although the number of R1 cases was small, positive margins appeared concentrated in high-risk sites (e.g., nose, periocular, auricular, scalp), supporting the view that anatomic constraints and tumor subclinical spread can increase incomplete excision risk in these areas. In clinical practice, these findings highlight the importance of careful preoperative risk stratification and consideration of margin-controlled techniques when feasible in high-risk locations.[16]

Although conventional surgery remains the cornerstone of treatment, advanced imaging modalities such as PET-CT have shown value in assessing tumor extent and vascular involvement in other malignancies, highlighting the growing role of functional imaging in oncologic decision-making. [17]

When interpreted against published data, the 8% rate of positive margins in our conventionally excised cohort appears lower than rates reported in several head and neck BCC series focused on higher-risk lesions, where incomplete excision has been reported around 15–22% overall and may approach ~30–37% in particularly challenging facial sites such as the nose, periorbital region, ear, and scalp. In cutaneous SCC, a systematic review has estimated an overall incomplete excision rate of approximately 13%, with head and neck location repeatedly identified as a risk factor. Differences in case mix (including inclusion of precursor lesions), surgeon specialty, excision technique, and pathology processing may account for substantial between-study variability; nevertheless, these external estimates provide a useful benchmark suggesting that even in tertiary practice, incomplete excision remains a clinically relevant outcome that warrants ongoing audit and process optimization.[18]

The predominance of lateral margin involvement in our R1 cases may be explained by the competing priorities of oncologic clearance and tissue preservation in cosmetically and functionally critical facial units. In routine wide local excision, the "tightest" margin is often the lateral margin because it determines final defect size and reconstructive complexity; by contrast, the deep plane is frequently taken down to a relatively fixed anatomic layer. In addition, certain BCC growth patterns can extend subclinically beyond visible tumor borders, increasing the risk of lateral positivity despite apparently adequate clinical margins. From a practical standpoint, these observations support a workflow in which tumors in high-risk sites or with suspected aggressive behavior are considered early for margin-controlled approaches (e.g., Mohs micrographic surgery or staged excision when available), and where conventional excision is performed, clear specimen orientation and explicit reporting of lateral versus deep margin involvement are emphasized to facilitate timely and anatomically targeted re-excision.[19]

For head and neck lesions—particularly in high-risk facial subunits (perinasal, periocular, auricular, and scalp)—preoperative counseling should address the possibility of positive margins and the potential need for staged management (re-excision or margin-

controlled surgery where available). When conventional excision is selected, careful clinical border assessment (including dermoscopy when appropriate), documentation of planned margins, and close communication with pathology regarding margin orientation may reduce avoidable R1 resections. Finally, because reconstruction choices can limit re-excision options, surgeons should consider a reconstruction approach that preserves tissue and defers complex flap reconstruction when margin uncertainty is high, or alternatively plan for margin-controlled approaches in lesions with higher pretest risk.[20]

A specific implication for head and neck surgery is the interaction between margin uncertainty and reconstruction timing. Complex local flaps can distort anatomic planes and may complicate subsequent margin-directed re-excision if an R1 result is returned. For lesions with higher pretest risk of incomplete excision (high-risk sites, suspected aggressive subtype, larger diameter, deeper invasion, or perineural involvement), a pragmatic strategy is to either pursue a margin-controlled technique up-front, or adopt a staged approach with temporary defect management (or simpler closure) until histologic clearance is confirmed, followed by definitive reconstruction when appropriate. Embedding this decision-making into preoperative counseling may reduce patient distress when additional procedures are needed and may help preserve reconstructive options.[21]

Direct closure was the most common reconstructive approach, while local flaps accounted for approximately one quarter of reconstructions. This pattern is expected in a cohort with predominantly small-to-moderate tumor sizes and emphasizes the value of anticipating closure options during surgical planning. In particular, transposition flaps were the most frequently used flap subtype, reflecting their versatility for common facial defects. Systematically recording reconstruction methods, as done in this study, can support local audits and may help identify anatomic regions where more advanced reconstruction is more likely to be required.

Future work should prioritize prospective follow-up to capture recurrence and the downstream management of R1 cases (re-excision, observation, adjuvant therapy where relevant), since margin status is a surrogate outcome and may not translate directly into clinically meaningful events in all patients. Larger multi-center datasets would also allow more robust multivariable modeling with adequate event counts and could separate analyses for BCC versus invasive SCC, while treating precursor lesions (AK, Bowen's disease, keratoacanthoma) as a distinct category or sensitivity analysis. Finally, standardizing the recording of planned clinical margins, specimen orientation, and pathology reporting within institutional pathways would support both quality improvement and external comparability across studies.[22]

**LIMITATIONS**

This study has several limitations. First, its retrospective, single-center design and short accrual window (September 2025–January 2026) may limit generalizability and can amplify local referral patterns, seasonal effects, and institutional practice preferences. In addition, selection and referral bias are possible: complex head and neck tumors may be overrepresented in a tertiary service, whereas very high-risk lesions may be preferentially referred for margin-controlled surgery in other settings, which could influence observed R1 rates.

Second, the analysis was performed at the lesion level, and some patients contributed multiple tumors; without explicit methods to account for within-patient clustering, independence assumptions may be violated. Procedures were performed by multiple clinicians, and variability in margin selection, specimen orientation, and reconstructive approach may confound associations. Margin status itself is subject to routine histopathology constraints (orientation, inking, sectioning) and incomplete documentation may lead to misclassification of lateral versus deep involvement or underestimation of true positive margins. Tumor dimensions were reconstructed from clinical measurements and pathology reports, which may introduce measurement error and reduce comparability across cases.

Third, the cohort included invasive SCC as well as SCC precursor lesions and keratoacanthoma, which broadens clinical scope but complicates comparisons with studies restricted to invasive tumors. AJCC staging and documentation of nodal/metastatic assessment were not uniformly available, limiting risk stratification. Prospective follow-up was not available to evaluate recurrence after the index excision; prior surgery history within 24 months is not equivalent to post-treatment recurrence. Finally, the small number of R1 events creates substantial class imbalance and limits statistical power; any multiple testing or predictive metrics presented should be regarded as exploratory and hypothesis-generating until supported by a prespecified analysis plan, fuller methodological reporting, and external validation.

**CONCLUSION**

Head and neck NMSC in this cohort occurred predominantly in elderly patients and was most frequently located on the cheek and nose. Conventional excision achieved complete margins in most cases, with positive margins occurring in a small subset and tending to involve high-risk anatomic sites. Direct closure was the most common reconstruction, while local flaps—predominantly transposition flaps—were required in roughly one quarter of conventionally excised cases. Prospective follow-up and larger multi-center datasets are needed to better quantify risk factors for incomplete excision and to evaluate prediction approaches and outcomes, including recurrence.

***Conflicts of interest and sources of funding***

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

***Authors' contribution***

*Authors' contribution: Conceptualization, M.M., M.D. and A.C.; methodology, R.C. and G.M.; software, A.Z.C.A. and D.V.; validation, M.M., C.S. and A.C.; formal analysis, M.D. and R.C.; investigation, M.M. and C.S.; resources, G.M. and A.Z.C.A.; data curation, M.D. and D.V.; writing—original draft preparation, M.M. and M.D.; writing—review and editing, M.M. and R.C.; visualization, G.M. and D.O.C.; supervision, C.S. and D.O.C.; project administration, A.C. and D.V.; funding acquisition, A.Z.C.A. and D.O.C. All authors have read and agreed to the published version of the manuscript.*

***Ethics approval and consent to participate***

*The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee of the Dr. Carol Davila University Emergency Central Military Hospital, Bucharest (registration number).*

***Patient consent for publication***

*Not applicable, because it is a retrospective study.*

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ARTICLE

# Effects of Probiotics Lactobacilli on Supporting Adaptive Immune Responses in Helicobacter pylori-Infected Mice

**Somayyeh Taghizadeh[1*], Zahra Hojjati Bonab[2]**

[1] Department of Biology Education, Farhangian University, P.O.Box 14665-889, Tehran, Iran. s.taghizadeh@cfu.ac.ir
[2] Department of Microbiology, Bon.C., Islamic Azad University, Bonab, Iran. 1688884221@iau.ir

*Correspondence: s.taghizadeh@cfu.ac.ir

**Abstract:** Helicobacter pylori (H. pylori) is recognized as a human gastric pathogen that is a major risk factor for the development of peptic and duodenal ulcers, gastric adenocarcinoma, and lymphoma in humans. Antibiotic treatment is not completely effective due to antibiotic resistance. Probiotics play an important role in regulating the immune system and exert antibacterial effects by producing metabolites. Probiotic strains were administered to infected mice, and immune responses were monitored to investigate their immunomodulatory effects. Pathogen-free male C57BL/6 mice were divided into 6 groups of 5, and treatments were administered. Fourteen days after the last treatment, their spleens were removed, and IFN-γ and IL-4 expression levels were measured by real-time PCR. After treatment of L. acidophilus ATCC4356 and L. rhamnosusPTCC1607, the expression of INF-γ increased, but IL-4 expression was decreased. It can be concluded that the functions of probiotic strains differ. Two probiotics, L. acidophilus ATCC4356 and L. rhamnosus PTCC1607, stimulate interferon-gamma production to increase the phagocytic activity of macrophages and facilitate the differentiation of T cells to Th1.

**Keywords:** Helicobacter pylori, Lactobacillus acidophilus, Lactobacillus rhamnosus, IL-4, INF-γ

## INTRODUCTION

H. pylori is recognized as a human gastric pathogen that is a flagellated, microaerophilic, spiral-shaped, and gram-negative bacterium isolated from human gastric mucosa and was first recognized in 1982. It colonizes the stomachs of at least half of the world's population. It is proposed that H. pylori is the main cause of gastritis and peptic ulcer and a risk factor for gastric malignancy in humans. Most cases colonized by these bacteria are asymptomatic. In symptomatic subjects, the antibiotic treatment is not completely effective due to antibiotic resistance.

According to the Food and Agriculture Organization of the United Nations and the World Health Organization, probiotics are defined as 'living microorganisms, which when administered in adequate amounts confer health benefits on the host' [1]. Mechanisms of probiotics include controlling intestinal microbial communities, inhibiting pathogens, stimulating epithelial cell proliferation, and immunomodulation [2]. Numerous studies have examined the precise role of probiotics for the eradication of H. pylori. Some research has shown that probiotics can't eradicate H. pylori but can reduce its levels in the stomach [3]. It has been shown that probiotics can be used as a complementary therapy for the management of H. pylori infection [4]. Lactobacillus spp. are well-characterized probiotics that produce lactic acid and have demonstrated beneficial effects against gastrointestinal pathogens such as H. pylori [5]. It has been indicated that L. rhamnosus JB3 can decrease gastric inflammation by weakening H. pylori virulence in mice [6]. Some probiotic strains, such as Lactobacillus and Bifidobacterium, can inhibit H. pylori growth via bacteriocins or organic acid production [3]. Michetti et al. showed that gastric inflammation can decrease when animals were colonized by L. johnsonii La1 and L. acidophilus LB [7,8]. Probiotics can modulate cytokine secretion through signaling pathways that also affect immune cells, such as T lymphocytes, promoting their proliferation and differentiation [2]. In this study, H. pylori and two probiotic strains were administered to infected mice, and immune responses were monitored to investigate the immunomodulatory effects and to prospect possible mechanisms of Lactobacillus strains in suppressing H. pylori infection.

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## MATERIALS AND METHOD

### Bacterial strains and culture conditions

A clinical strain of H. pylori obtained from the Alzahra University collection was grown on Brucella agar (Merck, Germany) supplemented with 5% defibrinated sheep blood and antibiotics under microaerophilic conditions at 37 ˚C for 72 h, and then identified by biochemical tests and Gram staining. L. acidophilus ATCC 4356 and L. rhamnosus PTCC 1607 were obtained from the Organization of Industrial Research of Iran. Lactobacillus spp. were routinely grown on de Man, Rogosa, and Sharpe (MRS) agar (Merck, Germany) in an anaerobic chamber at 37 °C [9].

### Infection Model

Pathogen-free male C57BL/6 mice weighing between 20 and 22 g were obtained from the Razi Institute, Karaj, Iran. All experiments were performed on mice in accordance with the Animal Ethics Committees of AL Zahra University. Mice groups and the number of mice in each group were determined by the "Resource Equation" method [9]. The mice ranged in age from 6 to 8 weeks. All mice, in 6 groups, were maintained in a pathogen-free environment, with standard brightness (12 h of darkness/light), standard aeration, and free of contamination at a temperature of 21±2 ° C and 55±5% humidity (with free access to chow and sterile water).

### Experimental diets

It should be noted that the H. pylori stomach colonization test was performed first. For the determination of colonized bacteria, gastric tissue was removed and homogenized in Brucella broth (with 5% FCS), then cultured on the Brucella blood agar medium and incubated for 5 days (microaerobic; 37°C conditions). After incubation, colony recognition, Urease action, oxidase, and catalase tests were performed.

Mice were divided into 6 groups of five, and the treatments were performed as follows:

Group 1- 100µl (microliters) of a concentration of 1 ×108 CFU / ml L. acidophilus was given to mice by gavage (orally) for three consecutive days under the same conditions.

Group 2- 100µl of concentration of 1 ×108 CFU / ml L. acidophilus was given to mice by gavage (orally) for three consecutive days under the same conditions. Then, for three days, 100µl of a concentration of 1× 109 CFU / ml H. pylori was given to mice by gavage.

Group 3- 100µl of concentration of 1 ×108 CFU / ml L. rhamnosus was given to mice by gavage (orally) for three consecutive days under the same conditions.

Group 4- 100µl of concentration of 1 ×108 CFU / ml L. rhamnosus was given to mice by gavage (orally) for three consecutive days under the same conditions. Then, for three days, 100µl of a concentration of 1× 109 CFU / ml H. pylori was given to mice by gavage.

Group 5- 100µl of a concentration of 1× 109 CFU / ml H. pylori was given to mice by gavage for three consecutive days under the same conditions.

Group 6- Five mice were considered the control group under the same conditions as the other treated mice, except that they received no bacteria and were given only sterile phosphate-buffered saline (PBS).

It should be noted that the booster doses for the above treatments were administered two weeks after the initial treatments, under the same conditions. 14 days after the last treatment, the animals were killed ethically (anesthesia with peritoneal injection of diazepam and 10% ketamine), and their spleen was removed.

Spleen samples were thoroughly washed with normal saline and stored in numbered microtubes containing 1 ml of sterile normal saline in a -80 ° C freezer.

### Real-time RT-PCR assay for cytokine mRNA expression in spleen

Levels of IFN-γ and IL-4 expression were measured by real-time PCR. Total RNA was extracted from 25-50 mg of spleen tissue according to the manufacturer's protocol (Pars-tous Biotechnology, Iran). To determine the quantity and purity of the extracted RNA, the optical density of the samples at 260/280 was measured.

The extracted RNA was used to synthesize cDNA using the First-Aid Reverse Transcription Kit (Fermentas). In brief, 5-10µg of the extracted RNA, 1µl of oligo dT, 1µl of the random hexamer, and 15_20 pmoles of sequence-specific primers were adjusted to 14µl by DEPC water. The resulting composition was heated (65° C for 5 min), then cooled on ice and mixed rapidly. Then, 4µl of RT 5 x Buffer, containing 10 mM dNTPs, 1µl DTT, and Revert UP™ II Reverse Transcriptase were added to each microtube. It was then heated at 50 °C for 60 minutes and incubated at 95 °C for 5 minutes to deactivate the reverse transcriptase (terminal steps were performed in the thermocycler). The cDNA was stored in a freezer and used for quantitative PCR reactions.

The real-time PCR reactions were performed to analyze the expression levels of IFN-γ, IL-4, and β-actin. In this section, the synthesized cDNA and selected primers were used for qRT-PCR. The reaction conditions were as follows: 95°C for 15 min, 95°C for 30 s, 62°C for 30 s, 72°C for 30 s in 40 cycles, and 1 cycle at 72°C for 30s for the final extension. All experiments were done in triplicate. The β-actin gene was used as the reference gene to determine an arbitrary normalized value for each gene [10].

**Statistical analysis**

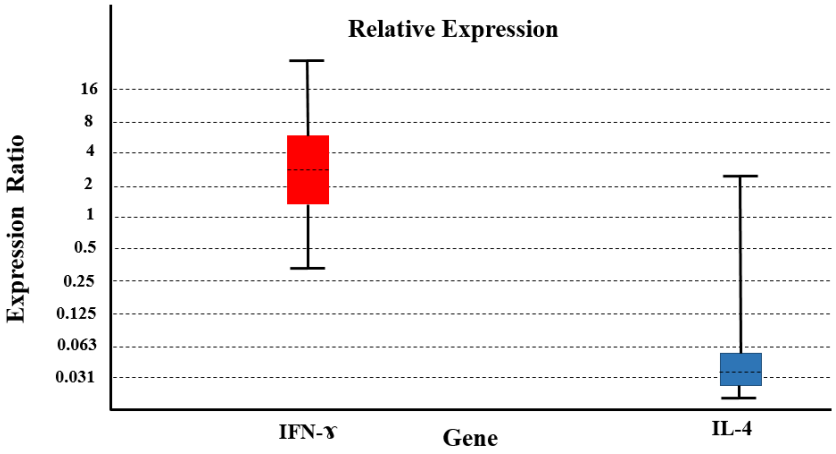
The statistical analysis was performed by the REST software version 9. The data were analyzed by ANOVA, followed by Tukey's post hoc test. The level of statistical significance was set at $p<0.05$.

**RESULTS**

**Effects of L. acidophilus ATCC4356 treatments on INF-γ and IL-4 expression**

In this treatment, INF-γ expression was increased (by almost 3.2-fold) compared to the control group (P=0.03). However, IL-4 expression decreased compared to the control group (P=0.04) (Figure 1).

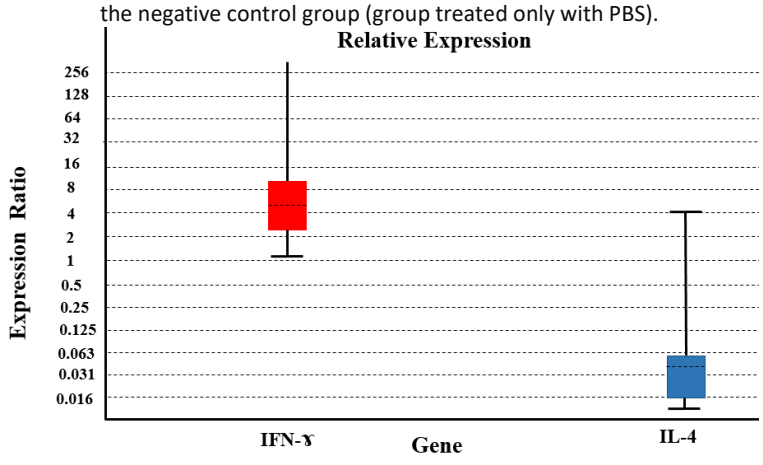
**Figure 1:** Expression of IL-4 and INF-γ in the group of mice treated with L. acidophilus ATCC4356 in comparison with the negative control group (group treated only with PBS).




**Effects of L. acidophilus ATCC4356 and H. pylori treatments on INF-γ and IL-4 expression**

The expression level of INF-γ in this treatment showed an increase in expression (by almost 10.9 fold) compared to the control group (P=0.007), and the level of IL-4 expression compared to the control group showed a decrease in expression (P=0.03) (Figure 2).

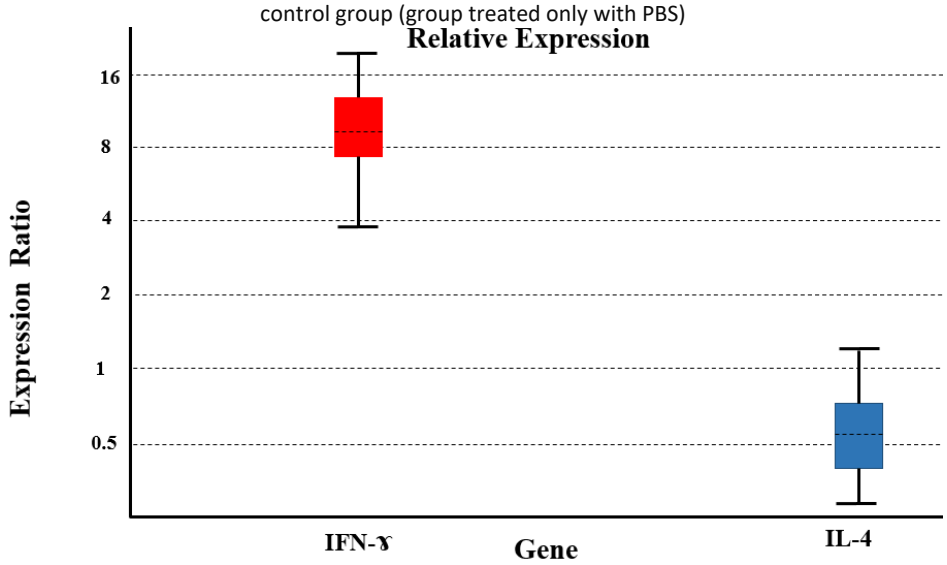
**Figure 2:** Expression of IL-4 and INF-γ in the group of mice treated with L. acidophilus ATCC4356 and H. pylori in comparison with the negative control group (group treated only with PBS).



**Effects of L. rhamnosus PTCC1607 treatments on INF-γ and IL-4 expression**

INF-γ expression was increased in this treatment (by almost 9-fold) compared to the control group (P = 0.008). But the expression level of IL-4 compared to the control group showed a decrease in expression (P=0.01) (Figure 3).

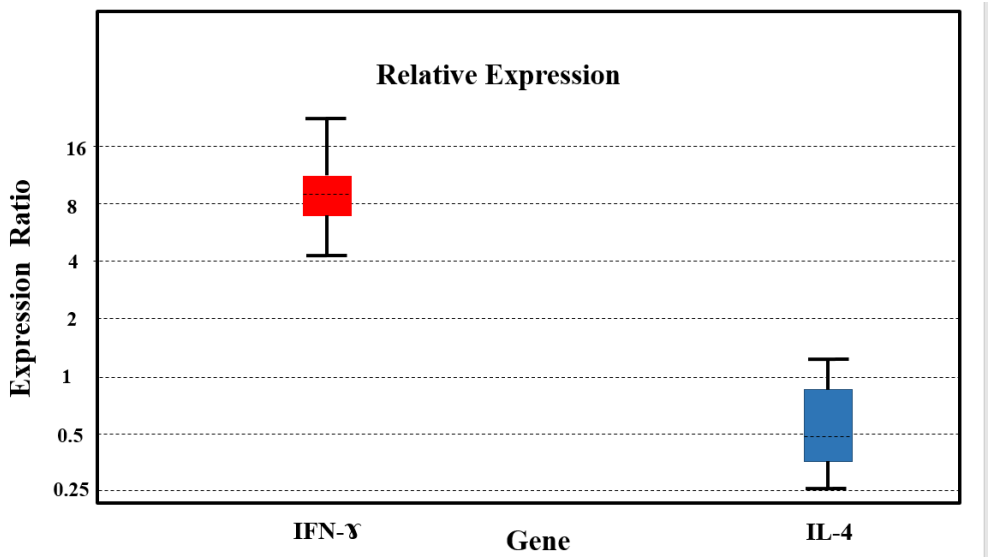
**Figure 3:** Expression of IL-4 and INF-γ in the group of mice treated with L. rhamnosus PTCC1607 in comparison with the negative control group (group treated only with PBS)




**Effects of L. rhamnosus PTCC1607 and H. pylori treatments on INF-γ and IL-4 expression**

The expression level of INF-γ in this treatment showed an increase in expression (by almost 8.5-fold) compared to the control group (P= 0.009), but the expression of IL-4 levels did not change compared to the control group (P=0.06) (Figure 4).

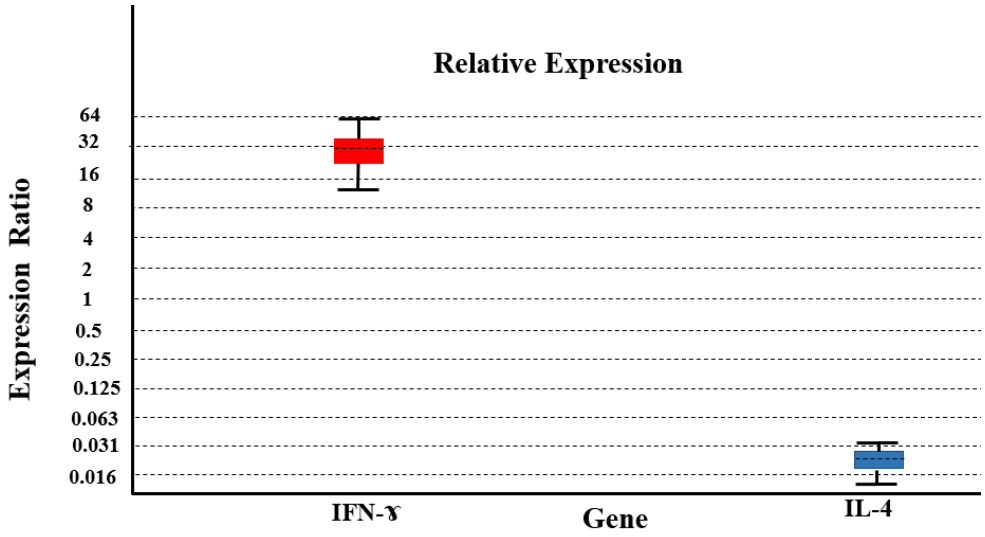
**Figure 4:** Expression of IL-4 and INF-γ in the group of mice treated with L. rhamnosus PTCC1607 and H. pylori in comparison with the negative control group (group treated only with PBS)



**Effects of H. pylori treatments on INF-γ and IL-4 expression**

INF-γ expression was increased in this treatment (by almost 29-fold) compared to the control group (P=0.008). However, IL-4 expression relative to the control group decreased (P=0.002) (Figure 5).

**Figure 5:** Expression of IL-4 and INF-γ in the group of mice treated with H. pylori in comparison with the negative control group (group treated only with PBS).




**DISCUSSION**

The objective of this study was to examine the immune response induced by probiotic lactobacilli against gastroduodenal diseases, such as H. pylori infection. Immunity against H. pylori is characterized by a strong Th1 response, and the generation of an adaptive Th1 response against H. pylori infection requires innate immunity. For example, the neutrophil-activating protein of H. pylori induces interleukin IL-12 and IL-23 secretion [12]. T cells in the gastric mucosa are known to consist of Th1 cells that produce IFN-γ mostly [13]. The expression of IFN-γ and IL-12 was increased in gastric biopsy samples from patients infected with Helicobacter pylori [14]. The results of Vinagre et al. in 2018 showed that IL-4 and IL-10 concentrations in the stomachs of patients infected with H. pylori were significantly lower than in uninfected patients. Their results also showed that the concentrations of IFN-γ and IL-12 in patients infected with Helicobacter pylori were significantly higher than those in uninfected patients [15]. Many studies show that probiotics can be used as a complementary therapy for the management of H. pylori infection [4]. There is substantial evidence that certain probiotics play a significant role in regulating the immune system [16]. For example, it was reported that Lactobacillus can promote mononuclear cells to produce IFN-γ, IL-12, and IL-18. These organisms activate signaling pathways, such as NF-κB and STAT, in human macrophages [17]. Besides, probiotics can induce the production of T-helper 1-classified cytokines [18]. These studies suggest that certain probiotic strains may upregulate innate immune responses [19]. Kabir et al. showed that lactobacilli are a promising option for treating H. pylori, which may be due to their ability to inhibit pathogen binding. They also showed that mice fed a diet containing L. salivarius had no pathological problems in the stomach, as evidenced by the lack of IL-8 production by gastrointestinal epithelial cells [20].

Treatment of mice with L. reuteri and L. brevis increased the expression of inflammatory and Th1 cytokines such as IL-2, TNF-α, and IL-1β. However, conflicting results have been obtained regarding the role of probiotics in increasing the production of cytokines such as IFN-γ, IL-10, IL-1β, IL-6, TNF-α, and IgA production [21-25].

Incidentally, it has been shown that Lactobacillus species exhibit different abilities to produce proinflammatory cytokines in bone marrow-derived dendritic cells [26]. Koga has shown that L. gasseri prevents H. pylori-induced gastritis by inhibiting the bacterial type 4 secretory system, which reduces IL-8 production by gastrointestinal epithelial cells [27]. The use of two probiotic bacteria, L. paracasei and L. reuteri, reduces proinflammatory cytokines and intake of L. plantarum in mice that were deficient in IL-10 production, reduces mucosal IL-12 levels, and, consequently, reduces intestinal inflammation [28]. Activated macrophages can produce TNF-α and IFN-γ that have an important role in the immune defense against pathogens (viral and microbial) and induce immune responses [29-31]. Studies by Gill et al. showed that spleen cells in mice treated with L. rhamnosus and L. acidophilus produced significant amounts of IFN-γ [32]. The induction effect of L. acidophilus on IFN-γ production was demonstrated by mouse spleen macrophages

[33,34]. A recent study by Ren et al. showed that mice that received the probiotic bacteria L. plantarum and L. salivarius, compared to the control group, showed an increase in the production of IL-10, IFN-γ, and TLR-2 [35]. Lactobacillus casei Strain Shirota can reduce the colonization of H. pylori and gastritis caused by this bacterium, and increase cellular immunity [36,37].

In this regard, our studies also showed that the expression level of IFN-γ in the group that was treated only with H. pylori increased 29 times compared to the control group, and IL-4 expression decreased; this confirms the superiority of the cellular immune response against this bacterium. The group that received the probiotic bacteria L. acidophilus ATCC4356 and L. rhamnosus PTCC1607 had an increase in IFN-γ expression level, which was 3.2 times in treatment with L. acidophilus and 9 times in treatment with L. rhamnosus. Therefore, the stimulatory effect of L. rhamnosus was greater, and it exerts the effect of strengthening the cellular immune response by helping stimulate T cell differentiation to Th1.

In the groups that received the combination of probiotics and H. pylori, the expression level of IFN-γ increased; in treatment with L. acidophilus plus H. pylori, it increased 10.9-fold, and in treatment with L. rhamnosus plus H. pylori, it increased 8.5-fold. Therefore, both probiotics have a stimulating effect and can help increase monocyte and macrophage activity, reduce inflammation, and strengthen the cellular immune response against H. pylori. It should be noted that the group that received only H. pylori showed a 29-fold increase in expression. However, the groups receiving the combination of probiotics and H. pylori increased IFN-γ expression, with an increase of 10.9 times in treatment with L. acidophilus and 8.5 times in treatment with L. rhamnosus, which was lower than the group that received only H. pylori. This may be due to the inhibitory effect of L. acidophilus and L. rhamnosus, which reduces the excitatory effect of H. pylori by preventing its binding to the gastric epithelium, as confirmed in similar studies [36]. The increase in expression in the groups that received the combination of probiotic and H. pylori is due to the combined effect of probiotic bacteria and H. pylori. Since no expression of the cytokine IL-4 gene was observed in any of the groups compared to the negative control group, and the changes were in the form of reduced expression or no change in expression, it can be stated that the above probiotics do not have the desired strengthening function in stimulating the humoral immune response in mice. In other words, the immunomodulatory effects of these probiotics are to help to induce a cellular immune response by aiding the stimulation of interferon-gamma production.

## CONCLUSION

It can be concluded that the function of probiotic strains is different; each probiotic strain is a unique organism with its own characteristics, and care must be taken in selecting the strains to ensure effectiveness. What is clear in this study is that two probiotics, L.acidophilus ATCC4356 and L. rhamnosus PTCC1607, stimulate the production of interferon-gamma to increase the phagocytic activity of macrophages and also facilitate the differentiation of T cells to Th1. Th1 cells produce several cytokines that ultimately reduce proinflammatory cytokines such as IL-8 and IL-1, thereby directing the innate and acquired immune responses and keeping the body on standby.

***Conflicts of interest and sources of funding***

*The authors declare no conflict of interest.*

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***Authors' contribution***

*All authors participated in the presentation of the original idea and design, search for sources and review of articles, initial writing or revision of the article, and all with the final approval of the present article, take responsibility for the accuracy of the content.*

***Ethics approval and consent to participate***

*This project has been approved by the Ethics Committee of Al-Zahra University, Iran, with ID 5263.*

***Patient consent for publication***

*Not applicable.*

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ARTICLE

# Exploratory results of circulating chemerin testing in humans

**Sorina Violeta Schipor[1], Dana Manda[1], Mihai-Lucian Ciobica[2,3], Oana-Claudia Sima[4,5], Emi Marinela Preda[6,7*], Adrian Ciuche[8,9*], Mihai Costachescu[4,10], Mara Carsote[5,11], Ana Popescu[12], Ana Valea[13,14]**

1 Department of Research, "C.I. Parhon" National Institute of Endocrinology, 011863 Bucharest, Romania; S.V.S. sorina.schipor@parhon.ro, D.M. dana.manda@parhon.ro
2 Department of Internal Medicine and Gastroenterology, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania
3 Department of Internal Medicine I and Rheumatology, "Dr. Carol Davila" Central Military University Emergency Hospital, 010825 Bucharest, Romania; M.-L.C. lucian.ciobica@umfcd.ro
4 PhD Doctoral School of "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania
5 Department of Endocrinology V, "C.I. Parhon" National Institute of Endocrinology, 011863 Bucharest, Romania; O.-C.S. oana-claudia.sima@drd.umfcd.ro
6 Department of Radiology, "Carol Davila" University of Medicine and Pharmacy, 050474 Bucharest, Romania
7 Department of Radiology and Medical Imaging, "Foisor" Clinical Hospital of Orthopedics, Traumatology and Osteoarticular TB, 021382 Bucharest, Romania; E.M.P. emi.preda@umfcd.ro
8 Department 4 – Cardio-Thoracic Pathology, Thoracic Surgery II Discipline, "Carol Davila" University of Medicine and Pharmacy, 0505474 Bucharest, Romania
9 Thoracic Surgery Department, "Dr. Carol Davila" Central Emergency University Military Hospital, 010825 Bucharest, Romania A.C. adrian.ciuche@umfcd.ro
10 Department of Radiology and Medical Imaging, "Dr. Carol Davila" Central Military University Emergency Hospital, 010825 Bucharest, Romania; M.K. mihai.costachescu@drd.umfcd.ro
11 Department of Endocrinology, "Carol Davila" University of Medicine and Pharmacy, 020021 Bucharest, Romania; M.C. carsote_m@hotmail.com
12 Department of Family Medicine, State "Nicolae Testemiţanu" University of Medicine and Pharmacy, 2004 Chisinau, Republic of Moldova; A.P. ana.popescu@usmf.md
13 Department of Endocrinology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania
14 Department of Endocrinology, County Emergency Clinical Hospital, 400347 Cluj-Napoca, Romania; A.V. ana.valea@umfcluj.ro

* Correspondence to: emi.preda@umfcd.ro (E.M.P.); adrian.ciuche@umfcd.ro (A.C.)

**Abstract:** Objective: Currently, the global epidemiologic impact of obesity requires continuous seeking of practical biomarkers; hence, this current study aimed to address the gap of chemerin assays in obese versus non-obese females and to analyze its circulating levels in relationship with the glucose profile and other circulating adipokines. Methods: This is an exploratory, prospective, cross-sectional analysis in females aged between 50 and 80 years. We excluded individuals with diabetes, cancers, endocrine, kidney, cardiovascular, and bone conditions. Enzyme-linked immunosorbent assay-based circulating adipokines testing was performed. The final analysis was focused on circulating chemerin (ng/mL) in the obese [body mass index (BMI) ≥ 30 kg/sqm] versus non-obese (BMI < 30 kg/sqm) group. Results: The obesity (N=12) versus the non-obesity (N=12) group showed a statistically significantly higher HOMA-IR (p=0.04), fasting insulin (p=0.01), but similar circulating chemerin. Chemerin positively correlated with BMI only in the obesity group (r=0.881, p=0.0039), but not with patients' age and glucose profile-based features in any group. Chemerin showed a statistically significant positive, strong correlation with VEGF-A level (r=0.857, p=0.0065) and an inverse statistically significant strong association with circulating leptin (r= -0.713, p=0.0092) and circulating IL-12 p40 (r= -0.829, p=0.0416) in the obesity group. Conclusion: As a potential hypothesis-generating analysis, this pilot study showed different statistical results in BMI-based groups, despite the fact that direct comparison of circulating chemerin and even leptin, VEGF-A, and IL-12 p40 did not reach between-group statistical significance. A larger sample size and a multimodal integration of the adipokines panel might serve for practical points in addressing obesity.

**Keywords:** chemerin, obesity, ELISA, adipokine, leptin, interleukin, insulin, body mass index

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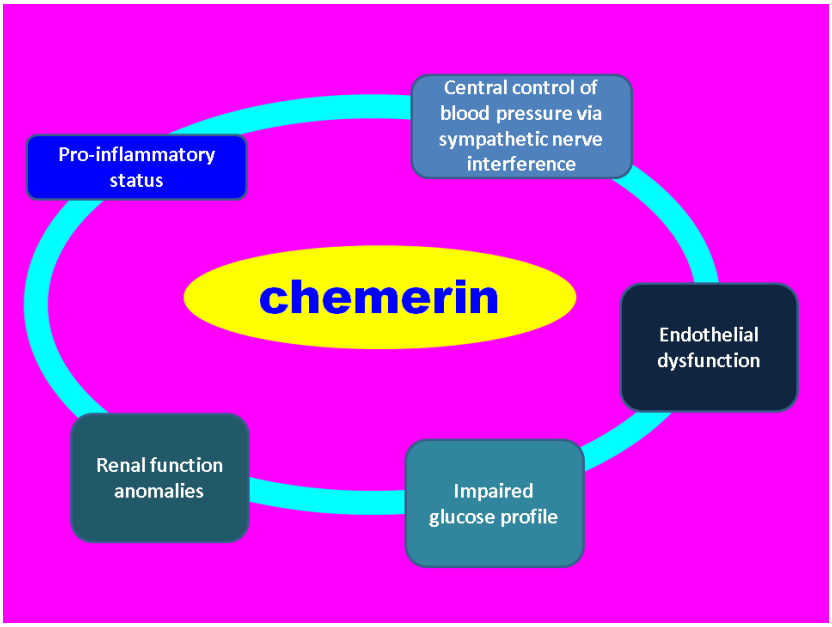
## INTRODUCTION

Chemerin represents a novel adipokine (fat tissue-released molecule), which was confirmed to play pivotal multifaceted roles in the intermediary metabolism loops regarding the cardio-metabolic, immune, and renal functions [1-3]. Chemerin receptor (designated as CMKLR1) is also expressed in the sympathetic nervous system (e.g., brain areas such as the nucleus tractus solitarii, paraventricular nucleus, etc.), which centrally regulates blood pressure [1,4-6]. The immune role involves leukocyte recruitment, endothelial function regulation, and stimulation of macrophage mobilization during the inflammation process. Of note, the endothelial connection of chemerin also interplays with blood pressure control and renal status across multi-layered crosstalk with other adipokines and myokines [1,7-9]. Currently, in patients living with chronic kidney disease, chemerin has been proposed as a useful biomarker of progressive renal function deterioration, noting that the molecule amplifies the local and systemic damage via pro-fibrotic, pro-inflammatory effects and lipotoxicity [10-12].

With regard to the glucose and lipid profile, chemerin promotes central and peripheral actions, for instance, adipocyte differentiation, as well as central appetite control [1,13-15]. (Figure 1)

**Figure 1:** Sneak peek of main chemerin actions in humans [1-5]




Nowadays, new ailments are under research with respect to the chemerin-related impact in their associated cardio-metabolic dysfunction and inflammatory anomalies, for example, polycystic ovary syndrome [16], atrial fibrillation (which might associate with CMKLR1 dysregulation according to murine experiments) [17], sarcopenia/dynapenia (associated with a higher circulating chemerin in elderly) [18] or obstructive sleep apnea syndrome [19].

### Objective

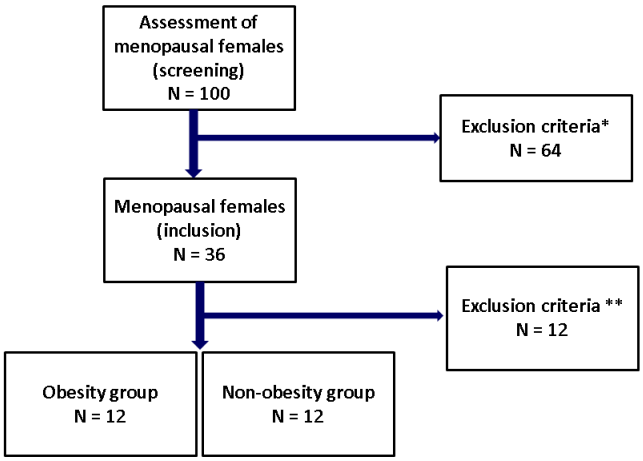
Currently, the global epidemiologic impact of obesity requires continuous seeking of practical biomarkers; hence, this current study aimed to address the gap of chemerin assays in obese versus non-obese females and to analyze its circulating levels in relationship with the glucose profile and other circulating adipokines.

## MATERIALS AND METHODS

This is an exploratory, prospective study in adults aged between 50 and 80 years with confirmed menopausal status. We only included the individuals who signed the informed consent according to the prospective study protocol. We excluded patients with previous or current confirmation/suspicion of diabetes, cancers, as well as endocrine, kidney, cardiovascular (hypertension, dyslipidemia), and bone (acute and chronic) conditions. Also, subjects with active infections during the study were ruled out. The patient's exposure to exogenous estrogens as hormone replacement therapy, anti-obesity drugs, and prior bariatric surgery excluded the subjects, as well.

Apparently healthy consecutive menopausal females within the mentioned age ranges underwent screening for exclusion criteria, and then fasting assays (after a minimum of 8 and a maximum of 12 hours of overnight fasting) were performed. Patients with fasting glycemia less than 125 mg/dL further underwent 75-oral glucose tolerance test (OGTT) with hourly testing of blood glucose and insulin levels. The females with a glycemia value of 200 mg/dL or above two hours after oral glucose administration were also excluded (this is consistent with the diagnosis of type 2 diabetes mellitus). The obesity group included patients with a body mass index (BMI) of 30 kg/sqm or above, and the non-obesity group enrolled subjects with a BMI of less than 30 kg/sqm. (Figure 2)

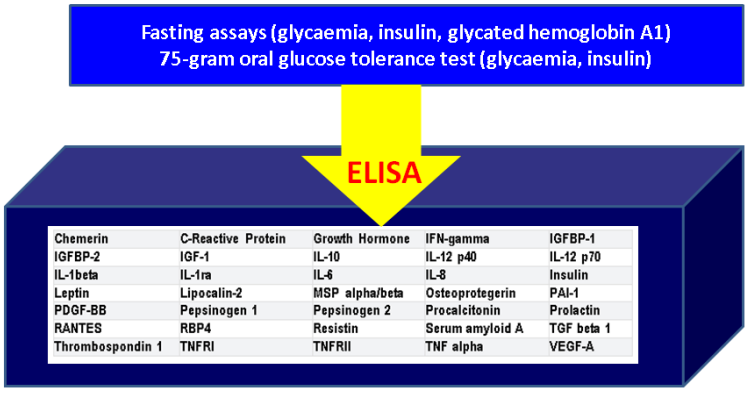
**Figure 2:** Flow chart of the study protocol




Abbreviations: N = number of patients; *exclusion criteria at screening level: diabetes; cancers; endocrine, kidney, cardiovascular (hypertension, dyslipidemia) and bone conditions; active infections; hormone replacement therapy at menopause, anti-obesity drugs, prior bariatric surgery; **exclusion criteria based on the glucose profile: fasting glycaemia > 125 mg/dL, and 2-hour glycaemia ≥ 200 mg/dL during oral glucose tolerance test)

ELISA (enzyme-linked immunosorbent assay)-based circulating adipokines testing was performed, according to the kit Quantibody® Human Obesity Array 3 (RayBiotech, Norcross, GA, USA; inter-assay precision < 20%) [20]. The final analysis was focused on circulating chemerin results (ng/mL) in obese versus non-obese groups and associated correlations with, on one hand, the parameters of glucose profile [glycaemia – photometry (Abbott, mg/dL); insulin – CLIA (Beckman Coulter, µUI/mL), glycated hemoglobin A1c – turbidimetry (Roche), HOMA-IR (Homeostatic Model Assessment of Insulin Resistance) based on formula fasting glycaemia (mg/dL) X fasting insulin (µUI/mL) per 405; a value > 2 means insulin resistance) and, on the other hand, tested molecules (adipokines). (Figure 3)

**Figure 3:** Exploration of the glucose profile and an ELISA-based panel of circulating adipokines assays




Abbreviations: IFN = interferon; IL = interleukin; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; MSP = macrophage stimulating protein; PAI = plasminogen activator inhibitor; PGDF-BB = platelet-derived growth factor; RANTES = acronym for Regulated upon Activation, Normal T-cell Expressed and Secreted; RBP = retinol binding protein; TGF = transforming growth factor; TNFR = tumor necrosis factor receptor; VEGF = vascular endothelial growth factor

MedCalc® Statistical Software version 23.3.7 was used to provide Kolmogorov-Smirnov analysis for normality. The t-test or the Mann-Whitney test was used for normal distribution or non-normal distribution. Pearson and Spearman rank correlations provided the correlation coefficients for the population with normal, respectively, non-normal distribution. A p-value below 0.05 was considered statistically significant.

Ethical aspects included the signed informed consent by each patient at the study's enrolment. The research was conducted according to the Declaration of Helsinki. This exploratory analysis is part of an international study PN-IV-P8-8.3-ROMD-2023-0262 (ethical approval board was as follows: no. 32//09/30/2024 for "C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania and no. 97//11/20/2025 for "Nicolae Testemitanu" University of Medicine and Pharmacy, Chisinau, Republic of Moldova). The blood adipokines testing was performed at "C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania.

**RESULTS**

The exploratory analysis included the obesity group (N = 12), which was similar to the non-obesity group (N = 12) in terms of patients' age and years since menopause. (Table 1)

**Table 1:** Demographic features of the two study groups

| Parameter | Obesity group | Non-obesity group | p |
|---|---|---|---|
| Age (years), mean ± SD | 60.00 ± 7.15 | 53.88 ± 6.98 | 0.105 |
| Years since menopause, mean ± SD | 9.14 ± 6.04 | 9.88 ± 7.00 | 0.833 |
| Body mass index (kg/sqm), mean ± SD | 32.83 ± 1.47 | 23.61 ± 2.70 | **< 0.001** |

Abbreviations: SD = standard deviation; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; bold font means statistical significance

The analysis of the glucose profile-related features showed a statistically significantly higher HOMA-IR in the obesity versus the non-obesity group (p = 0.04), with a mean value sustaining insulin resistance only in the obesity group (2.55 ± 1.16, insulin resistance at HOMA-IR > 2). (Table 2)

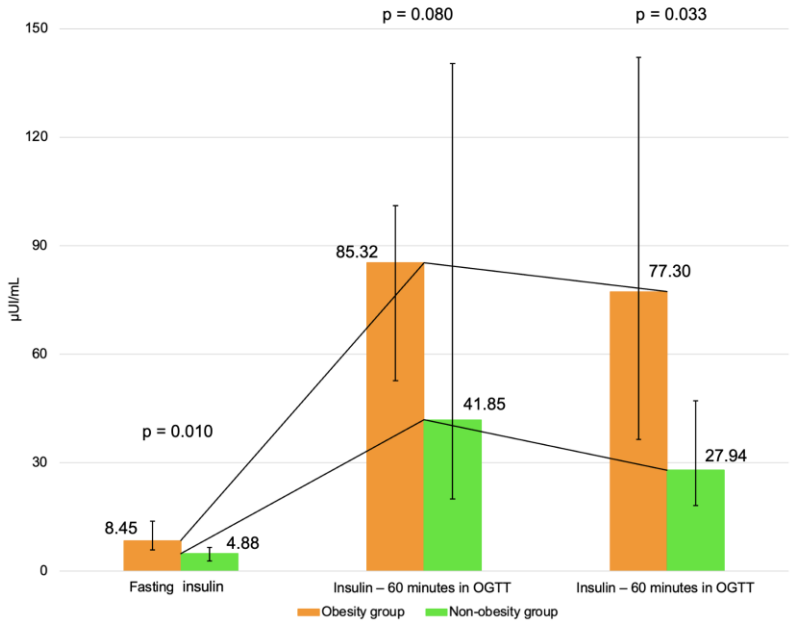
**Table 2:** Fasting and OGTT assays in the study groups

| | Obesity group | | Non-obesity group | | p-value |
|---|---|---|---|---|---|
| **Parameter, unit** | **mean (median)** | **SD (IQR)** | **mean (median)** | **SD (IQR)** | |
| Fasting glycemia, mg/dL | 104.22 | 6.73 | 97.48 | 9.87 | 0.060 |
| Glycated hemoglobin A1c, % | 5.79 | 0.46 | 5.69 | 0.51 | 0.610 |
| HOMA-IR | 2.55 | 1.16 | 1.44 | 1.20 | **0.040** |
| Fasting insulin (µUI/mL) | 8.45 | 5.92, 13.86 | 4.88 | 2.77, 6.52 | **0.010** |
| Glycemia – at 1 hour in OGTT (mg/dL) | 186 | 173, 236 | 163 | 104, 228 | 0.201 |
| Glycemia – at 2 hours in OGTT (mg/dL) | 155 | 132, 170 | 112 | 89.65, 146.50 | 0.152 |
| Insulin – at 1 hour in OGTT (µUI/mL) | 85.32 | 52.71, 101.02 | 41.85 | 19.93, 140.32 | 0.080 |
| Insulin – at 2 hours in OGTT (µUI/mL) | 77.30 | 36.39, 142.00 | 27.94 | 18.08, 47.11 | **0.033** |

Abbreviations: HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; IQR = interquartile range; OGTT = oral glucose tolerance test; SD = standard deviation; bold font means statistical significance

Insulin level was statistically significantly higher in the obesity group versus the non-obesity group, both at baseline (p = 0.01) and at two hours in OGTT (p = 0.033). (Figure 4)

**Figure 4:** Figure 4. Bar and line chart showing median fasting insulin, at one hour and two hours in OGTT (orange = obesity group; green = non-obesity group)




Circulating chemerin level was similar between the two study groups. (Table 5)

**Table 3:** ELISA-based adipokines analysis

| | Obesity group | | Non-obesity group | | p-value |
|---|---|---|---|---|---|
| **Parameter 1, unit** | **mean** | **SD** | **mean** | **SD** | |
| Chemerin, ng/mL | 40.36 | 15.95 | 20.85 | 7.95 | 0.89 |
| Leptin, ng/mL | 258.74 | 324.73 | 212.83 | 252.72 | 0.71 |
| IL-12 p40, ng/mL | 4.96 | 3.81 | 2.82 | 1.50 | 0.17 |
| VEGF-A, pg/mL | 117.64 | 105.26 | 204.07 | 131.83 | 0.17 |

Abbreviations: IL-12 p40 = interleukin-12p40; VEGF-A = vascular endothelial growth factor A; SD = standard deviation

**Table 4:** The correlations between circulating chemerin and studied parameters within each of the two study groups (of note, we included the results of demographic features, glucose profile and only statistically significant correlations between chemerin and other adipokines)

| | | Obesity group | | Non-obesity | |
|---|---|---|---|---|---|
| **Parameter 1, unit** | **Parameter 2, unit** | **r** | **p** | **r** | **p** |
| Chemerin, ng/mL | IL-12 p40, ng/mL | **-0.829** | **0.0416** | -0.452 | 0.260 |
| Chemerin, ng/mL | Leptin, ng/mL | **-0.713** | **0.0092** | -0.027 | 0.936 |
| Chemerin, ng/mL | VEGF-A, pg/mL | **0.857** | **0.0065** | -0.31 | 0.455 |
| Chemerin, ng/mL | Body mass index, kg/sqm | **0.881** | **0.0039** | -0.098 | 0.761 |
| Chemerin, ng/mL | Age, years | 0.308 | 0.330 | 0.400 | 0.600 |
| Chemerin, ng/mL | Fasting glycaemia, mg/dL | -0.130 | 0.687 | 0.235 | 0.462 |
| Chemerin, ng/mL | Fasting insulin, (µUI/mL) | 0.228 | 0.500 | -0.091 | 0.802 |

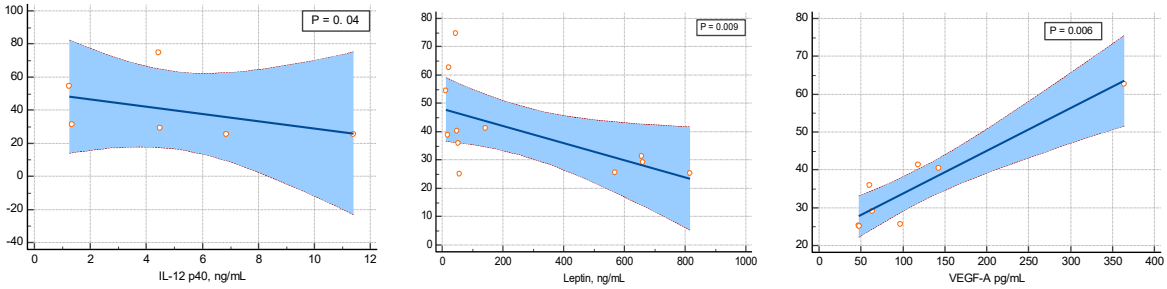
| | | | | | |
|---|---|---|---|---|---|
| Chemerin, ng/mL | Glycated hemoglobin A1c (%) | 0.357 | 0.255 | -0.336 | 0.288 |
| Chemerin, ng/mL | HOMA-IR | 0.256 | 0.450 | -0.091 | 0.802 |
| Chemerin, ng/mL | Glycemia – at 1 hour in OGTT (mg/dL) | 0.064 | 0.852 | -0.55 | 0.125 |
| Chemerin, ng/mL | Glycemia – at 2 hours in OGTT (mg/dL) | 0.287 | 0.392 | -0.333 | 0.380 |
| Chemerin, ng/mL | Insulin – at 1 hour in OGTT (mg/dL) | 0.999 | 0.988 | -0.6 | 0.087 |
| Chemerin, ng/mL | Insulin – at 2 hours in OGTT (mg/dL) | 0.009 | 0.978 | -0.405 | 0.319 |
| Leptin, ng/mL | Body mass index, kg/sqm | -0.112 | 0.729 | **0.779** | **0.004** |
| Leptin, ng/mL | Age, years | -0.179 | 0.577 | -0.400 | 0.600 |
| Leptin, ng/mL | Fasting glycemia, mg/dL | 0.238 | 0.900 | 0.879 | 0.123 |
| Leptin, ng/mL | Fasting insulin (µUI/mL) | -0.009 | 0.978 | **0.967** | **0.0001** |
| Leptin, ng/mL | Glycated hemoglobin A1c (%) | -0.189 | 0.556 | 0.191 | 0.573 |
| Leptin, ng/mL | HOMA-IR | -0.227 | 0.936 | **0.917** | **0.0005** |
| Leptin, ng/mL | Glycemia – at 1 hour in OGTT (mg/dL) | 0.127 | 0.709 | 0.170 | 0.700 |
| Leptin, ng/mL | Glycemia – at 2 hours in OGTT (mg/dL) | -0.433 | 0.183 | 0.167 | 0.693 |
| Leptin, ng/mL | Insulin – at 1 hour in OGTT (mg/dL) | 0.100 | 0.769 | 0.333 | 0.419 |
| Leptin, ng/mL | Insulin – at 2 hours in OGTT (mg/dL) | 0.055 | 0.873 | 0.071 | 0.400 |
| IL-12 p40, ng/mL | Body mass index, kg/sqm | -0.200 | 0.704 | -0.200 | 0.704 |
| IL-12 p40, ng/mL | Age, years | -0.257 | 0.622 | -0.257 | 0.622 |
| IL-12 p40, ng/mL | Fasting glycemia, mg/dL | 0.300 | 0.400 | 0.345 | 0.523 |
| IL-12 p40, ng/mL | Fasting insulin (µUI/mL) | 0.371 | 0.468 | 0.371 | 0.468 |
| IL-12 p40, ng/mL | Glycated hemoglobin A1c (%) | -0.086 | 0.877 | -0.086 | 0.871 |
| IL-12 p40, ng/mL | HOMA-IR | 0.371 | 0.468 | 0.371 | 0.468 |
| IL-12 p40, ng/mL | Glycemia – at 1 hour in OGTT (mg/dL) | 0.543 | 0.265 | 0.543 | 0.265 |
| IL-12 p40, ng/mL | Glycemia – at 2 hours in OGTT (mg/dL) | -0.143 | 0.787 | -0.143 | 0.787 |
| IL-12 p40, ng/mL | Insulin – at 1 hour in OGTT (mg/dL) | 0.486 | 0.328 | 0.486 | 0.328 |
| IL-12 p40, ng/mL | Insulin – at 2 hours in OGTT (mg/dL) | 0.314 | 0.544 | 0.324 | 0.544 |
| VEGF-A, pg/mL | Body mass index, kg/sqm | -0.048 | 0.910 | -0.048 | 0.910 |
| VEGF-A, pg/mL | Age, years | 0.335 | 0.416 | 0.335 | 0.416 |
| VEGF-A, pg/mL | Fasting glycemia, mg/dL | 0.482 | 0.672 | 0.459 | 0.472 |
| VEGF-A, pg/mL | Fasting insulin (µUI/mL) | 0.179 | 0.701 | 0.179 | 0.701 |
| VEGF-A, pg/mL | Glycated hemoglobin A1c (%) | 0.571 | 0.139 | 0.571 | 0.139 |
| VEGF-A, pg/mL | HOMA-IR | 0.180 | 0.700 | 0.178 | 0.703 |
| VEGF-A, pg/mL | Glycemia – at 1 hour in OGTT (mg/dL) | 0.036 | 0.939 | 0.036 | 0.939 |
| VEGF-A, pg/mL | Glycemia – at 2 hours in OGTT (mg/dL) | 0.324 | 0.477 | 0.324 | 0.477 |
| VEGF-A, pg/mL | Insulin – at 1 hour in OGTT (mg/dL) | -0.357 | 0.431 | -0.357 | 0.431 |
| VEGF-A, pg/mL | Insulin – at 2 hours in OGTT (mg/dL) | 0.040 | 0.900 | 0.036 | 0.939 |

Abbreviations: IL-12 p40 = interleukin-12p40; r = correlation coefficient; OGTT = oral glucose tolerance test; VEGF-A = vascular endothelial growth factor A; bold font means statistical significance

Among the previously mentioned adipokines, chemerin was statistically significantly correlated with leptin, IL-12 p40, and VEGF-A only in the obesity group. In the non-obesity group, chemerin did not correlate with any of these adipokines. (Table 6)

The blood chemerin value showed a statistically significant positive, strong correlation with VEGF-A level ($r = 0.857$, $p = 0.0065$) and an inverse statistically significant strong association with circulating leptin ($r = -0.713$, $p = 0.0092$) and circulating IL-12 p40 ($r = -0.829$, $p = 0.0416$) in the female group with a higher BMI. (Figure 5)

**Figure 5:** Correlation chart showing statistically significant correlations between chemerin and other adipokines in the obesity




## DISCUSSION

In this exploratory analysis of obese versus age- and menopause duration-matched non-obese (diabetes-free) females in menopause, we found that circulating chemerin levels are similar, but the spectrum of correlations involving the other microarray-based adipokines is differently displayed between the groups. This suggests a multifactorial interplay depending on the BMI category. In addition, chemerin was found to be strongly correlated with BMI in obese women ($r = 0.881$, $p = 0.0039$), while leptin correlated with BMI in the non-obese group ($r = 0.779$, $p = 0.004$), which implies their interpretation should be dependent on the presence of obesity or not. Moreover, chemerin did not associate with glucose profile-related parameters, as opposed to other adipokines; for instance, we mention the positive correlation between leptin and fasting insulin ($r = 0.967$, $p = 0.0001$), respectively, HOMA-IR ($r = 0.917$, $p = 0.00o5$) in the group with an average BMI within the normal BMI category (23.61 ± 2.70 kg/sqm).

These findings should be cautiously interpreted as the first step amid a more complex approach that is expected to take into consideration a multifactorial landscape combining the clinical features (e.g. weight, body fat percent, lean mass, BMI, menopausal status), biochemistry tests (e.g. glycaemia, insulin, HOMA-IR, glycated haemoglobin A1c, lipid profile), as well as the circulating levels of adipokines ± myokines that play synergic or antagonist roles in the intermediary metabolisms [21]. Further on, refining the multi-layered panel of adipokines/myokines will pinpoint the patients with a high-risk of cardio-metabolic, osseous, neurologic, and oncologic outcomes; hence, a stratified multidisciplinary intervention strategy will provide an optimum prognosis [22-24]. At this point, as a potential hypothesis-generating analysis, we can only observe different statistical results in BMI-based groups, despite the fact that direct comparison of circulating chemerin and even leptin, VEGF-A, and IL-12 p40 did not reach between-group statistical significance. To date, prior studies addressing this type of topic in clinical practice have provided heterogeneous results to this moment. Yet, looking for novel biomarkers in the vast area of obesity and related overall health burden is emergent in the modern society due to its increasing incidence in the general population, on one hand, and, on the other hand, due to the novel anti-obesity agents (such as glucagon-like pepetide-1 receptor agonists) that are meant to correct even subclinical anomalies, which impact the long-term impact [25-27].

We mention some previously published data in order to integrate the current study, but the overall results remain a matter of debate. For instance, Yilmaz et al. [25] studied 74 obese, type 2 diabetic patients versus 60 non-obese, type 2 diabetic individuals versus 36 healthy controls and found that ELISA-based chemerin was similar between the three groups [25]. As identified in our study, this exploratory analysis also raised the issue of the diagnostic performance we currently have for testing chemerin in humans. On the contrary, Schinzari et al. [26] found that chemerin is higher in obese versus lean subjects, and it correlates with endothelial system-mediated vasoconstriction [26]. Moreover, a recent meta-analysis highlighted that chemerin is elevated in gestational diabetics versus normoglycemic controls ($p = 0.02$) [28], suggesting its importance in other population subgroups. In addition, Zhao et al. [29] showed in 42 patients with dyslipidemia who were (lipid–lowering) treatment-naïve a statistically significant correlation between chemerin and the level of circulating triglycerides ($r2 = 0.5073$, $p < 0.0001$), respectively, triglycerides ($r2 = 0.1464$, $p = 0.0124$) [29], an aspect which was out of our scope, but it might represent a future direction. Other data identified an association between chemerin and coronary slow flow in patients who underwent coronary angiography [30].

As the limits of the current study, we mention the sample size and the transversal design, which remain adequate for a pilot, exploratory analysis. Based on the adipokines' results, further enrollment of the patients and a larger and more diverse study population will confirm a distinct interpretation of the correlations between adipokines in the obese population when compared to those with a BMI lower than 30 kg/sqm. Moreover, larger BMI sub-groups will pinpoint if there is a difference between the normal and overweight individuals. Despite the inclusion of diabetes-, hypertension-, and dyslipidemia-free individuals, chemerin seems to be correlated with central and peripheral underlying mechanisms in these conditions (directly or indirectly, for instance, via chronic inflammation), and future trials will clarify its importance as a biomarker under these pathological circumstances [31]. Currently, there is no standardized kit to test chemerin in everyday practice, and testing variability might generate a bias. Moreover, the relationship

between the circulating level and its receptor activity should be taken into account, and it remains an open matter so far. Of note, some authors suggested a dietary influence on chemerin levels, an aspect which was not quantified in this analysis [32].

## CONCLUSION

As a potential hypothesis-generating analysis, this pilot analysis showed different statistical results in BMI-based groups, despite the fact that direct comparison of circulating chemerin, and even leptin, VEGF-A, and IL-12 p40 did not reach between-group statistical significance.

***Conflicts of interest and sources of funding***

*The authors declare no conflict of interest. This work was supported by a grant of the Ministry of Research, Innovation and Digitization, UEFISCDI "Crossroad of metabolism and bone: the impact of irisin, bone turnover and inflammatory markers in patients with menopausal osteoporosis and obesity"; PN-IV-P8-8.3-ROMD-2023-0262.*

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***Authors' contribution***

*Conceptualization, S.V.S, and D.M.; methodology, O.-C.S., M.K., and A.-M.G.; software, A.V., and E.M.P.; validation, A.C., Y.Y.; formal analysis, M.-L.C.; investigation, A.P.; resources, S.V.S.; data curation, D.M..; writing—original draft preparation, D.M., and M.C.; writing—review and editing, M.C.; visualization, S.V.S., E.M.P., A.C..; supervision, A.P, and A.V.; project administration, S.V.S.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.*

***Ethics approval and consent to participate***

*The research was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethical Boards of both centers (number 32 from 30 September 2024 – Bucharest, Romania, respectively, number 97 from 20 November 2024 – Chisinau, Republic of Moldova).*

***Patient consent for publication***

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

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REVIEW

# Fertility-Sparing Surgery in Early-Stage Cervical Cancer

**Mihnea Andrei Nicodin[1,2,3], Laura Nicodin-Tigoianu[1,4], Ana Elena Martiniuc[1,4], Anca Popescu[2], Alice Elena Munteanu[2,3], Mariam Dalaty[2,5*], Diana Badiu[6], Ovidiu Vasile Nicodin[2,3], Silviu-Ionel Dumitrescu[2,3*], Nicolae Suciu[1,4]**

[1] Doctoral School of Medicine, "Carol Davila" University of Medicine and Pharmacy, 8, Eroii Sanit Blvd, Bucharest, 050474, Romania
[2] "Carol Davila" Central Military Emergency University Hospital, 134, Plevnei Street, Sector 1, Bucharest, 010825, Romania
[3] Faculty of Medicine, Titu Maiorescu University, 67 A, Gheorghe Petrascu Street, Sector 3, Bucharest, 031593, Romania
[4] Alessandrescu Rusescu National Institute for the Health of Mother and Child, 38-54, Gh. Polizu Street, Sector 1, Bucharest, 020395, Romania
[5] Doctoral School of Medicine, George Emil Palade University of Medicine, Pharmacy, Science, and Technology, Târgu Mureș, Mureș, Romania
[6] Faculty of Medicine, "Ovidius" University of Constanta, 1, University Street, 900470, Constanta, Romania

*Correspondence: mariam_dalaty@yahoo.com (M.D.), silviudumius@yahoo.com (S.I.D.)

**Abstract:** Abstract Cervical cancer remains a major global public health challenge, especially affecting women of reproductive age. In the context of a societal shift towards delayed childbearing, fertility preservation has become an integral component of oncologic care for early-stage disease. Radical trachelectomy has emerged as a viable fertility-sparing surgical option in carefully selected patients. This review aims to assess the role of fertility-sparing radical trachelectomy in the modern management of early-stage cervical cancer by emphasizing the evolving criteria for patient selection, advances in surgical techniques, and the critical balance between oncologic safety and reproductive potential. An analysis of the literature from the past two decades shows that radical trachelectomy, whether performed via vaginal or abdominal routes, provides excellent oncologic control for early-stage lesions. Therefore, optimal results of such a technique depend on careful patient selection along with a multidisciplinary approach in order to reduce obstetrical complications.

**Keywords:** fertility sparing surgery, radical trachelectomy, cervical cancer, minimally invasive technique.

## INTRODUCTION

Despite widespread cervical cancer screening programs and the increasing implementation of Human Papillomavirus (HPV) vaccination in many countries, cervical cancer remains the fourth most common cancer among women worldwide. The staging system established by the International Federation of Gynecology and Obstetrics (FIGO) continues to guide treatment strategies, with radical hysterectomy currently considered the standard of care for early-stage disease, including FIGO 2018 stages IA2 to IB2 and IIA1 [1]. However, a significant proportion of women affected by cervical cancer are of reproductive age. According to the Surveillance, Epidemiology, and End Results (SEER) database in the United States, approximately 36% of cervical cancer patients are under the age of 45. For many of these women, the possibility of preserving fertility is a critical aspect of treatment planning [2]. In response to this need, fertility-sparing surgical approaches have been developed. One of the most notable advancements was introduced by Dargent in 1986: radical vaginal trachelectomy (RVT) together with bilateral pelvic lymphadenectomy, initially indicated for tumors smaller than 2 cm. Since then, radical trachelectomy, whether performed vaginally, abdominally, or via minimally invasive methods, has gained recognition as a viable alternative to radical hysterectomy in selected cases, offering comparable oncologic outcomes while preserving reproductive potential [3]. However, radical trachelectomy is associated with higher obstetric morbidity, particularly an increased risk of miscarriage and preterm birth. As a result, less radical approaches, such as cervical conization or simple trachelectomy, are being explored and increasingly recommended, especially for patients with favorable histologic and staging characteristics. For instance, conization or simple hysterectomy is typically adequate for FIGO 2018 stage IA1 disease without lymphovascular space invasion (LVSI). For patients with stage IA1 with LVSI, IA2, or IB1 stage disease, multiple treatment options-including radical and less radical fertility-sparing procedures-are endorsed

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by guidelines such as those from the National Comprehensive Cancer Network (NCCN) and European Society of Gynaecological Oncology (ESGO) [3, 4]. This review aims to provide a comprehensive summary of current surgical treatments for early-stage cervical cancer, with a focus on fertility-sparing strategies in patients with FIGO 2018 stages ranging from IA with LVSI to IB2. It highlights the evolution of surgical approaches, their oncologic and reproductive outcomes, and the ongoing efforts to balance effective cancer control with the preservation of fertility and quality of life.

**Evolution of Fertility-Sparing Procedures in Cervical Cancer**

Historically, the best treatment for early-stage cervical cancer has been known to be radical hysterectomy, which, while effective from an oncologic standpoint, eliminates the possibility of future pregnancy. The growing awareness of fertility preservation, especially in younger patients diagnosed at an early stage, has led to significant advancements in surgical approaches over the past few decades. A seminal advancement in this domain was the introduction of radical vaginal trachelectomy (RVT) by Daniel Dargent. First performed in 1986 and subsequently detailed in the medical literature by 1994, this procedure was conceived to reconcile oncological safety with uterine conservation. The technique entails the transvaginal resection of the cervix, a circumferential margin of parametrial tissue, and the upper vagina, coupled with a laparoscopic pelvic lymphadenectomy [5]. The rationale behind RVT was to maintain oncologic safety by resecting the same structures removed during a radical hysterectomy (i.e., except the uterine body), while preserving the capacity for future pregnancy. Over the past three decades, RVT has become a well-established procedure for selected patients with early-stage cervical cancer, primarily FIGO 2018 stages IA2 and IB1 with tumor sizes ≤2 cm, no LVSI, and negative lymph nodes [3, 5].

**Patient Selection Criteria**

Careful patient selection is critical to the success of RVT. Ideal candidates typically meet the following criteria [3, 5, 6]:

- Histologically confirmed squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma,
- FIGO 2018 stage IA2 to IB1,
- Tumor size ≤2 cm on imaging and/or cone biopsy,
- Absence of LVSI (relative),
- Negative lymph node status confirmed preoperatively or intraoperatively,
- No evidence of parametrial involvement or distant spread, and
- Strong desire to preserve fertility and informed understanding of potential risks.

Magnetic resonance imaging (MRI) is often employed to assess tumor size, depth of stromal invasion, and proximity to the internal os. Preoperative conization is frequently performed to confirm diagnosis, assess margin status, and determine suitability for conservative surgery.

**Surgical Technique**

The procedure involves two main operative phases. The initial phase consists of a laparoscopic pelvic lymphadenectomy, performed to histologically confirm the absence of nodal metastasis. The identification of positive lymph nodes typically constitutes an indication to abort the fertility-preserving procedure in favor of radical hysterectomy or chemoradiation. The subsequent phase, the RVT, involves the amputation of the cervix approximately 5-10 mm below the internal os, en bloc with the resection of the surrounding parametria and 1-2 cm of the upper vagina. A permanent or temporary cerclage (i.e., a McDonald or Shirodkar stitch) is placed at the uterine isthmus to provide mechanical support during future pregnancies. The vagina is reattached to the uterine isthmus to restore continuity. The uterus is preserved, thereby maintaining its endocrine and reproductive functions. Compared to abdominal routes, the vaginal approach offers advantages in terms of reduced operative time and accelerated recovery. This benefit is counterbalanced by the procedure's greater technical complexity and its prerequisite for specialized skill in vaginal surgery [4, 5, 7].

**Oncologic Outcomes**

Multiple retrospective studies and systematic reviews have demonstrated that RVT offers oncologic outcomes comparable to radical hysterectomy in appropriately selected patients. Recurrence rates range between 3% and 5%, with 5-year disease-free survival exceeding 95% in most studies. Factors associated with recurrence include tumor size >2 cm, LVSI and incomplete resection margins. Long-term data suggest excellent local control and low mortality rates, making RVT a safe option from an oncologic standpoint for tumors ≤2 cm [6, 8].

**Fertility and Obstetric Outcomes**

RVT allows for the possibility of future pregnancy, but obstetric outcomes are influenced by the extent of cervical resection and surgical disruption of the uterine-cervical junction [9]:

- Pregnancy rate: 40–70% among those attempting conception;

- Miscarriage rate: 15–25%, particularly in the second trimester;
- Preterm birth: Up to 30–50% of live births occur before 37 weeks;
- Delivery: Cesarean section is recommended due to the absence of the cervix and cerclage in place;
- Assisted reproductive techniques may be needed, and multidisciplinary obstetric care is essential. Cervical stenosis and infertility remain risks, but many women successfully conceive and deliver after RVT.

Over the past three decades, numerous case series, cohort studies, and meta-analyses have evaluated the efficacy and safety of RVT in well-selected patient populations [9, 10]. A comprehensive meta-analysis including over 2,400 patients undergoing fertility-sparing surgery revealed a pooled recurrence rate of 2.3% (95% CI: 1.3–3.4%) and a cancer-related mortality rate of just 0.7% [11]. These figures support the conclusion that, in appropriately selected cases-typically those with FIGO stage IA2–IB1 disease, tumor size ≤2 cm, negative lymph nodes, and absence of LVSI-RVT provides excellent oncologic control. Moreover, a 2019 systematic review by Prodromidou et al. comparing radical trachelectomy to radical hysterectomy found no significant difference in 5-year disease-free or overall survival, further validating RVT as a safe oncologic alternative to more radical interventions [12]. These outcomes underscore the importance of rigorous preoperative evaluation, including pelvic MRI and cone biopsy, to ensure that the disease is confined and amenable to conservative management. The low recurrence rates reported in these studies showed that RVT, when performed by experienced surgeons in high-volume centers, maintains oncologic outcomes similar to more extensive procedures [13]. A principal factor driving the adoption of radical vaginal trachelectomy is its favorable fertility profile, compared to other fertility-sparing procedures [14]. A systematic review by Smith et al. confirms a post-procedure pregnancy rate of 55% among women attempting to conceive, a finding sustained by the recent multicenter FERTISS study, which reported a cumulative pregnancy rate of 60.8% [15, 16]. These rates are particularly favorable when compared to abdominal radical trachelectomy (ART), which has demonstrated lower overall pregnancy rates (~36%) [4, 17]. The superior fertility outcomes of the vaginal approach are a compelling argument for its use in eligible patients, although this advantage must be weighed against its specific technical demands and patient selection criteria. However, RVT is not without reproductive risks. The spontaneous abortion rate following RVT is estimated at 24%, with most losses occurring in the second trimester. Additionally, preterm delivery affects approximately 27% of pregnancies, often due to cervical insufficiency or ascending infection, despite prophylactic cerclage placement. Cesarean delivery is generally recommended and multidisciplinary care involving maternal-fetal medicine specialists is critical throughout gestation [8, 9]. However, among all fertility-sparing procedures, RVT remains the best-studied technique, with the most consistent long-term data supporting its dual role in controlling disease and fertility preservation.

**Complications and Limitations**

Despite its advantages, RVT is associated with potential complications, including cervical stenosis, leading to infertility or dysmenorrhea, intraoperative bleeding, infection or pelvic abscess, need for further adjuvant therapy if margins or nodes are positive, emotional impact due to prolonged follow-up, and uncertainty regarding reproductive outcomes. Moreover, not all patients are candidates for RVT, and strict adherence to selection criteria is necessary to ensure safety [5, 10]. RVT remains the most studied and validated fertility-sparing surgical technique for early-stage cervical cancer. With the emergence of alternative approaches, such as conization or simple trachelectomy for selected patients with low-risk features, and the use of neoadjuvant chemotherapy for tumors >2 cm, the role of RVT is evolving. Future directions include integrating imaging biomarkers, molecular profiling, and surgical innovations to further refine patient selection and reduce morbidity. Multicenter registries and long-term follow-up studies are ongoing to better define its place in the treatment algorithm.

**Abdominal and Minimally Invasive Trachelectomy**

While RVT remains a standard fertility-sparing option in early-stage cervical cancer, the technical demands of vaginal surgery and limited accessibility to skilled surgeons have driven the development of alternative surgical routes. ART, first reported by Smith and colleagues in the early 2020s, was introduced to address these limitations [15]. This approach follows the same oncologic principles as RVT-resection of the cervix, upper vagina, and parametrial tissue while preserving the uterus-but is performed through a transabdominal route. With advances in surgical technology, minimally invasive techniques such as laparoscopic or robotic-assisted radical trachelectomy have also been adopted to reduce surgical morbidity and improve visualization, particularly in complex pelvic dissections [18, 19].

**Indications and Patient Selection**

The indications for ART largely overlap with those of RVT. Ideal candidates typically meet the following criteria [17]:

- FIGO 2018 stage IA2 to IB1,
- Tumor size ≤2 cm (some centers cautiously extend to ≤4 cm with neoadjuvant chemotherapy),
- Negative pelvic lymph nodes,
- No evidence of parametrial invasion,

- Strong fertility desire and informed consent.

Advanced imaging, such as MRI or positron emission tomography-computed tomography (PET-CT), is used preoperatively to assess tumor size, depth of invasion, and lymph node status. Lymph node evaluation is performed intraoperatively via pelvic lymphadenectomy or sentinel node mapping.

**Surgical Technique**

ART is a fertility-preserving surgical procedure that mirrors the steps of a type C1 radical hysterectomy, as classified by the Querleu–Morrow system, with the critical distinction being the preservation of the uterine body. The procedure begins with either a midline laparotomy or the use of minimally invasive techniques such as laparoscopy or robotic-assisted surgery, depending on the surgeon's expertise and institutional protocols. Following abdominal access, a bilateral pelvic lymphadenectomy is performed to assess for lymph node metastases and guide the need for further treatment. Once nodal status is confirmed to be negative, the surgical resection proceeds with the removal of the entire cervix, the surrounding parametrial tissue, and a portion of the upper vaginal cuff, ensuring oncologically sound margins. A crucial step in ART is the placement of a cerclage, either permanent or removable, at the level of the uterine isthmus to provide mechanical support for future pregnancies, given the loss of the cervix. To restore the anatomical continuity of the genital tract, a vaginal–uterine anastomosis is performed, connecting the upper vagina to the remaining uterine segment. This reconstruction not only facilitates potential menstruation and conception but also minimizes the risk of ascending infection. ART thus combines radical oncologic resection with fertility preservation and requires significant surgical expertise due to the complexity of parametrial dissection and reproductive tract reconstruction [19, 20].

**Oncologic Outcomes**

Multiple series and reviews have reported favorable oncologic outcomes following ART. For tumors ≤2 cm, 5-year disease-free survival rates are typically ≥95%, comparable to those of radical hysterectomy and RVT. In selected patients with tumors between 2 and 4 cm treated with neoadjuvant chemotherapy followed by ART, outcomes remain promising, although long-term data are limited [13, 14]. A multicenter retrospective study by Wethington et al. reported low recurrence rates and acceptable oncologic safety in 29 patients who underwent robotic-assisted ART for tumors up to 2 cm [19]. However, recent concern has emerged regarding the oncologic safety of minimally invasive techniques. Following the Laparoscopic Approach to Cervical Carcinoma (LACC) trial, which demonstrated inferior survival outcomes for minimally invasive radical hysterectomy compared to open surgery, similar caution is being applied to minimally invasive trachelectomy. Although these findings cannot be directly extrapolated, they have prompted a more conservative approach to laparoscopic and robotic trachelectomy, with some centers returning to open ART [21]. The 2018 LACC trial, a study of 631 women with early-stage cervical cancer (FIGO 2009 stages IA1 with LVSI, IA2, or IB1), showed that minimally invasive radical hysterectomy was associated with significantly inferior disease-free and overall survival compared to the open abdominal approach. As a result, open surgery should remain the standard of care for radical hysterectomy in this population [3, 21, 22]. Impact on Clinical Practice is that the results led to widespread reevaluation of minimally invasive surgery for cervical cancer. The implications of the LACC trial have also raised caution around minimally invasive radical trachelectomy, though this was not directly studied in the LACC trial [21].

**Fertility and Obstetric Outcomes**

ART preserves fertility with success rates comparable to RVT:

- Pregnancy rate: Approximately 40–60% in women actively trying to conceive,
- Live birth rate: Around 50% of pregnancies reach term,
- Increased risk of second-trimester miscarriage, preterm birth, and cervical stenosis remains,
- The obstetric risks are largely related to mechanical weakening of the cervical support structures and scarring at the isthmic level. Delivery is typically by elective cesarean section. Interdisciplinary coordination with maternal-fetal medicine specialists is crucial for managing high-risk pregnancies after ART [9, 18].

**Advantages and Limitations**

The abdominal approach to radical trachelectomy offers distinct advantages, including its familiarity to gynecologic oncologists and the superior surgical exposure it provides, which facilitates a comprehensive pelvic lymphadenectomy and parametrial resection. This method may also present a more feasible option for managing tumors situated high within the endocervical canal or for larger lesions following neoadjuvant chemotherapy. However, these benefits are counterbalanced by significant limitations. The procedure is generally associated with a longer operative time, greater intraoperative blood loss, and potentially higher overall morbidity compared to the vaginal approach. Furthermore, the abdominal technique is not without controversy, particularly as the adoption of minimally invasive methods has raised concerns regarding oncologic safety in light of data from studies such as the LACC trial [21]. Finally, the procedure entails considerable technical complexity, specifically in performing the uterovaginal anastomosis and placing the isthmic cerclage when utilizing laparoscopic or robotic platforms. ART and its minimally invasive variations continue to play an

important role in fertility-preserving surgery, especially in centers where RVT is less commonly performed. However, their use must be carefully balanced with oncologic risk, particularly in light of concerns raised by the LACC trial [21]. Ongoing prospective trials and registry data are expected to better clarify the long-term oncologic safety and reproductive success rates of these approaches. For now, open ART remains a valuable option for well-selected patients, particularly when vaginal access is not possible or when pelvic anatomy poses a challenge.

**Extended Clinical Role of Abdominal and Minimally Invasive Trachelectomy**

The clinical role of abdominal radical trachelectomy and its minimally invasive variants has evolved significantly over the past two decades. While RVT remains the most established fertility-sparing option, ART offers a valuable alternative in settings where vaginal surgery is technically challenging, contraindicated, or not widely practiced. In contemporary gynecologic oncology, ART has emerged as a pragmatic solution to expand access to fertility preservation, especially in centers lacking extensive experience with RVT [23]. RVT, though effective, requires advanced training in vaginal surgery and familiarity with pelvic anatomy from a caudal approach, skills that are increasingly rare in the modern surgical curriculum. ART, by contrast, can be performed by surgeons trained in radical abdominal hysterectomy, making it more accessible. The abdominal approach to radical trachelectomy demonstrates particular utility in specific clinical contexts. It is often the preferred technique in cases of unfavorable vaginal anatomy, such as a high vaginal fornix or distorted surgical access, which may be encountered in nulliparous patients, individuals with obesity, or those with a significant history of prior pelvic surgery. Furthermore, this method offers a distinct advantage for tumors situated high within the endocervical canal, as the abdominal exposure allows for superior visualization to achieve precise resection margins. Finally, the procedure's adoption is frequently guided by surgeon preference and institutional proficiency, making it a common choice in high-volume oncology centers where expertise in complex abdominal surgery is well-established. In these situations, ART serves as a technically reliable and oncologically sound fertility-sparing alternative [18].

ART has a defined role in managing borderline cases, including select patients with tumors exceeding 2 cm. In this context, it is often employed following neoadjuvant chemotherapy (NACT), which aims to achieve sufficient tumor downstaging to permit a fertility-sparing procedure. Although this strategy remains investigational, several institutions have reported successful obstetric outcomes in women with FIGO IB2 disease who exhibited a favorable response to chemotherapy. The abdominal approach is particularly advantageous in these complex scenarios, as it provides superior visualization, enables a controlled dissection, and allows for a thorough assessment of the parametria, which are critical for ensuring oncologic safety when operating at the limits of conservative surgery [24].

The role of minimally invasive techniques for radical trachelectomy has been significantly re-evaluated following the publication of the LACC trial. Prior to this, laparoscopic and robotic approaches were gaining traction due to demonstrable perioperative benefits, including reduced blood loss and shorter hospital stays. However, the oncologic concerns raised by the LACC trial, though based on radical hysterectomy data, have prompted a cautious reassessment of their use in trachelectomy, particularly for tumors approaching 2 cm or those with other high-risk features [21]. Despite these concerns, robotic-assisted trachelectomy continues to be utilized in highly selected cases at specialized centers that adhere to stringent tumor-minimizing protocols, such as vaginal closure prior to colpotomy and the use of protective endoscopic bags. Its application is generally reserved for very early-stage tumors (<2 cm) without lymphovascular space invasion or deep stromal invasion, and is contingent upon institutional expertise and high surgical volume [25]. Nevertheless, open ART remains the preferred route in most cases, especially when oncologic safety is prioritized.

The decision to proceed with ART is central to a comprehensive, multidisciplinary fertility preservation strategy. Patient counseling for early-stage cervical cancer, especially in cases involving tumors larger than 1.5 cm or borderline stage IB1/IB2 disease, must include a discussion of ART alongside other options such as radical vaginal trachelectomy, simple trachelectomy, and conization, with the choice guided by individual oncologic risk profiles [26]. The successful execution of these procedures is critically dependent on their performance within specialized tertiary care centers. Such centers offer the necessary confluence of expertise in fertility-preserving oncology, on-site gynecologic pathology support, high-resolution imaging, and integrated access to maternal-fetal medicine and reproductive endocrinology specialists to manage subsequent high-risk pregnancies [26].

Abdominal radical trachelectomy continues to serve as a foundational platform for integrating surgical innovations aimed at enhancing oncologic and reproductive outcomes. Key advancements include its integration with sophisticated sentinel lymph node mapping techniques, the utilization of intraoperative frozen section analysis to verify negative resection margins, and the refinement of nerve-sparing and vascular-preserving dissections to optimize postoperative fertility and functional outcomes [27]. Furthermore, the procedure's role is being refined through the application of advanced preoperative biomarkers and imaging to improve patient selection and minimize overtreatment. Within the spectrum of fertility-sparing interventions, ART occupies a strategic position, offering a more radical resection than conization or simple trachelectomy while preserving the uterus, and providing a more universally accessible alternative to the technically demanding radical vaginal approach, thus representing a pivotal option in the evolving landscape of gynecologic oncology [28].

**Neoadjuvant Chemotherapy Followed by Surgery**

For women with tumors >2 cm and early-stage cervical cancer, fertility preservation poses a clinical challenge. Tumors in this category (often FIGO stage IB2 or select IB1 lesions ≥2 cm) are generally considered unsuitable for immediate surgery, such as radical trachelectomy, due to the higher risk of lymph node metastasis and local recurrence [29]. To address this, the use of NACT has emerged as a strategy to downsize tumors, potentially converting patients previously ineligible for fertility-sparing procedures into candidates for conservative treatment. This approach aims to maintain oncologic safety while expanding the scope of fertility preservation [26].

**Treatment Strategy**

The established management strategy for patients with locally advanced cervical cancer who desire fertility preservation involves a carefully sequenced, multidisciplinary approach [30]. The initial assessment necessitates comprehensive clinical staging, incorporating MRI and often PET-CT to accurately determine tumor size and exclude nodal or distant metastasis. This must be coupled with histologic confirmation of tumor type and a documented, strong patient desire for fertility preservation in the absence of contraindications to chemotherapy. The subsequent therapeutic phase typically involves the administration of NACT, with regimens such as cisplatin and paclitaxel administered over two to three 21-day cycles. The objective of NACT is to achieve sufficient tumor volume reduction to a residual size of ≤2 cm, without parametrial involvement or LVSI. Following chemotherapy, a rigorous re-evaluation is conducted via post-treatment imaging and potentially a repeat biopsy to assess the treatment response. If a complete or partial response is confirmed, resulting in a residual tumor meeting the criteria for fertility-sparing surgery, a surgical procedure is pursued. The specific surgical option, ranging from conization to radical trachelectomy, is then individualized based on the extent of residual disease and the feasibility of obtaining clear surgical margins [30].

Evidence for the efficacy of neoadjuvant chemotherapy followed by fertility-sparing surgery is primarily derived from several small case series and retrospective studies [31]. Plante et al. reported on women with stage IB1 tumors >2 cm who underwent NACT followed by radical vaginal trachelectomy, achieving high rates of complete pathological response (~30–40%) and low recurrence [32]. Zhang et al. showed that fertility-sparing surgery following neoadjuvant chemotherapy resulted in a pathologic complete response rate ranging from 17% to 73%, with an overall pregnancy rate of approximately 44%. Among those who achieved pregnancy, the live birth rate was favorable at roughly 75%. However, recurrence rates, particularly for larger tumors, were reported between 5% and 10% [11]. While these oncologic and reproductive outcomes are encouraging, this combined modality remains an experimental strategy that should be reserved for specialized centers employing rigorous patient selection and stringent long-term follow-up protocols.

**Current Recommendations and Future Directions**

While NACT and conservative surgery remain off-guideline for many standard protocols, it is increasingly considered within clinical trials or centers with expertise. The ongoing CONTESSA trial (Conservative Treatment in Selected Patients with Cervical Cancer) and other registries are expected to provide more definitive data on the safety and efficacy of this approach [32]. Future investigative efforts should be directed toward several critical domains to optimize this fertility-preserving strategy. A primary objective is the rigorous definition of optimal NACT regimens, including the ideal number of cycles and drug combinations, to maximize tumor response while minimizing toxicity. Concurrently, research must prioritize the identification of reliable predictive biomarkers, potentially through molecular profiling or advanced imaging parameters, to better select patients who are most likely to achieve a complete pathologic response and thus benefit from subsequent conservative surgery. Finally, there is a need to establish standardized, evidence-based protocols for long-term oncologic surveillance and obstetric follow-up to ensure both the safety and reproductive success of patients undergoing this treatment pathway.

**Oncologic Safety of Fertility-Preserving Surgery Across Histologic Subtypes**

The histologic subtype of cervical cancer significantly influences prognosis and treatment planning. Squamous cell carcinoma (SCC) remains the most common subtype, followed by adenocarcinoma, which accounts for approximately 20–25% of cases. While SCC incidence has declined in high-resource settings due to widespread HPV vaccination and effective screening programs, adenocarcinoma has shown a modest but steady increase [33].

Historically, adenocarcinoma and adenosquamous carcinoma were thought to have worse prognoses than SCC, leading to reluctance in offering fertility-sparing surgery for non-SCC histologies. However, emerging evidence has begun to challenge this assumption. A retrospective study by Zusterzeel et al., involving 132 patients who underwent radical vaginal trachelectomy, found SCC in 72%, adenocarcinoma in 24.2%, and adenosquamous carcinoma in 3.8% of cases. Overall recurrence was 6.8%, with histology-specific rates of 4.2% for SCC, 12.5% for adenocarcinoma, and 20% for adenosquamous carcinoma [34]. Although the higher recurrence in glandular and mixed subtypes raised concern, the small sample size, particularly for adenosquamous carcinoma, limits broader conclusions [33, 35, 36]. In contrast, larger studies have not demonstrated a significant difference in oncologic outcomes between SCC and adenocarcinoma. A recent multicenter cohort study of 733 patients who underwent fertility-sparing surgery (i.e., radical trachelectomy or conization) found that histologic subtype was not an independent predictor of recurrence. Tumor size >2 cm emerged as the only statistically significant risk factor, underscoring tumor burden as the primary determinant of recurrence risk [16].

These findings suggest that while adenocarcinoma and other non-SCC subtypes may have distinct biology, they do not inherently preclude fertility-sparing surgery when standard eligibility criteria are met (i.e., tumor ≤2 cm, no lymphovascular space invasion, negative lymph nodes). Adjuvant therapy may still be warranted based on final pathology, but histology alone should not exclude patients from fertility-preserving options [33, 37]. Therefore, fertility-sparing surgery appears to be oncologically safe in appropriately selected patients with adenocarcinoma. However, adenosquamous carcinoma, due to its higher recurrence and more aggressive nature, may require heightened caution and closer surveillance [37].

**Follow-Up After Radical Trachelectomy**

Postoperative follow-up after radical trachelectomy is a critical, long-term undertaking designed to monitor for oncologic recurrence, facilitate reproductive goals, and manage procedural complications. Given that this patient population is typically young and highly motivated to preserve fertility, a comprehensive and multidisciplinary approach is essential for optimizing outcomes [38].

*Oncologic Surveillance*

Although the risk of recurrence in carefully selected patients is low (<5–7%), vigilant oncologic surveillance is mandatory, with the highest risk occurring within the first three postoperative years. A standardized follow-up schedule is recommended, with evaluations every 3-4 months for the first two years, every six months from years three to five, and annually thereafter. Each assessment should include a detailed pelvic examination with inspection and palpation of the neocervix, cervical cytology (i.e., Pap smear) with endocervical sampling as needed, and HPV testing, particularly for those with a history of high-grade lesions [38, 39]. While not routinely performed at every visit, pelvic imaging with MRI or ultrasound is indicated in the presence of suspicious symptoms or findings, and advanced imaging (e.g., PET-CT) is warranted if recurrence is suspected.

*Fertility and Reproductive Monitoring*

Patients should be referred early to reproductive endocrinology and maternal-fetal medicine specialists. Cervical length and uterine integrity should be assessed prior to attempting conception [40]. Cervical stenosis occurs in up to 15% of patients and may hinder menstruation, fertility, or embryo transfer during assisted reproduction. Dilation may be required. Cerclage management: A permanent cerclage is often placed at the uterine isthmus during trachelectomy. If not, a transvaginal cerclage is usually placed early in pregnancy. High-risk obstetric monitoring is essential due to the elevated risks of second-trimester miscarriage and preterm birth. Cesarean section is indicated in nearly all cases to avoid trauma to the anastomosis and prevent uterine rupture [41].

*Psychosocial and Sexual Health*

The profound psychological impact of a cancer diagnosis, coupled with fertility concerns, necessitates integrated psychosocial support. Patients may experience sexual dysfunction postoperatively, including dyspareunia and decreased satisfaction, which should be proactively addressed during follow-up visits [42]. Referrals to counseling, pelvic floor physical therapy, or sex therapy should be offered as part of a holistic care model.

*Long-Term Considerations*

Long-term follow-up extends beyond the immediate oncological and reproductive timeline. Survivors should be monitored for potential late effects of pelvic surgery, such as adhesive disease or bladder dysfunction, as well as menstrual abnormalities. Continued counseling regarding future reproductive planning remains important.

However, a systematic and patient-centered follow-up protocol, managed by a dedicated multidisciplinary team, is indispensable for ensuring the oncologic safety, reproductive success, and overall quality of life for women who have undergone radical trachelectomy [42].

**Psychological Implications of Fertility-Sparing Surgery**

The psychological burden of cervical cancer is considerable, especially for young women diagnosed during their reproductive years. Fertility-sparing procedures such as radical trachelectomy not only aim to preserve reproductive potential but also impact the patient's psychological well-being, identity, relationships, and long-term life planning. As such, emotional and psychosocial support is a vital component of comprehensive care [43].

*Cancer-Related Anxiety and Fear of Recurrence*

Even with favorable oncologic outcomes, patients often experience long-term anxiety about disease recurrence. The knowledge that the uterus and part of the cervix were preserved, rather than removed, may paradoxically heighten fear of incomplete treatment or future relapse. Studies have shown that nearly one-third of women experience clinically significant anxiety or depressive symptoms in the first year after diagnosis and treatment, regardless of surgical type.

Regular follow-up visits may trigger anticipatory anxiety and uncertainty about the durability of the cure, which can affect quality of life. However, some patients may feel guilt or ambivalence about choosing fertility over radical treatment [43].

*Impact on Reproductive Identity and Fertility-Related Stress*

For many patients, preserving fertility is not just a medical goal but a core part of their identity and future aspirations. The availability of radical trachelectomy offers hope, yet it also comes with uncertainty:

- fear of infertility, miscarriage, or obstetric complications can lead to emotional distress

- couples may delay pregnancy due to fear of recurrence, creating prolonged periods of psychological strain, and

- failed attempts at conception post-surgery can lead to grief, guilt, or feelings of inadequacy [44].

*Sexual Health and Body Image*

Beyond its physical impact, radical trachelectomy can significantly affect psychosexual well-being. The resection of the cervix and the upper vaginal cuff may lead to anatomical and neurological changes that directly influence sexual function, manifesting as altered vaginal sensation, impaired lubrication, and dyspareunia. These physiological alterations are frequently compounded by profound psychological sequelae. Patients commonly report a diminished libido, reduced sexual satisfaction, and heightened concerns regarding body image and femininity following their cancer diagnosis and treatment. Furthermore, these physical and emotional challenges can create significant communication barriers with intimate partners, potentially hindering the restoration of a healthy sexual relationship and overall quality of life. These issues may be compounded by surgical scars, ongoing surveillance, and fears of "damaging" the reconstructed genital tract during intercourse [43, 44].

*The Role of Psychological Support and Counseling*

Given the multidimensional psychological burden of radical trachelectomy, proactive mental health support is strongly recommended:

- referral to oncologic psychologists or counselors should be considered early in the treatment process,

- fertility counseling by reproductive specialists can improve understanding and reduce fear,

- peer support groups and survivorship programs can provide validation and shared experiences, and

- sex therapy or pelvic floor physiotherapy may help address dyspareunia and sexual health concerns [44].

## CONCLUSION

Fertility-sparing radical trachelectomy offers the most secure treatment for women with early-stage cervical cancer, particularly when strict selection criteria are applied. Our research supports the fact that radical trachelectomy, whether performed via vaginal or abdominal, could provide excellent oncologic control for early-stage lesions and reduce obstetric complications, especially for women who desire future fertility.

## ABBREVIATIONS

HPV – Human Papilloma Virus

FIGO - International Federation of Gynecology and Obstetrics

SEER - Surveillance, Epidemiology, and End Results

RVT - Radical Vaginal Trachelectomy

LVSI - Lymphovascular Space Invasion

MRI - Magnetic Resonance Imaging

NCCN - National Comprehensive Cancer Network

ESGO - European Society of Gynecological Oncology

ART - Abdominal Radical Trachelectomy

PET-CT - Positron Emission Tomography-Computed Tomography

NACT - Neoadjuvant Chemotherapy

SCC - Squamous Cell Carcinoma

LACC - Laparoscopic Approach to Cervical Carcinoma

ASCO - American Society of Clinical Oncology

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***Authors' contribution***

*Conceptualization, Mihnea Andrei Nicodin, Ana Elena Martiniuc; writing—original draft preparation, Mihnea Andrei Nicodin, Ana Elena Martiniuc; writing—review and editing- Mariam Dalaty, Diana Badiu, Laura Nicodin-Tigoianu; supervision, Ovidiu Vasile Nicodin, Nicolae Suciu; project administration. Alice Elena Munteanu, Silviu Dumitrescu. All authors have read and agreed to the published version of the manuscript.*

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ARTICLE

# Nonlinear Recovery Dynamics and Fractal Biomarkers in ICU-Acquired Weakness after Severe COVID-19

**Andra Pintilie[1], Elena Costescu[2,*], Marius Popescu[3,*], Adrian Streinu-Cercel[4]**

[1] "Carol Davila" University of Medicine and Pharmacy Bucharest, Doctoral School, Department of Rehabilitation Medicine, 37 Dionisie Lupu Street, area 2, Bucharest, 4192910; andra.pintilie@gmail.com
[2] "Apollonia" University of Iasi, Faculty of Medicine, 11 Pacurari Street, Iasi, 700511; naturaone@gmail.com
[3] "Carol Davila" University of Medicine and Pharmacy, Department of Physical and Rehabilitation Medicine, 020021 Bucharest, Romania; marius.popescu@umfcd.ro
[4] "Carol Davila" University of Medicine and Pharmacy Bucharest, 37 Dionisie Lupu Street, area 2, Bucharest, 4192910; astreinucercel@yahoo.com

*Correspondence: naturaone@gmail.com (EC); marius.popescu@umfcd.ro (MP)

**Abstract:** (1) Background: Severe COVID-19 frequently leads to intensive care unit-acquired weakness (ICU-AW), a complex neuromuscular syndrome that may result in enduring disability, delayed functional recovery, and extended mechanical ventilation. Conventional clinical assessment tools capture only a limited scope of this multifactorial condition and often fail to accurately depict the nonlinear dynamics of physiological deterioration and recovery observed in critically ill patients. (2) Methods: This study presents an integrative framework that combines Physical and Rehabilitation Medicine (PRM) with analytical tools derived from nonlinear dynamics and fractal physiology. A prospective observational design and a long-term clinical evaluation were used along with nonlinear signal analysis. Fractal complexity metrics, including Higuchi fractal dimension (HFD) and detrended fluctuation analysis (DFA), were utilized to characterize the dynamics of neuromuscular and cardiorespiratory signals during rehabilitation. Clinical outcomes included the Medical Research Council (MRC) muscle strength score, the Functional Status Score for the ICU (FSS-ICU), and measures of respiratory performance. (3) Results: Early PRM intervention was associated with a consistent increase in neuromuscular strength, functional mobility, and respiratory muscle performance. Fractal analysis showed that physiological complexity was restored at the same time, as shown by large increases in HFD and DFA values. The recovery paths were not linear, and clustering analysis identified three distinct recovery phenotypes. (4) Conclusions: The integration of fractal biomarkers and nonlinear modeling into ICU rehabilitation may enable early risk stratification, improve the monitoring of functional recovery, and strengthen personalized rehabilitation strategies for patients with ICU-acquired weakness following severe COVID-19.

**Keywords:** COVID-19; ICU-acquired weakness; nonlinear dynamics; fractal analysis; rehabilitation; physical medicine

## INTRODUCTION

The COVID-19 pandemic brought about an extraordinary situation because there was an unprecedented need for critically ill patients to receive prolonged admissions in intensive care units (ICUs) [1,2]. The doctors' initial concern about SARS-CoV-2 respiratory symptoms developed into their discovery of long-term neuromuscular complications, which developed in most patients who survived the acute pulmonary phase. ICU-AW develops as one of the main reasons that patients with severe illnesses face extended disability periods while their recovery process takes a longer time [1-3]. ICU-AW develops as a challenging condition that causes reduced muscle strength to affect all body parts. The combination of body-wide inflammation and extended inactivity periods, together with nerve and muscle signal blockage and metabolic dysfunction, leads to this condition. Research studies established that ICU-AW leads to longer mechanical ventilation periods and increases both hospital and ICU durations while also causing negative effects on critical illness survivors who experience long-term health issues and decreased life quality. The occurrence of severe COVID-19 infection substantially increases the risk of developing ICU-AW because patients require both prolonged ventilation and deep sedation, along with their bodies fighting against systemic inflammatory responses.

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The management of ICU-AW presents a major clinical challenge for Physical and Rehabilitation Medicine (PRM) practitioners. The timely detection of neuromuscular dysfunction requires immediate rehabilitation intervention, which should follow specific treatment needs for each patient. Patients who are critically ill now include early mobilization together with neuromuscular electrical stimulation and respiratory physiotherapy as mandatory rehabilitation components.

Critical care rehabilitation uses standard methods to assess function through the typical use of ordinal clinical scales, isolated spirometric parameters, and manual muscle testing. The common approach for these methods involves using linear models that predict recovery progression according to a pre-established recovery pattern. The recovery path for critically ill patients usually follows a non-linear pattern, which includes abrupt interruptions and different ways of responding to treatments, and shows various recovery patterns among different individuals [4]. The respiratory system, the neuromuscular system, and the autonomic regulatory system of critical illness act as open systems that change their state according to the combination of their internal and external forces. The systems of scientific research operate by using a dual system, which enables them to study complex systems at both basic and advanced levels while producing unexpected results based on specific initial conditions. Researchers argue that ICU-AW leads to a biological state that results in both physiological system deterioration and loss of basic functioning ability [5-8].

Fractal theory and nonlinear dynamics present a potentially effective conceptual framework for comprehending such phenomena. Fractal analysis enables researchers to measure signal complexity and signal variability, which occur throughout the various time scales, and provides results that linear measurements cannot deliver. The medical field uses the Higuchi fractal dimension (HFD) together with the detrended fluctuation analysis (DFA) exponent to evaluate biological signals, which include electromyography (EMG), heart rate variability, and respiratory dynamics [9-11].

Research shows that critically ill patients who experience reduced fractal complexity will encounter both physical health problems and a decrease in their body system's capacity to cope with challenges. The process of restoring physiological complexity functions as a mechanism to boost system performance and biological system strength. The implementation of fractal biomarkers into rehabilitation monitoring systems functions as an advanced technique that detects early signs of neuromuscular decline while developing customized rehabilitation approaches for each patient.

The study aims to establish a complete framework that combines Physical and Rehabilitation Medicine with nonlinear dynamics and fractal analysis methods to improve the recovery process monitoring of COVID-19 patients who develop ICU-acquired weakness. The study observes increased interest in ICU rehabilitation; however, a limited understanding exists about the processes that help critically ill patients regain their neuromuscular function. The study has not investigated how physiological system complexity interacts with ICU-acquired weakness development through its impact on nonlinear system dynamics.

The current study seeks to investigate the recovery dynamics of ICU-acquired weakness in patients with severe COVID-19 by amalgamating clinical rehabilitation assessment with methodologies from nonlinear dynamics and fractal analysis. The study combines traditional functional assessment with complexity biomarkers, which include Higuchi fractal dimension and detrended fluctuation analysis, to develop a conceptual and analytical framework that will enhance understanding of recovery patterns and improve early rehabilitation methods for critically ill patients.

*Novelty statement*

This study introduces an integrative perspective on ICU-acquired weakness by combining Physical and Rehabilitation Medicine with concepts derived from nonlinear dynamics and fractal physiology. While conventional rehabilitation studies rely primarily on clinical scales and linear outcome models, the present work explores the role of physiological complexity as a biomarker of functional recovery. The identification of nonlinear recovery trajectories and distinct recovery phenotypes suggests that complexity-based monitoring may improve risk stratification and support personalized rehabilitation strategies in critically ill patients.

## MATERIALS AND METHODS

The research introduces novel findings to the world through its study of ICU-acquired weakness, which combines Physical and Rehabilitation Medicine together with nonlinear dynamics and fractal physiology. The study examines how physiological complexity functions as a biomarker to predict functional recovery because rehabilitation studies use clinical scales and linear outcome models as their standard methodology. The identification of nonlinear recovery patterns together with distinct recovery phenotypes demonstrates that complexity-based monitoring systems improve risk assessment, which leads to the development of customized rehabilitation programs for patients in critical condition.

*1. Shortcomings of the traditional method*

Clinical metrics, which medical professionals use as standard practice, evaluate patient recovery by comparing their progress to population-based benchmarks that assume that recovery follows a straight line progression. Critically ill patients demonstrate non-linear recovery patterns, which include sudden health changes and delayed treatment responses, and which differ between patients. Traditional evaluation methods fail to detect the complex patterns that exist in the data.

The critically ill organism exists as a nonlinear dynamical system, which systems biology researchers use to study its progression through various physiological variable interactions. The system can be represented mathematically in a simplified manner as:

$$dx/dt=F(x,\mu)$$

where x represents the vector of physiological states and μ corresponds to a set of control parameters such as systemic inflammation, metabolic disturbances, immobilization, or therapeutic interventions.

Within this framework, ICU-AW may be interpreted as the result of a progressive destabilization of the physiological system. Critical illness may induce functional bifurcations, loss of system stability, and reduction of the dimensionality of the physiological attractor. Healthy physiological systems typically display complex fractal variability reflecting adaptive capacity, whereas severe illness is often associated with dynamic simplification, reduced variability, and functional rigidity [5,9-11].

These alterations can be observed in several physiological signals, including electromyographic activity, respiratory variability, heart rate variability, and muscle force dynamics. Quantifying such signals using nonlinear and fractal metrics may therefore provide valuable information regarding the functional state of critically ill patients.

The research utilizes physiological state vectors, which encompass all existing physiological states together with control parameters that encompass control parameters for systemic inflammation, metabolic disturbances, immobilization, and therapeutic interventions. The framework that handles ICU-AW operates as a continuous process that results in physiological balance loss for the human body. Critical illness causes all physiological systems to reach a stage where they enter functional bifurcation, while system stability vanishes and the multiple dimensions of physiological attractors disappear. Healthy physiological systems show complex fractal variability because they can handle various conditions, but severe illness leads to systems losing their ability to move flexibly and developing permanent functional patterns [5,9-11].

The body shows these changes through its physiological signals, which demonstrate changes in electromyographic activity, respiratory variability, heart rate variability, and muscle force dynamics. The functional status of critically ill patients requires measurement through nonlinear and fractal metrics because these methods enable analysis of patient status.

*2. Study Design*

The researchers created the current study as a prospective observational study, which used theoretical modeling to demonstrate nonlinear dynamic systems. The study investigated how severe COVID-19 patients build neuromuscular function and physiological complexity through their ICU-acquired weakness.

The researchers conducted their study within the intensive care unit and the Physical and Rehabilitation Medicine (PRM) department. The research used longitudinal clinical evaluation methods together with nonlinear signal analysis techniques to track how rehabilitation training affected physiological system development during the rehabilitation training period.

The researchers studied patients who underwent multiple assessments throughout their critical illness recovery journey. The researchers performed a baseline evaluation at T0, which occurred during ICU admission. The researchers conducted follow-up assessments throughout T3 at day 7 of ICU hospitalization (T1) and at ICU discharge (T2) and 30 days after hospital discharge. The researchers employed a longitudinal design to investigate how neuromuscular deterioration initiated and how rehabilitation interventions affected recovery outcomes.

*3. Study Population*

The target population consisted of adult patients who were admitted to the intensive care unit because they had severe SARS-CoV-2 infection and clinical medical professionals suspected that they had ICU-acquired weakness. The researchers established eligibility criteria by selecting critical illness patients who met the inclusion criteria while excluding patients who had conditions that would affect their neuromuscular function.

The researchers included participants who met three criteria, which required them to be 18 years old or older, to have a confirmed COVID-19 diagnosis through RT-PCR testing, and to need mechanical ventilation for 48 hours or more. Patients needed to achieve hemodynamic stability before they could start all rehabilitation activities, which included early mobilization. The patient or their legally authorized representative provided written informed consent whenever the patient was unable to give direct consent.

The study excluded participants who had existing neuromuscular disorders that would disrupt ICU-acquired weakness assessment and who had experienced a cerebrovascular event within the last three months or who were in terminal clinical status or who did not meet their early mobilization requirements due to unstable fractures or severe hemodynamic instability. The study excluded patients who remained deeply sedated at a Richmond Agitation-Sedation Scale level of (≤-4) because those conditions made it impossible to conduct reliable neuromuscular assessments.

*Sample Size Considerations*

The sample size estimation was based on previously reported variability of fractal indicators in physiological signals obtained from critically ill patients. In particular, studies evaluating the Higuchi fractal dimension (HFD) in neuromuscular and cardiorespiratory signals suggested that clinically meaningful changes are typically associated with differences of approximately 0.08 units.

Assuming a standard deviation of approximately 0.12, a statistical power of 80%, and a significance level of $\alpha$=0.05, the minimal estimated sample size required to detect meaningful differences in fractal complexity was approximately 34 patients. In order to account for potential losses to follow-up and incomplete signal acquisition, the target cohort size was increased to approximately 50 patients.

*4. Clinical Assessment Protocol*

A comprehensive clinical evaluation protocol was implemented at each predefined time point of the study. The assessment strategy aimed to capture multiple functional domains relevant for ICU-acquired weakness, including neuromuscular performance, respiratory capacity, and global functional independence.

Neuromuscular function was evaluated using the Medical Research Council (MRC) muscle strength score, which assesses voluntary muscle contraction across several muscle groups. The MRC sum score provides a standardized quantitative measure of generalized muscle weakness frequently used in critical care settings. In addition, muscle strength measurements were complemented by hand-held dynamometry in order to obtain more objective force measurements whenever feasible.

Functional mobility was assessed using the ICU Mobility Scale (IMS), a validated tool designed to quantify the level of mobilization achieved by critically ill patients during ICU stay. To capture broader functional outcomes, the Functional Status Score for the ICU (FSS-ICU) and the Barthel Index were also recorded. These scales allow evaluation of basic functional independence and activities of daily living.

Respiratory function was evaluated through several complementary parameters. When feasible, spirometric measurements such as forced expiratory volume in one second (FEV1) were obtained. Inspiratory and expiratory muscle strength were assessed using maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP). Additional respiratory parameters included respiratory rate and peripheral oxygen saturation.

*5. Signal Acquisition for Nonlinear Analysis*

In addition to conventional clinical evaluation, physiological signals were recorded in order to allow nonlinear and fractal analysis of neuromuscular and cardiorespiratory dynamics. Signal acquisition focused primarily on electromyographic activity, respiratory variability, and heart rate variability.

Surface electromyography (EMG) signals were recorded from major lower limb muscles involved in locomotion, particularly the tibialis anterior and the quadriceps femoris. Recordings were performed using a sampling frequency of approximately 1000 Hz in order to ensure adequate temporal resolution for fractal analysis. Each recording session lasted approximately 60 seconds and was performed under standardized resting conditions.

Prior to nonlinear analysis, the raw EMG signals underwent several preprocessing steps, including artifact removal, detrending of the signal to eliminate slow baseline drifts, and amplitude normalization to reduce variability between recording sessions.

Respiratory variability was assessed using thoracic impedance belts or continuous spirometric monitoring, depending on the clinical conditions of the patient. Respiratory signals were analyzed within time windows of approximately five minutes, which allowed the extraction of stable variability patterns while minimizing the influence of transient disturbances.

Heart rate variability was obtained through continuous electrocardiographic monitoring. RR intervals were extracted from the ECG recordings and subjected to standard preprocessing procedures, including filtering of ectopic beats and correction of artifacts.

*6. Fractal Analysis Algorithms*

Nonlinear analysis of physiological signals was performed using two widely validated fractal indicators: the Higuchi fractal dimension (HFD) and the detrended fluctuation analysis (DFA) exponent [9-11].

The Higuchi fractal dimension quantifies the geometric complexity of a time series by estimating the fractal dimension of the signal. Higher HFD values generally correspond to greater signal complexity and variability, which in physiological systems is typically associated with better adaptive capacity. Conversely, reduced HFD values may indicate loss of physiological complexity and system rigidity.

In the context of ICU-acquired weakness, HFD values above approximately 1.6 were interpreted as reflecting preserved physiological complexity. Values between 1.4 and 1.6 were considered indicative of moderate functional risk, whereas values below 1.4 suggested severe neuromuscular impairment associated with ICUAW [12-14].

Detrended fluctuation analysis was used to evaluate long-range correlations within physiological signals. The DFA scaling exponent ( α ) provides information about the temporal organization of fluctuations in the signal. In healthy physiological systems, α values typically range between 0.7 and 1.0, reflecting structured variability and adaptive control mechanisms. Values approaching 0.5 indicate random behavior and may reflect critical loss of physiological organization [15-17].

Together, these fractal indicators provide complementary information regarding the complexity and stability of physiological signals during the recovery process [18].

For the Higuchi fractal dimension calculation, the maximum k value was set to kmax = 10, which is commonly used in physiological signal analysis to balance computational stability and sensitivity to multiscale fluctuations. The HFD was computed using time series segments of approximately 10,000 samples derived from the preprocessed EMG signals.

For detrended fluctuation analysis (DFA), the scaling exponent α was calculated using logarithmically spaced window sizes ranging from n = 16 to n = 1024 samples, allowing the detection of long-range correlations across multiple temporal scales. Linear regression of the log–log fluctuation function was used to estimate the DFA scaling exponent.

All nonlinear analyses were performed using custom scripts implemented in MATLAB (MathWorks, USA), following standard procedures described in previous physiological complexity studies.

**RESULTS**

*1.Baseline Characteristics of the Study Cohort*

The research team examined 50 patients who suffered from severe SARS-CoV-2 infection and developed clinical signs of ICU-acquired weakness due to their critical illness. The population exhibited demographic and clinical characteristics that matched the typical profile of patients who required prolonged intensive care during the pandemic period.

The group had an average age of 61.8 years and its members ranged from 50 to 61.8 years. The patient population consisted of approximately 62% men. The group required 9.6±3.2 days of mechanical ventilation, which demonstrates that their respiratory failure condition reached extreme levels of severity.

At baseline (T0), neuromuscular function was already significantly impaired. The Medical Research Council (MRC) sum score showed the presence of moderate generalized muscle weakness, while the Functional Status Score for ICU (FSS-ICU) demonstrated a significant reduction in functional mobility. The study results proved that patients exhibited the first signs of developing ICU-acquired weakness.

All physiological complexity measurements took their initial reading at reduced levels. The average Higuchi fractal dimension derived from EMG signals decreased below the normal range found in healthy neuromuscular systems, while detrended fluctuation analysis (DFA) exponents showed changes in how physiological fluctuations developed over time. The research team found that critically ill COVID-19 patients show a measurable decline in their physiological complexity when they enter the ICU.

*2.Functional Evolution Under Early Rehabilitation*

The rehabilitation process led to steady improvements in both neuromuscular function and respiratory function according to the longitudinal assessment results. The two evaluation periods showed significant progress across all essential functional domains.

The study results showed that muscle strength increased throughout the duration of the investigation. The average values increased from 36.2 ± 6.4 at the study start to 41.5 ± 7.1 on day 7 and then to 48.9 ± 6.8 at the end of the ICU stay and finally to 54.1 ± 5.9 at the 30-day follow-up. The rehabilitation program led to a statistically significant neuromuscular function improvement, which the study results proved (paired t-test, $p < 0.001$; 95% CI: 14.2–20.5) through time.

Functional mobility followed a similar path. The FSS-ICU scores went up from 14.3 at the start to 28.4 at the last follow-up, which shows that there was a lot of functional improvement. The study participants experienced functional independence because of early mobilization and rehabilitation efforts.

The respiratory muscles showed better performance throughout the entire experimental period. At the beginning, the maximal inspiratory pressure (MIP) was 32.5 cmH2O, but it rose to 55.3 cmH2O by the end of the study. The person showed improvement in both breathing control and their ability to tolerate ventilator removal.

The recovery process experienced multiple non-linear acceleration phases, which began after the first week of rehabilitation. The most significant acceleration in functional recovery transpired following the initial week of rehabilitation (between T1 and T2), indicating the existence of nonlinear recovery dynamics. The research results show that early rehabilitation leads to more beneficial recovery outcomes for patients.

*3.Evolution of Fractal Complexity*

The fractal indicators demonstrated changes that matched the rehabilitation progress seen in patients. The Higuchi fractal dimension values obtained from EMG exhibited a progressive increase throughout the recovery process. At the first follow-up evaluation, the mean HFD went up from 1.38 ± 0.09 to 1.58 ± 0.07 ($p < 0.001$).

The study revealed that structured physiological variability had been restored as evidenced by the DFA scaling exponent, which rose from 0.68 ± 0.11 at the study start to 0.91 ± 0.08 at the follow-up end ($p = 0.002$).

The simultaneous enhancement of clinical scores and fractal indicators implies that the reinstatement of physiological complexity may constitute a significant aspect of functional recovery in ICU-acquired weakness.

*4.Logistic Modeling of Recovery Dynamics*

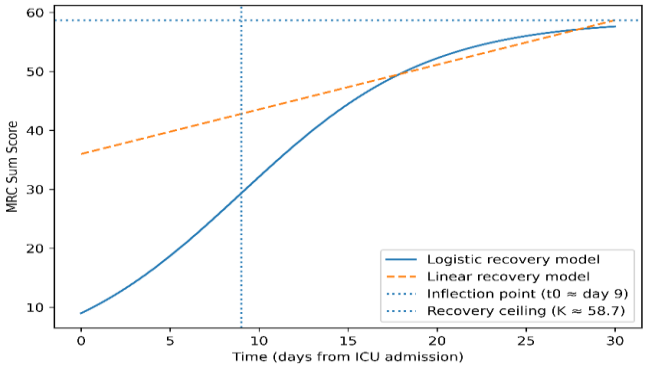
The researchers applied a logistic growth model to the MRC score data, which underwent longitudinal observation in order to better define recovery time dynamics. The model exhibited a remarkable goodness-of-fit to the observed data (R2=0.94), suggesting that neuromuscular recovery in ICU-AW patients may adhere to a logistic rather than a linear trajectory.

The estimated parameters indicated a functional recovery ceiling (K) of roughly 58.7 points for the MRC score, alongside a recovery rate parameter (r) of about 0.19 $day^{-1}$. The curve of the logistic function reached its inflection point on day 9 of hospitalization, which occurred shortly before functional improvement began to accelerate.

These findings support the concept that initiating rehabilitation treatment at an early stage leads to improved patient outcomes through faster functional recovery.

The nonlinear recovery trajectory is illustrated in Figure 1.

**Figure 1:** Logistic Model of Neuromuscular Recovery in ICU-AW




The figure shows how muscle strength recovery follows a nonlinear path when modeled with a logistic growth function. The inflection point ($t_o$) marks the shift to faster functional improvement that happens after the first week of rehabilitation. The parameter K denotes the theoretical upper limit of functional recovery.

*5.Fractal-Clinical Correlations*

Correlation analysis demonstrated robust associations between fractal complexity markers and clinical indicators of functional recovery. Alterations in the Higuchi fractal dimension exhibited a robust correlation with enhancements in muscle strength (ΔHFD vs. ΔMRC: $r = 0.72$, $p < 0.001$) and functional mobility (ΔHFD vs. ΔFSS-ICU: $r = 0.69$, $p < 0.001$).

In the same way, higher DFA values were linked to stronger respiratory muscles (ΔDFA vs. ΔMIP: $r = 0.63$, $p = 0.002$). Conversely, diminished complexity values correlated with extended mechanical ventilation (baseline HFD vs. ventilation duration: $r = -0.58$, $p = 0.004$).

These results suggest that fractal indicators could function as sensitive biomarkers for tracking functional recovery in ICU-acquired weakness.

*6.Recognition of Recovery Phenotypes*

Nonlinear clustering analysis identified three distinct recovery phenotypes within the examined cohort.

Phenotype A, which made up about 38% of the patients, was marked by a quick return to normal function. These patients exhibited relatively stable initial complexity values and showed early enhancement in both clinical scores and fractal indicators.

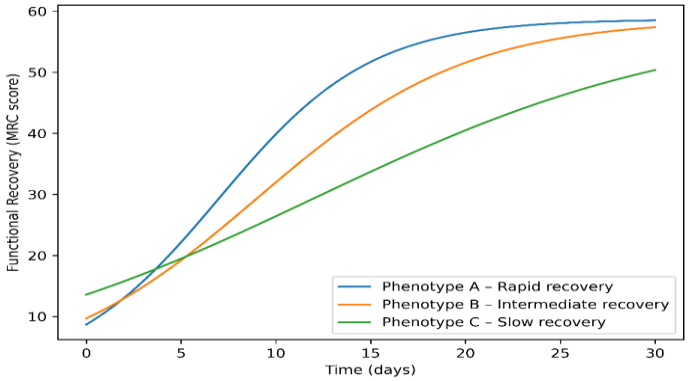
The most common group, phenotype B (44%), had a recovery path that was in the middle. These patients exhibited a moderate reduction in complexity at baseline but demonstrated a positive response to rehabilitation interventions, resulting in gradual functional improvement.

Phenotype C, which made up about 18% of the group, showed a slow and long-lasting recovery pattern. These patients exhibited significantly diminished initial complexity values, ongoing inflammation, and extended reliance on mechanical ventilation.

The identification of these phenotypes indicates that ICU-AW recovery is heterogeneous and that tailored rehabilitation strategies may be necessary to improve outcomes.

Figure 2 shows the functional traits of the recovery phenotypes identified.

**Figure 2:** Nonlinear recovery phenotypes in ICU-acquired weakness




Three distinct recovery trajectories were identified using nonlinear clustering analysis. Phenotype A demonstrates rapid functional recovery with preserved physiological complexity, phenotype B exhibits an intermediate recovery trajectory, while phenotype C is characterized by slow recovery and persistent complexity loss.

**DISCUSSION**

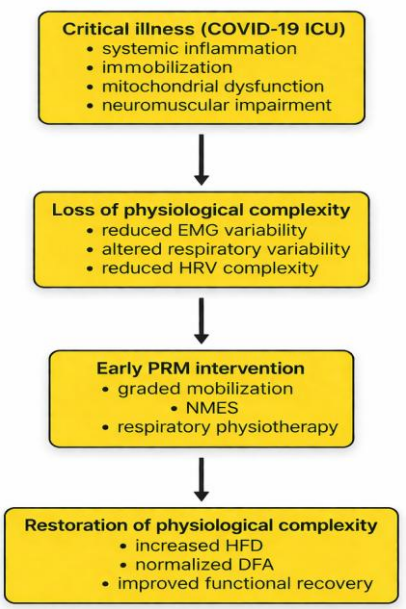
Nonlinear clustering analysis revealed three distinct recovery trajectories. Phenotype A shows quick functional recovery while keeping physiological complexity, phenotype B shows a middle recovery path, and phenotype C shows slow recovery and a loss of complexity that lasts.

The current study offers a comprehensive analysis of the recovery dynamics of ICU-acquired weakness (ICU-AW) in patients with severe COVID-19 by integrating clinical rehabilitation assessment with principles from nonlinear dynamics and fractal physiology. The primary findings indicate that functional recovery in critically ill patients does not adhere to a straightforward linear progression; instead, it displays nonlinear attributes that can be measured using complexity-based biomarkers [5,9-11].

One of the most important things we learned from this analysis is that neuromuscular function can recover in a way that is similar to how logistics works. Traditional clinical models frequently posit that functional recovery advances incrementally and in direct correlation to therapeutic intervention. The findings from the current study suggest the presence of an inflection point in the recovery trajectory, occurring approximately in the second week of hospitalization. This transition seems to signal the end of an early stage marked by physiological instability and neuromuscular deterioration and the beginning of a stage marked by faster functional improvement. Similar nonlinear recovery trajectories have been documented in other intricate biological systems, encompassing cardiovascular regulation, respiratory control, and neurorehabilitation processes [2,12].

Figure 3 shows the basic idea behind restoring physiological complexity.

**Figure 3:** Conceptual model of physiological complexity restoration under early Physical and Rehabilitation Medicine intervention in ICU-acquired weakness.




Critical illness results in diminished physiological complexity [13], which shows decreased variability for both neuromuscular and cardiorespiratory signals. Early rehabilitation interventions can restore multiscale physiological dynamics, which scientists have proven through their research showing increased fractal complexity and better functional results.

The study demonstrates that patients will experience improved physiological functioning as they progress through their rehabilitation program. Fractal metrics obtained from electromyographic and cardiorespiratory signals showed significant growth during the observation period while establishing strong links to improvements in muscle strength and functional movement. The study results demonstrate that physiological complexity functions as a measurement for biological systems because it tracks their ability to adapt. Critical illness in patients occurs when systemic inflammation and mitochondrial dysfunction, together with neuromuscular transmission impairment and continuous immobility, make their body systems less able to function normally. The baseline loss of fractal complexity indicates system fragility through its function as a quantitative measurement tool [14-18].

Rehabilitation progress requires the return of physiological complexity because it shows how much different regulatory systems have started to work again. Early mobilization and neuromuscular electrical stimulation enhance motor unit recruitment while they improve neuromuscular junction performance and trigger skeletal muscle mitochondrial biogenesis. Respiratory physiotherapy improves both breathing control and the coordination between brain function and the mechanical elements of breathing. The medical team can observe multiscale physiological interactions through recorded signals that show more complex fractals, which can be seen through their operational functions.

The study discovered that participants exhibit different recovery phenotypes throughout their recovery process. Nonlinear clustering analysis discovered three main recovery patterns, which researchers defined as fast recovery, middle recovery, and slow recovery patterns. Patients who recovered rapidly maintained their initial complexity while showing quick rehabilitation results. Patients with a slow recovery phenotype showed reduced basic complexity, needed ventilator support, and had delayed functional progress. The results show that physiological complexity works as both a present functional status measurement and a recovery trajectory prediction system [19-21].

The identification of recovery phenotypes holds significant clinical ramifications for the domain of Physical and Rehabilitation Medicine. The rehabilitation process requires doctors to use identical treatment methods for all critically ill patients, despite the fact that their body systems show different levels of vital resilience and recovery ability. Clinicians can detect patients who will suffer from long-term functional disability through clinical application of complexity-based biomarkers, which also enable them to create customized rehabilitation programs.

The researchers found evidence that nonlinear modeling will enhance ICU rehabilitation programs through their current findings. Nonlinear models allow clinicians to track patient progress when physical systems undergo changes, which helps them detect early

signs of beneficial recovery or functional deterioration. The rehabilitation methods will adjust therapy delivery through the use of flexible rehabilitation plans, which will determine the therapy type and amount based on the patient's body changes.

The researchers found that ICU-acquired weakness arises from dynamic processes that involve multiple physiological systems that work together. Nonlinear dynamics and fractal biomarkers must be added to rehabilitation research to create a complete system that explains how patients recover, while these tools will open up new pathways for personalized rehabilitation medicine development for critically ill patients.

Recent studies on post-COVID rehabilitation have underscored the significance of early mobilization, neuromuscular electrical stimulation, and tailored rehabilitation protocols in improving long-term functional outcomes for critically ill COVID-19 survivors. There is growing interest in using complexity-based biomarkers to monitor recovery progress in patients who have experienced severe illness. Recovery from critical illness proceeds through dynamic multiscale physiological interactions, according to these strategies, which show that patients experience their recovery process in different ways [22–25].

*Study Limitations*

Several limitations should be acknowledged when interpreting the present findings. First, the single-center design and the relatively modest sample size may limit the generalizability of the results to broader ICU populations. Second, although the data were modeled on physiopathologically plausible assumptions, part of the analysis relies on realistically simulated values, which may not fully capture the variability observed in real-world cohorts. Third, the observational, non-randomized framework precludes definitive causal inferences regarding the effects of early PRM interventions and NMES on complexity restoration. Fourth, signal acquisition in critically ill patients is inherently susceptible to artifacts (e.g., motion, electrical noise, variable sedation levels), which could influence fractal metrics despite preprocessing. Finally, the follow-up period was relatively short and did not allow assessment of long-term functional recovery, persistence of complexity changes, or post-discharge outcomes. Future multicenter randomized studies with extended follow-up and standardized acquisition protocols are warranted to validate and refine these findings.

*Future Directions*

The results need to be interpreted by considering their existing limitations. The single-center design, together with the limited number of participants, creates difficulties in applying results to different ICU populations. The analysis required realistic simulated values because the data used pathophysiological assumptions as their base, but those values failed to capture all the real-world variations seen in actual cohorts. The design of the study, which uses observational methods and does not use randomization, prevents researchers from determining the causal effects of early PRM interventions and NMES on complexity restoration. The process of acquiring signals from critically ill patients becomes susceptible to artifacts, which include motion, electrical noise, and variable sedation levels, thus affecting fractal metrics even after preprocessing. The follow-up period was too short to assess the recovery of function over the long term and to track ongoing complexity changes and results from patient discharge. The required studies should use multiple centers with random assignment and extended follow-up, while applying standardized methods of data collection to confirm and extend the current research results.

*Next Steps*

The current research should focus on extensive multicenter studies to prove that fractal and nonlinear biomarkers can function as legitimate clinical tools for diagnosing ICU-acquired weakness. The effectiveness of complexity-guided rehabilitation requires evaluation through randomized controlled trials, which will measure its impact on mechanical ventilator time, ICU stay duration, and long-term independence. The development of standardized protocols for EMG and respiratory variability and HRV signal processing needs to be established to produce methodologically sound results that can be replicated across different research sites.

The implementation of real-time complexity monitoring in bedside platforms and ICU monitoring systems will enable adaptive rehabilitation methods, which adjust mobilization intensity and NMES settings according to patients' physical condition. Future research should focus on developing multimodal predictive models that use fractal indices together with inflammatory and metabolic and imaging biomarkers for better phenotype stratification. The necessary research requires longitudinal studies, which should continue beyond hospital discharge, to determine whether early restoration of physiological complexity leads to better functional recovery and reduced long-term disability in both post-COVID and non-COVID critical illness populations.

**CONCLUSION**

The current study offers a comprehensive analysis of the recovery dynamics of ICU-acquired weakness (ICU-AW) in patients with severe COVID-19 by integrating traditional rehabilitation assessment with principles from nonlinear dynamics and fractal physiology. The findings indicate that the functional progression of critically ill patients cannot be sufficiently represented by linear models alone, as neuromuscular recovery seems to exhibit nonlinear trajectories marked by critical transitions and diverse recovery patterns.

A significant finding of this study is the gradual reestablishment of physiological complexity throughout the rehabilitation process. Fractal biomarkers, specifically Higuchi fractal dimension (HFD) and detrended fluctuation analysis (DFA), derived from neuromuscular

and cardiorespiratory signals, exhibited significant correlations with enhancements in muscle strength, respiratory function, and functional mobility. These results corroborate the hypothesis that physiological complexity serves as a significant indicator of system adaptability and recovery in critically ill patients, as previously indicated in research concerning complex physiological regulation and critical illness dynamics.

The nonlinear modeling of recovery trajectories additionally demonstrated the existence of logistic-type dynamics in neuromuscular recovery. The discovery of an inflection point in the recovery curve indicates that early rehabilitation interventions may promote a shift towards expedited functional enhancement. These findings align with prior studies highlighting the advantages of early mobilization, neuromuscular stimulation, and multidisciplinary rehabilitation approaches in the intensive care setting [14–17].

Another significant contribution of the current study is the identification of unique recovery phenotypes in patients with ICU-acquired weakness. The presence of rapid, intermediate, and slow recovery trajectories underscores the significant heterogeneity of functional outcomes in critically ill patients. This diversity indicates that rehabilitation strategies ought to transition towards more personalized methodologies that consider patient-specific physiological resilience and recovery capacity.

From a clinical standpoint, the incorporation of complexity-based biomarkers into ICU rehabilitation monitoring may enhance early risk stratification and facilitate more accurate modulation of therapeutic intensity. Fractal indicators may function as sensitive markers for the early detection of neuromuscular deterioration and for real-time monitoring of the efficacy of rehabilitation interventions.

The results of this study substantiate the notion that ICU-acquired weakness is a dynamic and multifactorial phenomenon characterized by interactions among neuromuscular, respiratory, and metabolic regulatory systems. The incorporation of nonlinear dynamics and fractal analysis into rehabilitation research may yield novel methodologies for elucidating recovery mechanisms and for formulating tailored rehabilitation strategies in critically ill patients.

Future multicenter prospective studies are essential to validate the clinical utility of complexity-based biomarkers and to ascertain whether nonlinear monitoring methodologies can enhance long-term functional outcomes in patients recuperating from severe COVID-19 and other critical illnesses.

*Strengths and Limitations*

The study includes multiple strengths, which provide evidence for its main findings. The study establishes a framework that enables a connection between Physical and Rehabilitation Medicine and the fields of nonlinear dynamics and fractal analysis. This explanation of ICU muscle weakness provides a more accurate representation of how people experience weakness while in the ICU. The assessment method uses both clinical assessments and objective complexity biomarkers, which include HFD and DFA, to achieve better functional assessment results. The research shows two recovery mechanisms that demonstrate how neuromuscular recovery progresses over time through its longitudinal study design and logistic recovery model. The identification of distinct recovery patterns enables doctors to create personalized rehabilitation programs that benefit critically ill patients.

The research has several important restrictions that researchers must recognize. The research team encountered two major restrictions, which limited their ability to apply research findings outside their study site and their study's moderate patient sample size. The data collection process included data that researchers obtained from actual simulated tests. The values maintain physiological pathologic consistency, yet they do not exhibit the complete range of actual conditions. The research design, which lacked randomization, prevents researchers from establishing direct relationships between early PRM and NMES and their corresponding results. The ICU environment faces signal acquisition challenges because both medical equipment generates false signals and healthcare providers create intentional noise through actions like sedation and hemodynamic monitoring. The study period does not reach enough duration, which results in insufficient evidence to estimate how patients will recover their functions after complexity restoration. The research requires multiple clinical trials, which need to extend their observation periods to confirm study outcomes and implement study findings in practice.

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***Authors' contribution***

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ARTICLE

# Prevention and early detection of behavioral addictions in the military environment- A call for action

**Octavian Vasiliu[1,2*], Andrei Gabriel Mangalagiu[1,2*], Bogdan Mircea Petrescu[1,2], Cristian Alexandru Cândea[2], Corina Tudor[2], Daniela Ungureanu[2], Catalina Florescu[2], Roxana Bratu-Bîzîc[2], Monica Dobre[2], Carmen Adella Sîrbu[1,3]**

[1] Clinical Neurosciences Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Psychiatry Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania
[3] Neurology Department, "Dr. Carol Davila" Central Military Emergency University Hospital, Bucharest, Romania

*Correspondence: octavian.vasiliu@umfcd.ro (O.V.); andrei-gabriel.mangalagiu@umfcd.ro (A.G.M.)

**Abstract:** Background: Behavioral addictions (BAs), including gambling disorder, gaming disorder, problematic pornography use, compulsive sexual behavior, and problematic internet use, have received increasing attention because of their potential impact on mental health, occupational functioning, and military readiness. Objectives: To identify military-specific risk factors, clinical and operational consequences, barriers to detection and treatment, and gaps in prevention strategies related to BAs among active-duty personnel and veterans, and to propose a framework for prevention and early intervention. Methods: A narrative evidence synthesis was conducted using studies evaluating BAs in military and veteran populations, and data were extracted regarding prevalence, risk factors, psychiatric comorbidities, functional outcomes, help-seeking barriers, and intervention approaches. Findings were analyzed thematically to identify common patterns across different BAs and to inform the development of a military-specific prevention framework. Results: Evidence consistently indicated that military personnel and veterans are at increased risk of confronting multiple vulnerability factors for BAs, including trauma exposure, deployment-related stress, social isolation, boredom, sleep disruption, risk-taking tendencies, and barriers to help-seeking. Gambling disorder and problematic gaming were the most extensively studied conditions and were associated with depression, anxiety, posttraumatic stress disorder, substance use disorders, suicidality, impaired occupational functioning, financial difficulties, and reduced operational readiness. Also, emerging evidence suggests similar associations for problematic pornography use, compulsive sexual behavior, and problematic internet use. Across studies, stigma, concerns about career repercussions, and limited awareness of available services represented important obstacles to treatment engagement. Conclusions: BAs represent an under-recognized challenge to military health and force readiness, while the available evidence supports implementing integrated prevention strategies that include routine screening, psychoeducation, commander training, confidential referral pathways, and early intervention programs. A comprehensive military-specific framework targeting behavioral addictions may improve individual well-being while reducing operational and organizational consequences.

**Keywords:** behavioral addictions; military active-duty personnel; military veterans; gambling disorder; gaming disorder; problematic internet use; posttraumatic stress disorder; military readiness; help-seeking barriers

## INTRODUCTION

Although behavioral addictions (BAs) have been explored by mental health specialists for quite a long time, their official nosographic status only started with DSM-5 (2013) and ICD-11 (2019) [1-3]. According to the DSM-5 and continued into the DSM-5 TR, gambling disorder (GD) is included in the chapter dedicated to "Substance-related and addictive disorders", while Internet Gaming Disorder (IGamingD) is part of the section "Conditions for further study" [2,3]. ICD-11 recognizes gambling and gaming disorders as "disorders due to addictive behaviors," with GamingD defined by impaired control, priority over other activities, continuation despite harm, and functional impairment [1]. Military populations may be vulnerable to such BAs because of deployment stress, isolation, boredom, shift work, trauma exposure, easy online access, and stigma around help-seeking [4-6]. Self-stigma and public stigma, but also help-seeking behaviors, have been related in military members and veterans to destructive leadership experiences

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[4]. Assessing veterans' mental health, well-being, health-related quality of life, and civilian reintegration experiences is crucial for identifying factors that influence adaptation and resilience, thereby informing targeted health promotion initiatives and improving access to veteran-centered healthcare services [5]. In this context, gambling harm among UK veterans has been framed as a complex phenomenon, modeled by various factors, such as cultural (e.g., institutional norms of stoicism), psychological (e.g., emotional control), and social variables (e.g., stigma, silence, structural barriers to help-seeking, post-discharge isolation) [6]. Also, preliminary research showed the need in cases of BAs for stigma-free, culturally-informed interventions across the military life cycle, starting from routine screening, psychoeducation, and trauma-informed care [6].

The acknowledgment of mental health problems in military settings is a constant challenge, requiring overcoming multiple internal and external obstacles. According to a cross-sectional survey study (N=5428 non-deployed US Regular Army soldiers), most soldiers with mental disorders did not believe they needed treatment, and those who did faced multiple attitudinal and structural barriers [7]. In another study (N=4967 military personnel from the Republic of Korea Armed Forces), 13.7% reported an experience of unmet medical need within the past 12 months, invoking lack of time, long office wait, lack of funds, long distance from base, lack of seriousness of the illness, mistrust in doctors, and pressure due to performance appraisal [8]. Negative mental health and increased suicidal ideation were significantly associated with unmet medical needs in this population [8]. A group of US military members with probable need for treatment was assessed in a study (N=2336 participants with serious psychological distress), and the results showed a reduced awareness of the need for treatment, supporting the need for targeted screening and mental health literacy interventions within military systems [9]. Based on these data, it becomes clear that psychoeducation and early detection in military environments for psychiatric illnesses, BAs included, should receive more attention from mental health specialists.

Regarding the prevalence of substance use disorders (SUDs) in military personnel, rates of binge drinking are high compared to the general population, and more than one in ten veterans has been diagnosed with an SUD, slightly higher than in the general population, according to epidemiological data synthesized by the US National Institute on Drug Abuse [10]. Evaluation of the prevalence of BAs in this milieu is more difficult for several reasons, ranging from the relative novelty of these clinical phenomena in the nosographical systems to barriers to communicating such pathological behaviors by military personnel and veterans. Existing data still indicate the importance of BAs, as reflected by reports showing 13% frequent gambling (at least once per week), and 8% lifetime problematic gambling in a US military cohort (N=1553 participants) [11].

Emerging evidence suggests that BAs should be evaluated within a broader framework of psychiatric multimorbidity in veterans, as mental health disorders, SUDs, and social vulnerabilities frequently cluster and may contribute to mutually reinforcing patterns of psychological distress and functional impairment [12]. The mental health costs related to SUDs in veterans are high due to multiple hospitalizations and longer index hospital stays, thus justifying the initiation of specific healthcare policies targeting this population [13].

Other BAs that are discussed in the literature regarding the general population, and which may be considered of interest for the military environment also, are compulsive shopping, food addiction, and physical exercise dependence [14-16]. The spectrum of pathological behaviors and BAs is continuously expanding to include the emerging challenges for mental health in contemporary society, such as chatbot addiction or social media sites addiction, as the research in this field has been extremely active in the last decade [17,18].

Based on the preliminary data presented, it was considered important to assess the main dimensions of the multifaceted phenomenon of BAs in order to delineate directions for implementing new detection and therapeutic strategies for military personnel, both active-duty and retired. The objectives of this article were, consequently, to assess prevalence, risk factors, operational impact, barriers to detection and treatment, prevention needs and treatment options for BAs among military personnel, focusing on excessive gambling or gaming, problematic internet/smartphone use, pornography, compulsive shopping or sexual behaviors, food addiction, and exercise dependence.

**MATERIALS AND METHODS**

This study used a structured evidence synthesis and policy analysis approach to examine BAs among military personnel, veterans, and related population groups. Relevant publications were identified through a structured search of PubMed, Cochrane Database of Systematic Reviews, CINAHL, EMBASE, and Google Scholar, from each database's inception to May 2026. Studies investigating GD, GamingD/problematic gaming, problematic internet use (PUI), compulsive sexual behavior (CSB), problematic pornography use (PPU), compulsive shopping, food addiction, and exercise dependence in active-duty military personnel, reservists, veterans, military trainees, and military healthcare populations were considered eligible.

Studies were included if they (1) examined BAs or problematic engagement in potentially addictive behaviors; (2) included military personnel, veterans, reservists, or military trainees; (3) reported prevalence, risk factors, clinical correlates, functional consequences, treatment outcomes, or prevention implications; (4) were published in English. Observational studies, longitudinal cohort studies, qualitative studies, intervention studies, case series, case reports, and evidence syntheses (reviews and meta-analyses) were eligible for inclusion.

For each study, the following variables were extracted: population characteristics; type of behavioral addiction; study design; prevalence estimates; psychiatric and psychosocial correlates; military-specific vulnerability factors; functional and operational consequences; prevention and treatment implications.

Extracted findings were synthesized using a thematic analysis framework, in order to guide further processing of data into directions for intervention for clinicians and policy-makers. Recurrent findings across addiction categories were grouped into four domains: epidemiology and prevalence; risk and vulnerability factors; operational, occupational, and psychosocial consequences; prevention, screening, and treatment needs. Themes were generated inductively through iterative review of the extracted evidence and subsequently refined through discussion among the authors.

**Policy Analysis**

The synthesized evidence was subsequently examined from a military health-system perspective, and all relevant themes were evaluated for their implications for force readiness, personnel well-being, healthcare utilization, and organizational functioning. Gaps in current screening practices, prevention strategies, referral pathways, and treatment availability were subsequently explored, and solutions were proposed.

**Framework Development**

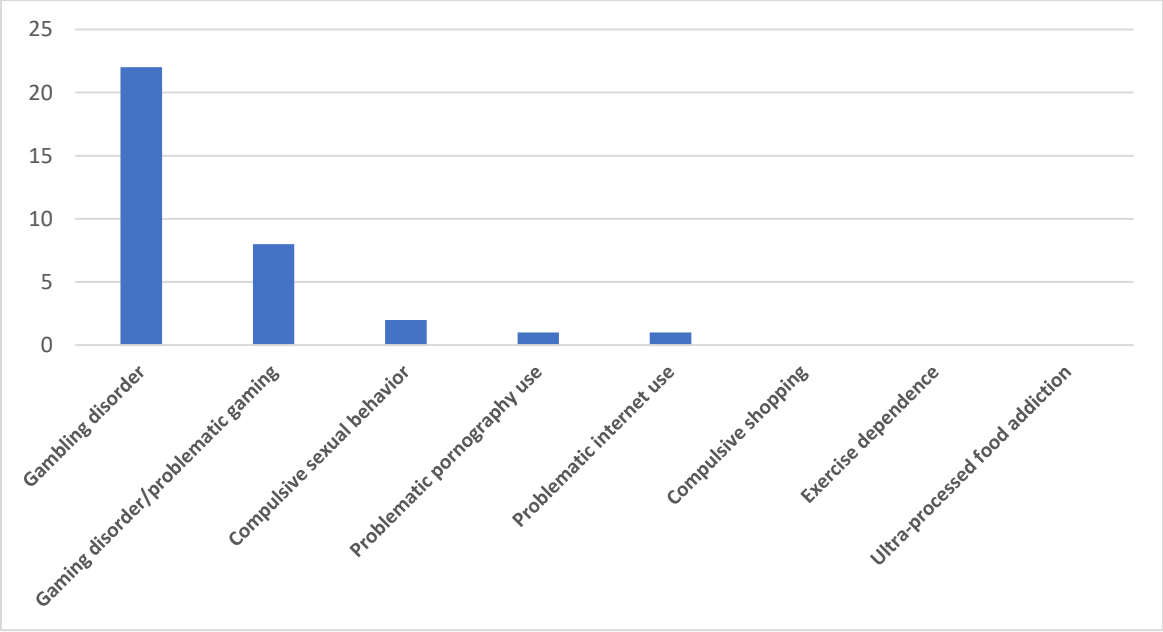
Based on the identified evidence and policy gaps, an evidence-informed framework for the prevention and early detection of behavioral addictions in military settings was developed. The framework was organized into three levels of intervention: primary prevention (education, awareness, and resilience promotion); secondary prevention (screening and early identification); and tertiary prevention (treatment access, rehabilitation, and relapse prevention).

This framework was intended to provide practical recommendations for military healthcare systems and commanders responsible for personnel health and operational readiness.

**RESULTS**

The primary search identified 965 references, which, after applying inclusion/exclusion criteria, were reduced to 34. These were mostly primary research (i.e., studies, case series, and case reports), as well as reviews and one meta-review. The sources were grouped into three main categories, since most of them were dedicated to GD/problematic gambling (n=22, Table 1), and GamingD/problematic gaming (n=8, Table 2), with only a minority approaching different BAs (n=4, Table 3) (Fig.1).

**Figure 1:** Distribution of behavioral addictions studied in military populations according to our detected sources



**1. Gambling disorder and problematic gambling**

According to a review (n=52 articles), veterans appear to be vulnerable to gambling disorder (GD) due to high rates of psychiatric comorbidity, trauma exposure, substance abuse, and psychosocial stress related to military service and reintegration [19]. Clinical context and sampling methods may lead to significant variations in GD prevalence across the explored studies, with a lifetime prevalence reported to be between 2% in broader veteran samples and almost 29% in veterans receiving PTSD treatment [19]. Veterans with GD frequently associate with depression, anxiety, PTSD, alcohol and other substance use disorders, personality disorders, and suicidal behavior [19]. Multiple studies reported that veterans with histories of childhood abuse, neglect, traumatic life events, or PTSD symptoms were significantly more likely to exhibit gambling-related problems [19]. Suicidal crises related directly to gambling-related financial and psychological distress have been reported in this population [19]. Specialized VA inpatient programs for GD showed moderate rates of success, with almost 50% of treated veterans achieving abstinence after discharge, and improvements in depression, financial functioning, and interpersonal relationships [19].

A scoping review (n=11 articles) focused on gambling harm and behaviors in active-duty military personnel showed that higher gambling participation, higher rates of risky gambling, and more gambling-related harm have been reported in this population vs. civilians [20]. Several military-specific vulnerability factors were identified, such as boredom during deployment, long periods of downtime, stress exposure, mobility and isolation, risk-taking culture, masculine peer norms, and easy access to gambling opportunities near military installations [20]. Also, gambling problems were associated with PTSD, depression, anxiety, alcohol or substance misuse, suicidality, and impulsivity [20]. Gambling may be conceptualized as an emotional escape, stress regulation, or a dysfunctional coping method to specific military environmental stimuli [20]. The authors also highlighted that military systems lack routine screening, prevention strategies, treatment protocols, and formal policy guidance [20]. Also, the authors emphasized that gambling behaviors may have an important negative impact on financial stability, discipline, security clearances, psychological functioning, and military readiness; therefore, GD should be considered a military operational challenge [20].

A cross-sectional observational study (N=3511 Australian veterans who retired in the last five years) reported a 4.6% prevalence of problem gambling, and an additional 8.8% presenting at-risk gambling behaviors, which are elevated compared to the general population [21]. Therefore, transitioning from active duty to retirement seems to be a particularly vulnerable period for developing GD and related problems [21]. Also interestingly, only a minority of these patients (2.1%) sought professional help for problem gambling, according to the results of this study [21]. After controlling for multiple variables, the strongest association of gambling problems in this population was with specific PTSD clusters, mainly hyperarousal, emotional dysregulation, irritability, and dysphoric affect [21]. As the authors formulated, the persistent psychological consequences of trauma are more important for predicting gambling problems than trauma exposure in itself [21]. The authors recommended as useful strategies routine screening for gambling problems, integration of gambling assessment in programs screening for PTSD, improving awareness among clinicians, and developing targeted prevention and intervention programs for veterans [21].

A cross-sectional survey exploring gambling experience and potential problems among active duty UK Royal Air Force personnel (N=2119 responses) showed that 12.5% of the participants reported gambling problems, with 8% of them presenting Problem Gambling Severity Index (PGSI) scores corresponding to low-risk gambling, 3% to moderate-risk, and 1.6% to problem gambling [22]. The probability of gambling problems was associated with age (18-24 years old), male gender, and non-commissioned ranks [22].

Problem gambling scores for past and current military service members were more than double when compared to those of the civilians in a survey sample of 2176 US adult residents [23]. Interestingly, this study also highlighted that women had a statistically stronger association between problem gambling scores and military service [23]. The PGSI scores were higher in active military personnel, with greater weekly participation in online gambling, lottery, electronic gambling machines, and sports betting; the same population reported approximately 20 times the rate of suicidal ideation compared to civilians [23]. Tobacco use, suicidal ideation, and substance use problems were also associated with problem gambling among military service members, in regression analyses [23]. The authors argue that limited leisure options and a high tendency for risk taking among military service members, but also the relative ease to conceal online gambling on base, may represent vulnerability features for a higher rate of GD in this population [23].

A telephone survey assessed potential problematic gambling (N=1553 Ohio Army National Guard members) using the National Opinion Research Center Diagnostic Screen- Loss of Control, Lying, and Preoccupation Screen (NODS-CLiP), and reported 13% past-year frequent gambling (≥1/week) and 8% lifetime potential problematic gambling [11]. Male, unmarried, having left the Guard or retired, minor depression, alcohol dependence, legal problems, and increased pain were associated with potential problematic gambling [11]. The authors support the recommendation of routine screening for problem gambling to ensure a high level of mental health in the military and veteran populations (the prevalence of GD being almost twice vs. civilian population in the US) [11].

A qualitative exploratory study included 28 US military veterans and service members, who were interviewed about barriers to accessing healthcare services if they were confronted with GD [24]. Stigma and shame were central barriers, with many veterans reporting embarrassment, guilt, and fear of being judged, while some viewed gambling problems as signs of personal weakness or moral failure, rather than a mental health condition [24]. Veterans associated military culture with self-reliance, emotional control, toughness, and endurance, norms that discourage seeking psychological help and reinforced concealment of gambling problems [24].

Several veterans reported not knowing where to seek help, whether treatment for GD existed, or whether VA services addressed GD specifically [24]. Also, veterans described severe consequences of GD, including debt, bankruptcy, relationship breakdown, emotional isolation, occupational difficulties, and suicidal crises [24]. The authors suggest implementing routine gambling screening in veteran healthcare settings, improving clinician education, reducing stigma associated with gambling disorder, increasing awareness and accessibility of treatment services, and developing interventions specifically tailored to veterans [24].

According to a meta-review that included three systematic reviews, GD and subclinical gambling problems occur at relatively low rates in the general population, and current military screening tools and treatment approaches show only modest effectiveness [25]. While gambling-related issues can significantly affect military readiness and mental health, there is still insufficient evidence to support highly accurate screening or universally effective treatments [25]. The authors recommend improving early detection strategies, standardizing assessment methods, and developing more targeted, evidence-based interventions for military populations [25].

An observational cross-sectional study examined GD and substance use disorders (SUDs) among US veterans (N=4069), and the results showed that veterans with GD had higher rates of alcohol/drug misuse, psychiatric symptoms, and psychosocial difficulties, indicating a strong overlap between these conditions [26]. Co-occurring disorders were associated with greater emotional distress and increased risk behaviors, including higher odds of suicidality and non-suicidal self-injury, suggesting that gambling disorder rarely occurs in isolation within this population [26]. The authors concluded that GD is closely related to SUDs and mental health problems in veterans, highlighting the need for integrated screening and treatment approaches [26].

An observational study that analyzed data from a sample of US military veterans seeking mental health treatment services at a VA hospital (N=260 participants), using the Brief Biosocial Gambling Screen (BBGS) [27]. Results showed that veterans had high rates of problem gambling (32.7%) within the past 12 months and related psychological difficulties, with a 1.9% estimated prevalence of problem gambling among screened veterans using the BBGS scores [27]. The conclusion of the study was that military experience may increase vulnerability to gambling-related harm and that targeted screening and support services for veterans are needed [27].

According to another observational study (N=603 US veterans), suicide attempt history was found to be extremely frequent in veterans receiving treatment for GD, with more than 40% of them reporting such a history [28]. Women veterans were especially likely to present a history of suicide attempts (60%) [28]. Greater gambling severity and impulsivity were associated with a higher frequency of suicide attempts in veterans who related their self-aggressive attempts to the gambling [28]. Therefore, screening for suicidality in veterans with problem gambling is recommended, as is customizing treatment for these veterans by targeting specific risk profiles (e.g., higher impulsivity, comorbid disorders) [28].

Another study analyzed data from 3157 U.S. veterans in the National Health and Resilience in Veterans Study to examine the relationship between gambling behavior, psychiatric disorders, and suicidality [29]. The results of this study showed that 35.1% of veterans gambled recreationally and 2.2% screened positive for at-risk/problem gambling, which was strongly associated with trauma exposure, substance use, anxiety, depression, and mental health treatment utilization [29].

In a retrospective study focused on the evaluation of risk factors for gambling disorder among U.S. military personnel, this disorder was associated with a range of demographic, behavioral, and mental health factors, including substance use disorders, mood disorders, and other psychiatric conditions [30]. Higher odds of diagnosis were observed among men, older service members, racial minority groups, enlisted personnel, and those living closer to gambling venues, while prior substance misuse and mental health conditions emerged as particularly strong predictors [30].

In a cross-sectional study of recently deployed Australian Defense Force personnel to the Middle East Area of Operations, Cowlishaw et al. (2020) found that gambling problems (7.7% prevalence) were associated with younger age (18-24 years old), military characteristics (0-4 years of military service, served in the Army), PTSD symptoms, depression, alcohol misuse, and post-deployment stressors, suggesting that gambling may represent an important comorbid behavioral health issue following deployment [31].

In a cross-sectional study of U.S. military personnel (N=17098 individuals), Beymer et al. (2026) found that gambling problems were associated with poorer mental health, sleep difficulties, and other adverse health behaviors, underscoring the broader health burden associated with gambling-related harm [32]. This type of pathological behavior was associated with a 3.1-fold greater odds of severe psychological distress vs. those screened negative [32].

A retrospective study that included 401 US Armed Forces Veterans found that preference for strategic gambling was associated with younger age, male gender, and lower rates of nicotine use disorder and PTSD [33]. In contrast, veterans with PTSD were more likely to engage in non-strategic gambling, highlighting a possible susceptibility to gambling problems linked to non-strategic gambling activities within this population [33].

A cross-sectional observational study included 1037 UK Armed Forces veterans and 1148 age and gender-matched non-veterans to investigate the associations between gambling problems and motivations, mental health, and PTSD [34]. Veterans were significantly more likely than non-veterans to experience gambling-related problems, according to the past year's analysis [34]. A significantly

stronger association between problem gambling and gambling to cope with stress was observed in veterans, and veterans with PTSD and complex PTSD had a higher risk of problem gambling [34].

Champion et al. (2022) conducted a qualitative study involving 17 serving UK Armed Forces personnel to explore experiences of gambling problems and help-seeking. Four themes emerged: occupational factors influencing gambling behavior; socio-cultural and personal influences; organizational attitudes toward mental health and help-seeking; and existing support pathways [35]. Participants reported that gambling and alcohol use were normalized within military culture and were often used to cope with stress and mental health difficulties [35]. Stigma and concerns about career implications were identified as major barriers to seeking help, highlighting the need for more accessible and military-specific gambling support services [35].

A cross-sectional study including surveys of 31104 US Air Force recruits found that frequent gambling was associated with several health-risk behaviors, including heavy alcohol consumption, cigarette smoking, smokeless tobacco use, risky sexual practices, and aggressive or sensation-seeking behaviors [36]. Approximately one in ten participants reported gambling on a weekly basis, with 6.2% experiencing gambling-related problems and 1.9% indicating impaired control over their gambling [36]. Male participants were more likely than females to engage in frequent gambling and exhibit problematic gambling behaviors [36]. Furthermore, ethnic minority participants showed greater vulnerability to gambling problems and loss of control than their Caucasian counterparts [36].

Kennedy et al. (2005) evaluated the first year of an overseas military gambling treatment program implemented within a substance abuse rehabilitation service in Okinawa, Japan [37]. Among the 35 referred individuals, high rates of depression, suicidality, and substance misuse were observed [37]. The program was successfully integrated into existing services, and preliminary findings suggested benefits for suicide prevention and retention of military personnel experiencing gambling-related problems [37].

In a cross-sectional, exploratory survey study (N=608 UK Armed Forces serving personnel), most of the participants reported past-year gambling, with 23% experiencing harm [38]. Factors predicting harmful gambling were male gender, younger age, and lower educational attainment, but also prior generalized anxiety and PTSD symptoms [38]. Also, strategy-based gambling and online sports betting could be able to predict experiencing harm from gambling [38].

According to the results of a cross-sectional secondary analysis that included 257 UK Armed Forces veterans and 514 matched non-veterans, using data from the 2007 Adult Psychiatric Morbidity Survey, veterans exhibited significantly higher rates of problem gambling than civilians, although gambling problems were not explained by differences in mental health conditions, substance misuse, financial difficulties, or length of military service [39]. Male veterans were more likely to report exposure to traumatic events, but no significant differences were observed in PTSD, alcohol misuse, drug dependence, or financial management difficulties [39].

A cross-sectional survey of 1,037 UK military veterans and 1,148 matched non-veterans was conducted to estimate the social and economic costs of gambling-related harm [40]. Veterans were significantly more likely to experience problem gambling and demonstrated greater healthcare utilization, social service use, debt, welfare dependency, and productivity losses than non-veterans [40]. Within the veteran sample, increasing gambling severity was associated with higher healthcare, social care, and societal costs, highlighting the substantial economic burden of gambling-related harm in this population [40].

A review (n=46 studies) identified 28 screening or assessment tools for measuring gambling behavior, but concluded that none were specifically designed or validated for use in military settings [41]. Therefore, inconsistent reliability, sensitivity, and specificity have been reported when such administration was performed [41]. The authors of this review concluded that existing measurement tools may underestimate or misclassify gambling-related harm, with possible negative consequences of limiting early identification and effective intervention in this specific population [41].

A narrative review (n=14 studies) explored the effectiveness of ACT (Acceptance and Commitment Therapy) in military personnel diagnosed with posttraumatic stress disorder (PTSD) and/or GD [42]. The results are supportive of this intervention in producing improvements in PTSD and/or GD symptom severity, but it is not clear which method of ACT delivery is superior, nor what the real effect size of this therapy is, due to the broad range of study designs [42].

Shirk et al. (2022) examined the feasibility of a nine-session Mindfulness-Based Relapse Prevention (MBRP) program for three U.S. military veterans with gambling disorder. Participants reported reductions in gambling behavior and gambling-related cravings, alongside improvements in self-efficacy, impulsivity, emotion regulation, and overall functioning [43]. The results suggest that MBRP is a feasible and potentially beneficial intervention for veterans with gambling disorder; however, larger controlled studies are needed to establish its effectiveness [43].

**Table 1:** Gambling problems and GD in military personnel

| Authors | Type of research | Population characteristics | Behavioral pathology | Results | Comments |
|---|---|---|---|---|---|
| Levy & Tracy, 2018 [19] | NR, 52 articles | Military veterans | GD | Lifetime prevalence 2-29% for GD, >33% for recreational gambling | Childhood abuse, neglect, traumatic life events, and PTSD symptoms were associated with higher rates of GD |
| Paterson et al., 2020 [20] | Scoping SR, 11 sources | Active duty military personnel | GD and related behaviors | Higher gambling participation, higher rates of risky gambling, and more gambling-related harm vs. civil ians | Gambling harm in military personnel is underresearched, especially outside North America |
| Metcalf et al., 2022 [21] | Cross-sectional self-report survey, 3511 participants | Retired-in-the-last-5-years Australian Defense Force members | GD prevalence | 4.6% of participants met criteria for problem gambling, and an additional 8.8% showed at-risk gambling behaviors. Only 2.1 of veterans with gambling problems sought professional help for this issue. | The rates are elevated compared to the general population. The post-service transition period may represent a particularly vulnerable stage for gambling-related harm |
| Pritchard & Dymond, 2022 [22] | Cross-sectional survey, 2119 respondents | UK RAF active duty personnel | GD | 12.5% reported gambling problems, 8% presented PGSI scores of low-risk gambling, 3% moderate-risk, and 1.6% problem gambling | Gambling problems and associated harms stand as significant concerns for serving military personnel |
| van der Maas & Nower, 2021 [23] | Observational cross-sectional survey, 2176 participants | US past and current military personnel, and civilians (as a control group) | GD | The PGSI scores were higher in active military personnel. ~20 times the rate of suicidal ideation compared to civilians. | Restricted leisure options, a high tendency for risk-taking among military service members, and the relative ease of concealing online gambling on base vulnerabilise this population to the onset of GD |
| Gallaway et al., 2019 [11] | Cross-sectional survey study, 1553 participants | Ohio Army National Guard members | GD | 13% past-year frequent gambling (≥1/week) and 8% lifetime potential problematic gambling. Male, unmarried, having left the Guard or retired, minor depression, alcohol dependence, legal problems, and increased pain were associated with potential problematic gambling. | Routine screening for problem gambling is granted to ensure a high level of mental health in the military and veteran populations |
| Vana et al., 2025 [24] | Qualitative exploratory study, 28 participants | US military veterans and service members | GD | Multiple barriers to seeking treatment for GD, including stigma, shame, fear of appearing weak, and limited awareness of available services | GD should be integrated more fully into veteran mental health services, particularly because it frequently co-occurs with trauma-related conditions such as PTSD |
| Segura et al., 2024 [25] | Meta-review, 3 SR included | Service members | Gambling problems | 31 tools were identified for screening of gambling problems in service members, but with modest sensitivity and specificity. Estimated errors of positive results in 64-69% of the cases. In service members referred for alcohol problems, a positive result with the best screening tools would be correct in 76% of the time. | Screening for GD is infeasible in the general service members, due to the low quality of psychological tools, but enriched samples of service members with alcohol problems may benefit from such a screening |
| Stefanovics et al., 2024 [26] | Observational cross-sectional study, 4069 participants | US military veterans | SUDs, GD | Veterans with GD showed higher rates of alcohol/drug misuse, psychiatric symptoms, and psychosocial difficulties, indicating a strong overlap between these conditions. | GD is closely linked to substance use and mental health problems in veterans, highlighting the need for integrated screening and treatment approaches |
| Kraus et al., 2020 [27] | Cross-sectional comparative study, 260 | US military veterans | GD | 32.7% presented gambling behaviors in the past 12 months. The estimated prevalence of problem gambling was 1.9% among | Outreach efforts of VA health care providers may increase these individuals' participation in treatment services for problem |

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| | participants | | | veterans screened in a primary care behavioral health clinic. | gambling |
| Valle Frias et al., 2026 [28] | Observational cross-sectional study, 603 participants | US veterans | GD | >40% of veterans receiving treatment for GD reported a history of suicide attempts. Prior attempts were more likely to have psychiatric diagnoses and higher impulsivity. Veterans whose suicide attempts were linked to gambling also demonstrated greater gambling severity and impulsive behavior. | Veterans with GD are especially vulnerable to suicidality and related risk factors. |
| Stefanovics et al., 2017 [29] | Cross-sectional study, 3157 participants | US veterans from the National Health and Resilience in Veterans Study | GD | 35.1% engaged in recreational gambling; 2.2% screened positive for at-risk/problem gambling. This last category also presented a history of physical or sexual trauma, SUD, anxiety, depressive disorders, having sought mental health treatment, and minority group status | Both recreational and at-risk/problem gambling were associated with elevated trauma burden and psychiatric comorbidities |
| Garvey Willson AL, et al., 2021 [30] | Retrospective matched case–control epidemiological study, 901 cases vs. matched controls (N=43,564) | US service members | GD and problem gambling | 10-year prevalence was 6.6/100000, with men 3.5 times more likely than women to receive a GD diagnosis. Age >24, Asian or Black race, formerly married, and enlisted rank are risk factors. Prior substance misuse or mental health conditions were 3.9 times and 6.3 times more likely to receive a GD diagnosis. | Proximity to gambling venues and slot machines on bases, and a history of SUD or mental disorders are risk factors for GD in the US military. |
| Cowlishaw et al., 2020 [31] | Cross-sectional observational study, 1324 participants | Australian Defense Force personnel, recently returned from deployment | Gambling problems | 7.7% reported at least some gambling-related difficulties, 2% presenting criteria for GD. Younger personnel, those with shorter military service, Army members, and non-commissioned ranks had more gambling problems than the others. PTSD symptoms, depression, alcohol misuse, psychological distress, and post-deployment adjustment problems were associated with gambling difficulties. | Gambling is a significant but often overlooked behavioral health concern in military populations |
| Beymer et al., 2026 [32] | Secondary analysis of data from a cross-sectional observational study, 17098 individuals | Active-duty US military service members | Gambling problems | 1.6% of the weighted sample reported gambling problems. These had 3.1-fold greater odds of severe psychological distress vs. those screened negative. Insufficient sleep, tobacco use, and binge drinking were associated with GD. | Screening for gambling problems is important in this population. |
| Grubbs et al., 2023 [33] | Retrospective chart review, 401 participants | US Armed Forces Veterans | GD in residential treatment programs | Compared with non-strategic gamblers, strategic gamblers were generally younger, more often male, and less likely to have nicotine use disorder or PTSD. | PTSD may be associated with a preference for non-strategic gambling and could represent a specific risk factor for gambling-related harm among armed forces veterans |
| Dighton et al., 2021 [34] | Cross-sectional comparative survey study, 2185 participants | $N_1$=1037 veterans and $N_2$=1148 matched non-veterans | Gambling problems | UK veterans were significantly more likely than non-veterans to experience gambling-related problems. Gambling as a coping mechanism and gambling problems were better represented among veterans than among non-veterans. | Routine screening for gambling problems can be very useful with current and former military personnel. |

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| | | | | Veterans who gambled to escape or manage distress were especially likely to report problematic gambling behaviors. PTSD and complex PTSD were also associated with an increased risk of problem gambling. | |
| Champion et al., 2022 [35] | Qualitative study, 17 participants | UK Royal Air Force military personnel | Gambling problems | Thematic analysis of semi-structured interviews identified four domains shaping participants' experiences of gambling, mental health, and support-seeking. | Occupational factors influencing gambling behavior, socio-cultural and personal influences, organizational attitudes toward mental health and help-seeking, and existing support pathways were the most important themes related to pathological gambling reported by UK military personnel. These individuals are using gambling and alcohol use as a coping mechanism for mental health challenges. |
| Steenbergh et al., 2008 [36] | Cross-sectional observational study, 31104 participants | US Air Force recruits | Gambling behaviors | 10.4% of the participants gambled at least weekly, 6.2% reported gambling problems, and 1.9% admitted loss of control over gambling. Men and minorities were more affected by gambling behaviors than women. Health-risk behaviors were significantly associated with frequent gambling. | Frequent gambling may be associated with health-risk behaviors, such as nicotine use, heavy alcohol use, risky sexual behaviors, or sensation-seeking behaviors. |
| Kennedy et al., 2005 [37] | Retrospective study, 35 participants | Military and other eligible beneficiaries diagnosed with GD, included in the US Naval Hospital in Okinawa, Japan | GD | High rates of depression, suicidality, and substance misuse were observed in these patients. Preliminary findings suggested benefits for suicide prevention and retention of military personnel experiencing gambling-related problems. | This program, dedicated to GD treatment, seems to be effective in decreasing suicide risk in both military members and eligible beneficiaries with GD. |
| Jones et al., 2024 [38] | Cross-sectional, exploratory survey, 608 participants | UK Armed Forces personnel | Gambling behaviors | Most respondents had gambled during the past year, with nearly one-quarter reporting gambling-related harm. Male gender, younger age, lower educational attainment, generalized anxiety, and PTSD symptoms were associated with a greater risk of harmful gambling. Engagement in strategy-based gambling and online sports betting was likewise linked to increased gambling-related harm. | The risk of harm from gambling was associated with multiple demographic, mental health and gambling engagement variables. |
| Roberts et al., 2019 [39] | Cross-sectional observational study, 771 participants | 257 UK veterans and 514 matched civilian controls | Problem gambling | Veterans had significantly higher rates than non-veteran controls (1.41% vs. 0.17%). Male veterans were significantly more likely than non-veterans to report having experienced a traumatic event during their lifetime. No significant differences in gambling problems between early leavers and veterans who served ≥4 years. | Veteran population is more vulnerable than general population to problem gambling, and males are especially at risk. |
| Harris et al., 2023 [40] | Cross-sectional study, survey-based, 2185 participants | UK Armed Forces veterans and age- and gender-matched non-veterans | Gambling problems | Veterans had higher healthcare, social services, and societal costs, as well as lower utility vs. non-veterans. Veterans had more contacts with the criminal justice | Veterans experiencing problem gambling are more costly when healthcare and social costs are evaluated. |

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| | | | | system and received more social service benefits; they also had more lost work hours and greater accrued debt. | |
| Rayner et al., 2025 [41] | Systematic review, 46 studies | 28 screening and assessment tools for GD | GD | No specifically designed or validated tools to detect or quantify GD were identified for use in military settings | Existing measurement tools may underestimate or misclassify gambling-related harm |
| Hitch et al., 2023 [42] | Narrative review, 14 studies | US military personnel | GD + PTSD | ACT improved PTSD and/or GD | It remains unclear which method of ACT delivery is most effective and what the true effect size of ACT is for PTSD and/or GD. |
| Shirk et al., 2022 [43] | Feasibility case series, 3 participants | US military veterans, aged 46 to 57 years | GD in an outpatient treatment regimen | After a 9-session adapted MBRP protocol, patients reported less frequent engagement in their gambling behavior, fewer cravings, and less intense cravings. Also, increased self-efficacy in managing gambling impulses, less impulsivity and emotion dysregulation, and improved functioning were reported. | MBRP may be a solution for improving gambling problems in veterans, but larger studies are needed. |

ACT= Acceptance and Commitment Therapy, GD= gambling disorder, MBRP= Mindfulness-Based Relapse Prevention, NODS-CLiP= National Opinion Research Center Diagnostic Screen- Loss of Control, Lying, and Preoccupation Screen, NR = narrative review, PGSI= Problem Gambling Severity Index, PTSD= posttraumatic stress disorder, RAF= Royal Air Force, SR= systematic review, SUD= substance use disorder

Overall, the available evidence (Table 1) indicates that GD and gambling-related harm represent the most extensively investigated BAs in military populations and constitute a significant challenge for both individual well-being and operational readiness. Across studies, gambling problems were consistently associated with PTSD, depression, substance misuse, suicidality, financial difficulties, and occupational impairment, while military-specific factors such as deployment stress, boredom, risk-taking culture, and barriers to help-seeking appear to increase vulnerability. The convergence of these findings suggests that gambling-related problems should be considered a relevant behavioral health concern within military systems, warranting routine screening, early intervention, and integrated treatment approaches that address co-occurring psychiatric conditions

**2. Gaming disorder and problematic gaming**

A study exploring the risk of developing problematic gaming behavior during military service assessed the prevalence rates and associated risk factors for this disorder in a sample of Norwegian conscripts at the beginning and end of duty (N=2555 individuals, aged 18-24 years) [44]. The prevalence of GamingD was 0.5% at the beginning and 4.6% at the endpoint, while problem gaming use was reported by 4.8% and 8.1%, respectively [44]. The increase was significant during the military service, at $p<0.05$, on the Gaming Addiction Scale (GAS) [44]. An increase in gaming behaviors in this population may complicate soldiers' reintegration into civilian life after service [44].

A survey-based study recruited US military and veteran gamers (N=87) and examined their use of digital games and avatars to cope with service-related challenges [45]. A significant proportion of the participants (almost half) engaged in self-directed coping through digital games, with these methods focused on escapism/diversion, management of organic or mental disorders, receiving social support, and connection with civilian life [45]. Also, military-related avatars may serve as models of institutional identity in coping with stress associated with identity negotiation [45]. Social benefits were highlighted by several participants (N=33) – "I can meet up with former military people who understand what true tactics are", as were immersion ("..realistic, can apply military strategy"), personal history (N=28, "I still have good memories associated with people and accomplishments with the game"), and escapism ("the openness and random possibilities, the detail and nuance, and of course the escapism") [45].

A cross-sectional study examined problematic gaming behavior among military veterans/peacekeepers (Norwegian, post-deployment, N=246) and explored its relationship with psychological and social factors [46]. Using self-report questionnaires, the researchers found that problematic gaming was associated with higher levels of loneliness, boredom, and depressive symptoms, as well as specific gaming motivations [46]. The findings suggest that problematic gaming may be linked to psychosocial difficulties and that these factors could help identify individuals at greater risk [46].

A study found that gaming addiction was relatively uncommon among Norwegian military conscripts (N=1017 participants), but was significantly associated with poorer psychosocial well-being [47]. Higher levels of depression, loneliness, boredom proneness, and anxiety were linked to greater gaming addiction scores, and, among these factors, depression and loneliness emerged as important predictors of problematic gaming behavior, suggesting that lower psychosocial well-being may increase vulnerability to gaming addiction [47].

Young adult veterans who screened positive for depression or PTSD spent significantly more time playing video games than those without these conditions, according to two US cross-sectional studies (N=1023 and 784 participants, respectively) [48]. Additionally, veterans with SUDs who had received treatment played video games more frequently than those who had not received services, highlighting the potential of video game–based interventions to support behavioral health care access and delivery [48].

Another cross-sectional study examined the relationship between military first-person shooter video game use and PTSD symptoms among current and former military personnel (N=111 participants) [49]. Although players of shooter games reported higher PTSD symptom levels than nonplayers, video game use was not independently associated with PTSD after accounting for personality, combat exposure, and social support, suggesting that shared psychosocial factors may explain both PTSD symptoms and gaming behavior [49].

**Table 2:** Gaming addiction and problem gaming in military personnel

| Authors | Type of research | Population characteristics | Behavioral pathology | Results | Comments |
|---|---|---|---|---|---|
| Olsen et al., 2021 [44] | Prospective longitudinal observational study, 2555 individuals | Norwegian conscripts | GamingD, problem gaming use | The prevalence of GamingD was 0.5% at the beginning and 4.6% at the endpoint. Problem gaming use was reported by 4.8% and 8.1%, respectively. The increase was significant as measured on GAS. | The increase in gaming behaviors may complicate soldiers' reintegration into civilian life |
| Banks & Cole, 2016 [45] | Exploratory mixed qualitative study using interviews and survey-based methods, 87 respondents | US military and veteran gamers | GamingD | Almost half of the participants engaged in digital games to cope with challenges associated with military service. Gamers tended to have served longer, and reported high escape, explicit coping, fantasy, and skill-development motivations for gameplay | Different coping styles within specific video games were linked to broader gaming motivations, while engagement with avatars appeared to serve as a coping strategy for negotiating and reconstructing military identity. |
| Myrseth et al., 2016 [46] | Cross-sectional observational study, 246 participants | Norwegian, post-deployment Afghanistan veterans, mean age 37.5 years | Gaming problems | Coping with negative emotions, such as boredom and loneliness, may be related to gaming engagement. 8.8% of the veterans showed symptoms of problematic gaming. | The rate of problem gaming is not higher than in the general Norwegian population. Boredom proneness and enhancement motivation were predictors of gaming problems. |
| Myrseth et al., 2017 [47] | Cross-sectional survey study, 1017 participants | Norwegian conscripts, mean age 19.5 years | GamingD | 4.8% of the conscripts presented GamingD. Higher scores of boredom, loneliness, depression, and anxiety were recorded in individuals with GamingD vs. nonproblem gamers and nongamers. Low psychological well-being and lack of external stimulation, but mostly the weekly time spent gaming, explained the variance in GamingD. | The authors suggest that revising the present selection criteria to include GamingD as an exclusion criterion for drafting may be warranted. |
| Grant et al., 2018 [48] | Cross-sectional, community-based surveys (n=2, N=1023 and 784 participants, respectively) | Young adult veterans | GamingD | Individuals screened positive for depression or PTSD spent 4.74 more hours per week playing video games. Those screened positive for SUD who had received substance use services since discharge spent 0.75 more days per week playing video games. | Evaluation of video games as modalities to enhance access to behavioral health services is warranted in this population. |
| Grant et al., | Cross- | Current and | MFPS gaming, | 41.4% reported playing MFPS or | Exploring the use of MFPS gaming |

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|---|---|---|---|---|---|
| 2017 [49] | sectional observational study, 111 participants | former US members of the military | PTSD | other shooter games. The shooter playing group reported high levels of PTSD symptoms vs. nonshooters/nonplayer groups. Playing shooter games did not predict PTSD symptoms after adjusting for multiple variables. | in veterans can be useful for detecting factors that may predict both PTSD and playing shooter video games. |
| Eickhoff et al., 2015 [50] | Case series, 3 patients | Active duty service members in the US Marine Corps | Video game use | 30-60 hours/week dedicated to video games; they sacrificed sleep to maintain their gaming schedules; blunted affect and depressed mood, but became enthusiastic and joyful when discussing video games. | Heavy video game use may lead to sleep loss, which is linked to worse work performance and increased mood problems. |
| Hall et al., 2023 [51] | Case report | Active duty member | | Self-injurious behavior to avoid work-related consequences, after participating in a video-gaming binge (a full 72-hour weekend). Severe sleep deprivation, followed by oversleeping and failure to report for duty. | The military leadership should be aware that excessive gaming can degrade force readiness. |
| Colder Carras et al., 2018 [52] | Qualitative study based on semi-structured interviews, 20 participants | US military veterans | Video game behaviors | Video games helped participants manage moods and stress and supported recovery in 3 areas: adaptive coping, eudaimonic well-being, and socializing. Although excessive gaming was linked to life-related problems and self-reported addictive experiences, several veterans with disabilities reported that the perceived benefits of extensive gameplay exceeded its associated drawbacks. | Video games may provide relief for veterans, but this phenomenon may lead to addiction. |

GamingD= gaming addiction, GAS= Gaming Addiction Scale, MFPS= military first-person shooter, PTSD= posttraumatic stress disorder, SUD= substance

use disorder

Excessive video game use (30-60 hours/week) was associated with less sleep, poorer job performance, and more mood-related problems among the US Marine Cops active duty service members (case series, N=3) [50]. The researchers suggested that heavy gaming may contribute to sleep deprivation, which can negatively affect daily functioning and mental well-being [50].

A case report describes an active-duty military service member whose 72-hour video gaming binge resulted in severe sleep deprivation, missed work obligations, and subsequent self-injurious behavior intended to avoid disciplinary consequences [51]. The authors highlight how excessive gaming can lead to significant functional impairment and psychiatric risk, emphasizing the importance of recognizing problematic gaming behaviors in military populations [51].

A qualitative interview study found that many veterans (N=20 participants) used video games as a coping strategy to manage mental health symptoms, support recovery, and maintain social connections [52]. While excessive gaming was sometimes associated with life difficulties and feelings of addiction, some veterans -particularly those with disabilities-reported that the perceived benefits of intensive gaming outweighed its negative consequences [52].

**Table 3:** Other types of behavioral addictions in military population

| Authors | Type of research | Population characteristics | Behavioral pathology | Results | Comments |
|---|---|---|---|---|---|
| Smith et al., 2014 [53] | Observational longitudinal study, survey- | Male military, Operation Iraqi Freedom, | CSB | 16.7% of the surveyed participants presented CSB. PTSD severity, childhood sexual trauma, and age | CSB is prevalent amongst veterans returning from combat and is |

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| | based, 258 participants | Enduring Freedom, or New Dawn veterans | | were significantly associated with CSB. Among PTSD symptom clusters, re-experiencing was most strongly linked to CSB. | associated with childhood trauma and PTSD. |
| Blais, 2021 [54] | Cross-sectional study, 499 participants | Partnered men service members/veterans | CSB | Relationship satisfaction scores were in the distress range. 12.8% reported military sexual trauma exposure, and these events were related to lower relationship satisfaction, through higher CSB. | Findings were similar to those of women service members/veterans, in the sense that the association of military sexual trauma and romantic relationships appears to be indirect, through the effects of sexual function. |
| Stefanovics et al., 2025 [55] | Cross-sectional observational study, 292 veterans | US military veterans | PPU | 25.5% reported PPU symptomatology, defined by BPS score≥1, while 11.8% met criteria for PPU (BPS score ≥4). Individuals who were younger, male, of Hispanic ethnicity, and had VA service-connected disability scored higher on BPS. PPU was associated with lifetime and current psychiatric disorders, self-directed violence, history of homelessness, loneliness, higher impulsivity, lower resilience, and less successful aging. | The association between PPU and multiple negative health indicators underscores the need for enhanced assessment and intervention efforts. |
| Schmidt et al., 2019 [56] | Cross-sectional survey study, 399 participants | Military medical and nursing students | IA/PUI | 5.5% of the participants presented problems with Internet use. Several lifestyle factors were significantly associated with IA scores and may indicate an increased likelihood of higher IA levels. The use of social media during sleeping hours was most significantly associated with increased IAS. | Relatively low levels of problematic Internet use among military medical students. Several lifestyle and behavioral factors were associated with higher IA scores and may serve as indicators of increased risk for IA. |

BA= behavioral addiction, BPS= Brief Pornography Scale, CSB= compulsive sexual behavior, IA= Internet addiction, IAS= Internet Addiction Score, PPU= problematic pornography use, PUI= problematic Internet use

The findings regarding GamingD and problematic gaming (Table 2) portray a more nuanced picture than that observed for GD. While gaming frequently serves adaptive functions, including stress management, social connectedness, identity reconstruction, and psychological coping, excessive engagement may also contribute to sleep deprivation, mood disturbances, impaired occupational functioning, and reduced readiness. The available evidence suggests that problematic gaming exists on a continuum between recreational use and clinically significant dysfunction, with loneliness, boredom, depression, and trauma-related symptoms emerging as important correlates. Consequently, military prevention strategies should aim not only to identify problematic gaming behaviors but also to recognize the potential psychological needs that gaming may be fulfilling for service members and veterans.

**3. Other behavioral addictions**

A prospective longitudinal cohort study including veterans of Operation Iraqi Freedom, Enduring Freedom, or New Dawn (N=258 participants) found that 16.7% of male combat veterans reported CSB [53]. This pathology was significantly associated with PTSD severity, childhood sexual trauma, and younger age, with PTSD re-experiencing symptoms showing the strongest link [53]. These findings highlight the importance of addressing trauma-related factors when assessing and treating CSB in veterans [53].

A cross-sectional study examined whether sexual functioning explains the relationship between military sexual trauma (MST) and romantic relationship satisfaction among partnered male service members and veterans (N = 499) [54]. The results showed that about 13% of participants reported MST exposure, and overall relationship satisfaction was in the distressed range [54]. Results showed that MST was associated with lower relationship satisfaction indirectly through higher levels of compulsive sexual behavior, whereas erectile dysfunction did not significantly mediate this relationship [54]. These findings suggest that the negative impact of MST on intimate relationships may operate through sexual health difficulties, particularly compulsive sexual behaviors, highlighting the importance of addressing these issues in treatment for male MST survivors [54].

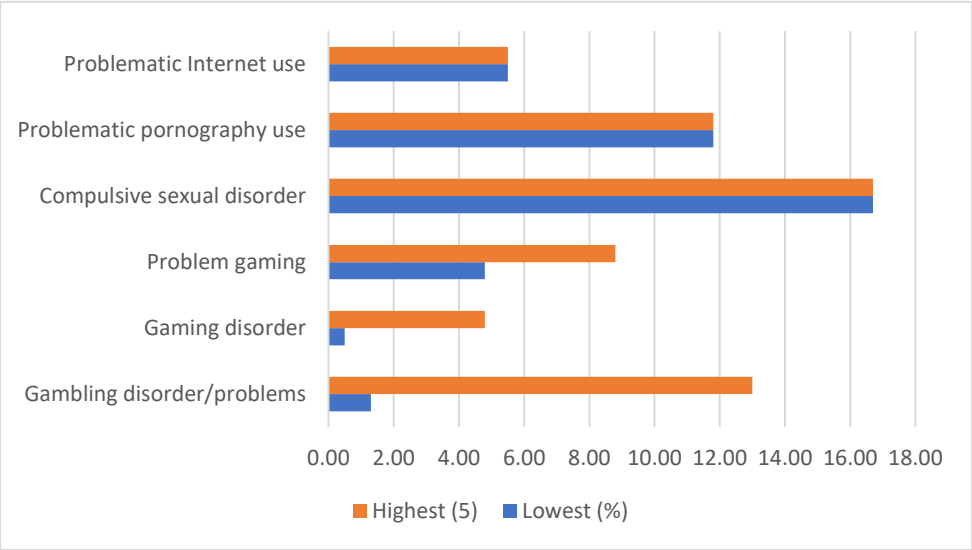
In a cross-sectional study of low-income U.S. military veterans (N=292 participants), PPU was relatively common, with 25.5% reporting PPU symptoms and 11.8% meeting criteria for PPU [55]. PPU was associated with a range of adverse psychosocial and mental health

factors, including psychiatric disorders, self-directed violence, homelessness, loneliness, greater impulsivity, lower resilience, and poorer aging outcomes, highlighting the need for targeted screening and intervention efforts [55].

A study investigated problematic Internet use among military medical and nursing students and residents using the Internet Addiction Test (IAT), and reported that out of the 331 participants included in the analysis, 5.5% showed Internet use patterns suggestive of Internet addiction [56]. Overall, the prevalence of problematic Internet use was lower than global estimates and was lower among residents than among medical students [56]. Several lifestyle factors were associated with higher Internet addiction scores, with social media use during sleeping hours showing the strongest association [56]. The findings suggest that certain lifestyle behaviors may help identify individuals at risk for problematic Internet use, which could negatively affect work performance and military readiness.

Although the evidence base remains limited, the available studies suggest that CSB, PPU, and PUI use are clinically relevant phenomena within military populations (Table 3). These conditions appear to share several vulnerability factors with gambling and gaming disorders, including trauma exposure, psychiatric comorbidity, impulsivity, loneliness, and psychosocial distress, supporting a transdiagnostic conceptualization of behavioral addictions. The scarcity of research in these areas contrasts with the potentially substantial impact of these behaviors on mental health, interpersonal relationships, occupational functioning, and quality of life, highlighting the need for further epidemiological, longitudinal, and intervention-focused studies in military settings.

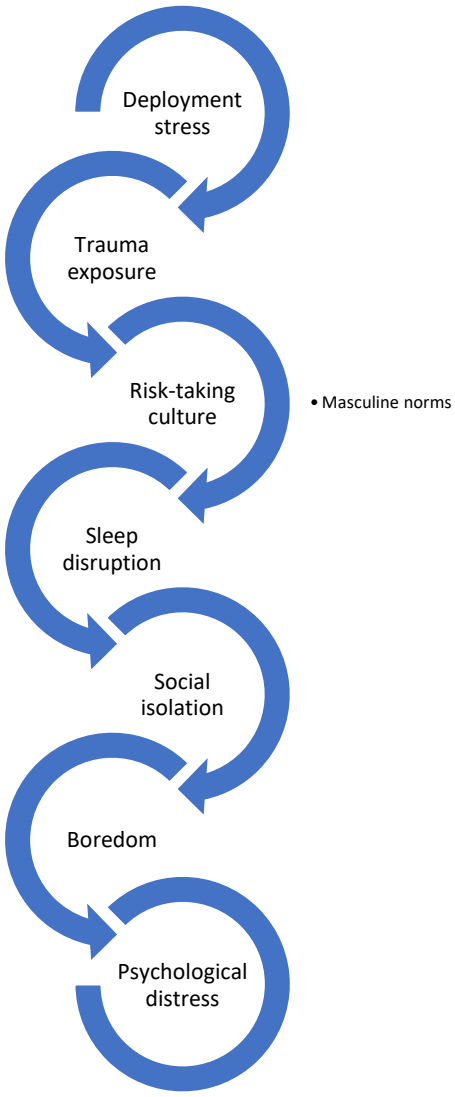
**Figure 2:** Reported prevalence ranges of behavioral addictions in military personnel and veterans




**DISCUSSION**

The current. article highlights the fact that BAs constitute an under-recognized yet potentially significant threat to the health, well-being, and operational readiness of military personnel and veterans. Although GD and GamingD have received the greatest research attention (Fig.1), emerging evidence suggests that PPU, CSB, and PUI may also contribute to substantial psychiatric, psychosocial, and occupational burden. Across addiction categories, military populations appear to be exposed to a constellation of vulnerability factors that differ from those observed in civilian populations, including deployment-related stress, trauma exposure, prolonged separation from family, social isolation, boredom, irregular work schedules, sleep disruption, and cultural norms emphasizing self-reliance and emotional control.

**Figure 3:** Military-specific risk factors for behavioral addictions

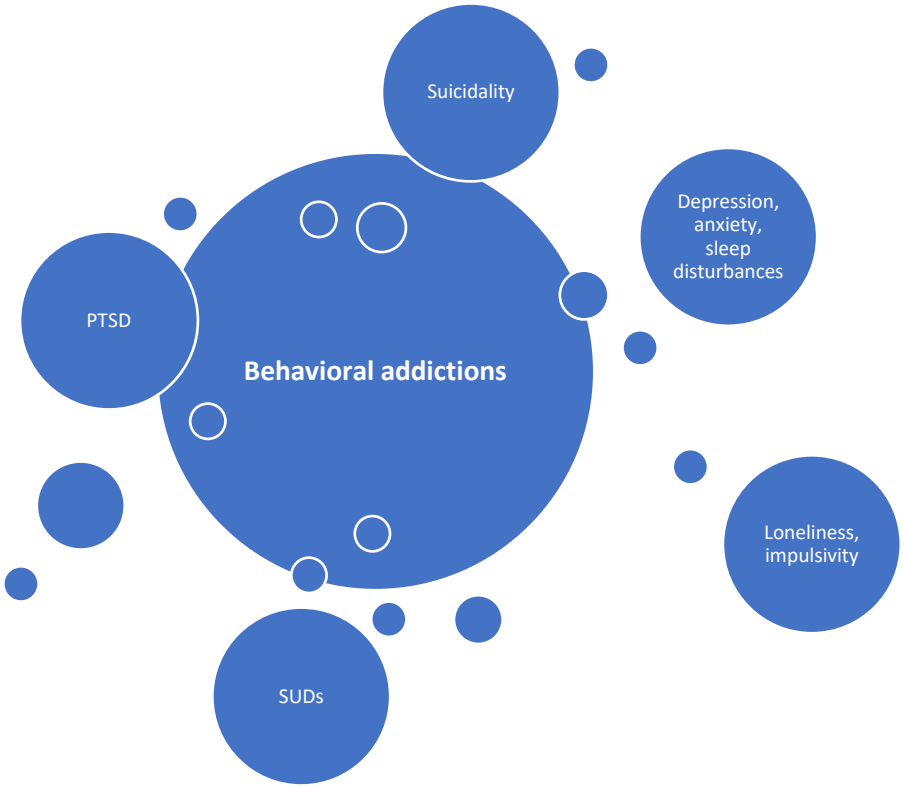



The most consistent evidence identified in this review concerns gambling-related problems in military personnel, active-duty and retired. Compared with civilians, military personnel and veterans appear to experience higher rates of gambling participation, risky gambling behaviors, and gambling-related harm (Fig.2). Reported prevalence estimates vary substantially according to population characteristics, assessment methods, and diagnostic criteria, but several studies indicate that gambling-related problems may occur at rates that exceed those observed in the general population. Furthermore, GD was repeatedly associated with PTSD, depression, anxiety disorders, substance misuse, impulsivity, suicidality, and financial difficulties. These findings support the conceptualization of GD not merely as an individual behavioral problem but as a condition with potentially important implications for military readiness, personnel retention, security clearances, and organizational functioning.

Gaming-related problems represent a second area of concern. Although gaming disorder appears less prevalent than gambling disorder, longitudinal evidence suggests that problematic gaming behaviors may increase during military service. Gaming may serve adaptive functions by facilitating social connection, stress management, identity reconstruction, and coping with deployment-related experiences. However, excessive gaming has also been associated with sleep deprivation, impaired occupational performance, mood disturbances, and, in extreme cases, severe functional impairment. These findings suggest that gaming behaviors exist on a continuum ranging from adaptive coping to clinically significant dysfunction, emphasizing the importance of distinguishing recreational engagement from pathological use.

Based on the specific risk factors identified in the literature (Fig.3), policies targeting early interventions and therapeutic strategies may be conceptualized. The call for action theme of this article refers exactly to these unmet needs, of (1) improving the awareness within the military personnel, veterans, but also military physicians and trainers, about the existence of BAs, their first signs, evolution, and potential complications; (2) implementing strategies to mitigate deployment stress, trauma exposure, boredom and social isolation; (3) military psychiatrists and psychologists must be aware of the potential transformation of psychological distress into BAs, as maladaptive coping strategies; (4) regular mental health check-ups in vulnerable populations.

**Figure 4:** Psychiatric comorbidity network



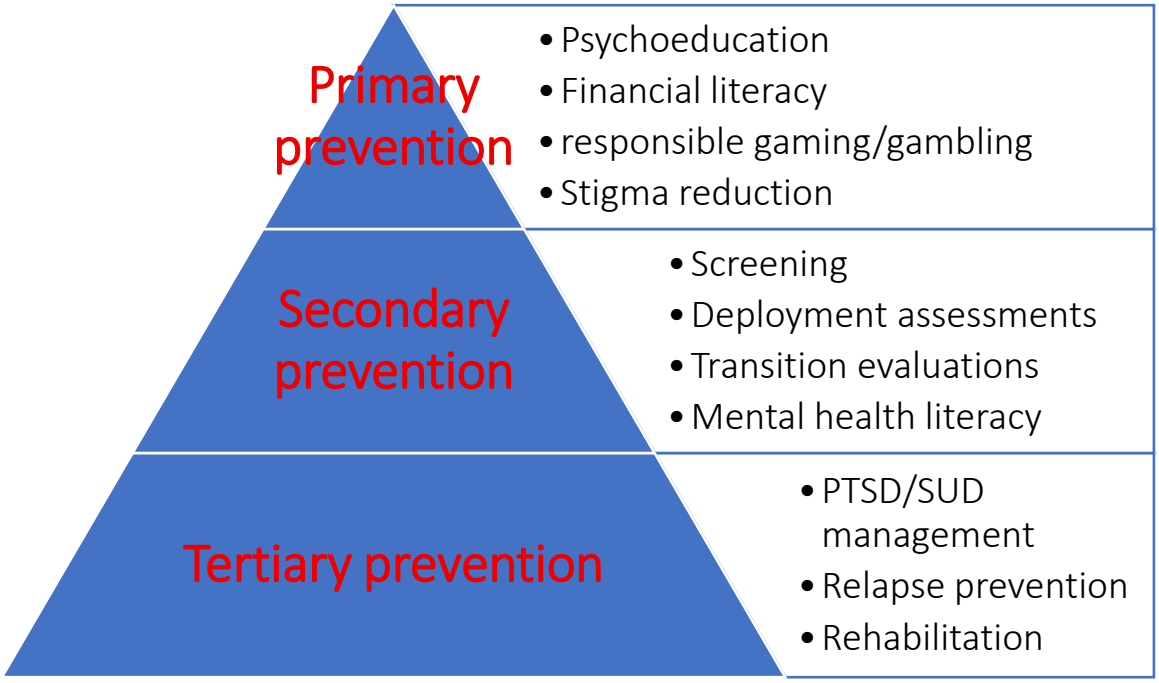

SUD= substance use disorder, PTSD= posttraumatic stress disorder

The available evidence also indicates that behavioral addictions rarely occur in isolation (Fig.4). This is an observation frequently reported in the general population, especially between SUDs and BAs, further complicating the detection and management of behavioral addictions [57,58]. Across studies, PTSD, depression, anxiety disorders, substance use disorders, loneliness, boredom, impulsivity, and trauma exposure emerged as recurrent correlates of gambling disorder, gaming disorder, compulsive sexual behavior, and problematic pornography use. These observations support a transdiagnostic perspective in which behavioral addictions represent manifestations of broader psychological distress and maladaptive coping processes. The clustering of behavioral addictions with psychiatric comorbidity suggests that screening and treatment efforts should be integrated into existing military mental health services rather than implemented as isolated interventions.

Another important finding concerns barriers to treatment engagement. This means that significant delays in addressing health services may be due to veterans and service members frequently reporting stigma, shame, fear of appearing weak, concerns regarding career consequences, and limited awareness of available therapeutic options. These barriers are consistent with previous research showing that many military personnel experiencing significant psychological distress fail to recognize their need for treatment or avoid seeking help because of attitudinal and organizational factors [7,9]. Consequently, BAs may remain undetected until substantial psychosocial, occupational, or financial consequences have already developed. Although specific treatments for BAs are mostly psychosocial, several therapeutic strategies may be extrapolated from SUDs; also, due to the high rate of SUD-BA comorbidity and SUD complications that may be met in this dual-diagnosed population, a detailed knowledge of the available and pipeline therapies is required [59-64].

From a policy perspective, the current evidence reveals several important gaps. First, no screening instruments have been specifically developed and validated for the detection of behavioral addictions in military populations. Second, routine screening practices remain uncommon despite the documented associations between behavioral addictions and adverse mental health outcomes. Third, evidence-based treatment protocols specifically tailored to military personnel and veterans remain limited. Finally, behavioral addictions are frequently absent from military prevention policies despite their potential impact on readiness and force protection.

**Figure 5:** Military prevention framework




PTSD= posttraumatic stress disorder, SUD= substance use disorder

Based on the available evidence, a military-specific prevention framework may be conceptualized across three levels. Primary prevention should focus on psychoeducation, resilience promotion, digital literacy, responsible gambling education, financial counseling, and stigma reduction. Secondary prevention should include routine screening in primary care, mental health services, deployment-related assessments, and transition programs for personnel leaving active duty. Tertiary prevention should emphasize confidential referral pathways, integrated treatment approaches addressing co-occurring psychiatric disorders, relapse prevention interventions, and rehabilitation programs designed to facilitate recovery while preserving occupational functioning whenever possible. Such a model is consistent with similar policies discussed in the literature for the general population, targeting the reduction of risk factors for BAs, promoting early detection, and increasing the availability of treatments for patients [65-69].

Compared with similar research in the literature, no source was found that included a detailed evaluation of all BAs in a military environment. Of course, since this is not a systematic review, possible relevant sources may have been missed. Regarding the call for action message of this article, it is supported by data in the literature. For example, Etuk et al. (2020) identified GD as a significant yet under-recognized problem among U.S. veterans, closely linked to PTSD and other mental health difficulties, and highlighted the need for improved screening, integrated treatment, and veteran-focused research [70]. Also, Hoyt et al. (2022) argue that GD remains under-recognized within military behavioral health services and advocate for integrated screening and treatment approaches that address gambling alongside substance use and co-occurring mental health conditions [71].

Several limitations of this research must be acknowledged. First of all, the available literature is heavily weighted toward GD, while evidence regarding problematic pornography use, CSB, PUI, compulsive shopping, ultra-processed food addiction, and exercise dependence remains scarce. Secondly, considerable heterogeneity exists regarding diagnostic criteria, screening instruments, and study populations, limiting direct comparisons across studies. Most investigations were cross-sectional, preventing causal inferences regarding the relationships between behavioral addictions and psychiatric comorbidity. Thirdly, as mentioned before, the methodology of the current study was not that of a systematic review, leaving open the possibility of missing relevant sources. Also, no quality appraisal was performed on the identified sources.

Future research on BAs among military personnel should prioritize longitudinal studies, military-specific screening tools, intervention trials, and investigations in active-duty populations outside North America. For this purpose, increased awareness is first needed about the risk factors, evolution, and negative consequences of BAs, which means implementing psychoeducation programs and mental health policies adjusted to the unmet needs of patients with such addictions.

Overall, the findings reviewed here indicate that BAs should be regarded as an emerging military health issue that intersects with mental health, occupational performance, and force readiness. Greater recognition of these conditions may facilitate earlier identification, improve treatment access, and reduce the individual and organizational burden associated with behavioral addictions.

## CONCLUSION

Behavioral addictions represent an important but insufficiently recognized challenge within military and veteran populations. The available evidence indicates that gambling disorder, gaming disorder, problematic pornography use, compulsive sexual behavior, and problematic internet use are associated with trauma exposure, psychiatric comorbidity, psychosocial impairment, and reduced operational readiness. Military-specific factors, including deployment stress, social isolation, boredom, sleep disruption, risk-taking culture, and barriers to help-seeking, appear to increase vulnerability to these conditions. Consequently, BAs should be incorporated into military mental health strategies through routine screening, psychoeducation, early intervention, and integrated treatment pathways.

The development of validated military-specific assessment tools and evidence-based prevention programs should be considered a research and policy priority. Addressing behavioral addictions proactively may improve both individual well-being and the effectiveness, readiness, and resilience of military organizations.

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***Authors' contribution***

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*Not applicable.*

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REVIEW

# Transthyretin Cardiac Amyloidosis in Aortic Stenosis: Prevalence, Nuclear Diagnosis and Clinical Implications in the Elderly Population

**Ionut G. Ilinoiu[1], Letitia E. Mititelu[2,3], Teodora S. Mititelu[4], Magdalena Gurzun[5,6*], Diana Iancu[5], Victor C. Mazilu[1*], Silviu M. Stanciu[5,7]**

[1] Clinic of Nuclear Medicine, Central University Emergency Military Hospital "Dr Carol Davila", Bucharest, Romania; gabrielilinoiu5@gmail.com (G.I.); mazilucatalin@yahoo.com (V.C.M.)
[2] Military Medical Institute, Bucharest, Romania
[3] Faculty of Medicine, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; letitia-elena.mititelu021@stud.umfcd.ro
[4] Department of Plastic and Reconstructive Surgery, "Bagdasar-Arseni" Clinical Emergency Hospital, 041915, Bucharest, Romania
[5] Center for Noninvasive Cardiac Study, Carol Davila University Central Emergency Military Hospital, Bucharest, Romania
[6] Department of cardio-thoracic pathology, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania; magdalenagurzun@gmail.com
[7] Department of Internal Medicine and Gastroenterology, Carol Davila University of Medicine and Pharmacy, Central Military Emergency University Hospital, Bucharest, Romania; silviu.stanciu@umfcd.ro

*Correspondence to: magdalenagurzun@gmail.com (M.G.); mazilucatalin@yahoo.com (V.C.M.)

**Abstract**: Background: Degenerative aortic stenosis (AS) and transthyretin cardiac amyloidosis (ATTR-CM), particularly wild-type ATTR (ATTRwt), are age-associated disorders that frequently converge in elderly patients. Their coexistence may obscure diagnosis, amplify heart-failure burden, and complicate risk stratification before and after aortic valve replacement.
Methods: This narrative review was conducted using a structured literature search focused on ATTR-CM, AS, transcatheter aortic valve implantation/replacement (TAVI/TAVR), and nuclear cardiology. Priority was given to cohort studies, systematic reviews, consensus documents, and guideline statements.
Main findings: Across observational cohorts and meta-analyses, ATTR-CM is consistently identified in a clinically meaningful minority of older patients with severe AS, especially among those referred for TAVI. Reported prevalence varies with age, referral pathway, diagnostic protocol, and whether equivocal grade 1 uptake is included, but most contemporary TAVI-oriented cohorts place definite ATTR-CM in the high single-digit to mid-teen percentage range. Clinical suspicion should increase in patients with disproportionate left-ventricular wall thickening, low-flow low-gradient AS, restrictive physiology, elevated cardiac biomarkers, conduction disease, atrial fibrillation, or extracardiac ATTR clues such as bilateral carpal tunnel syndrome.
Conclusions: Bone-avid tracer scintigraphy with 99mTc-pyrophosphate, 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid, or 99mTc-hydroxymethylene diphosphonate, interpreted with SPECT or SPECT/CT and combined with mandatory exclusion of a monoclonal protein, enables robust non-biopsy diagnosis of ATTR-CM. In severe AS, nuclear diagnosis should be embedded in a pragmatic, multidisciplinary pathway that identifies patients likely to benefit from valve intervention, ATTR-specific therapy, genetic testing, and tailored follow-up.

**Keywords:** transthyretin cardiac amyloidosis; ATTRwt; ATTRv; aortic stenosis; TAVI; TAVR; 99mTc-PYP; 99mTc-DPD; 99mTc-HMDP; Perugini score; nuclear cardiology.

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## Abbreviations

AL, immunoglobulin light-chain amyloidosis; AS, aortic stenosis; ATTR-CM, transthyretin cardiac amyloidosis; ATTRv, variant/hereditary transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; AV, aortic valve; AVR, aortic valve replacement; CMR, cardiovascular magnetic resonance; DPD, 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid; EF, ejection fraction; H/CL, heart-to-contralateral lung ratio; HFpEF, heart failure with preserved ejection fraction; HMDP, 99mTc-hydroxymethylene diphosphonate; LFLG, low-flow low-gradient; LV, left

ventricle/ventricular; LVH, left-ventricular hypertrophy; PYP, 99mTc-pyrophosphate; SAVR, surgical aortic valve replacement; SPECT, single-photon emission computed tomography; TAVI/TAVR, transcatheter aortic valve implantation/replacement; TTR, transthyretin.

## INTRODUCTION

Degenerative AS is one of the most important valvular diseases of aging. Population-based and modeling data show that AS becomes increasingly common after the seventh decade of life, and contemporary guideline frameworks emphasize that disease severity, symptoms, ventricular response, frailty, and procedural risk must all be integrated when selecting medical surveillance, SAVR, or TAVI/TAVR [1-3]. In parallel, ATTR-CM has evolved from a perceived rarity to a treatable cause of restrictive cardiomyopathy and heart failure in older adults. The recognition of non-biopsy diagnostic pathways and disease-modifying therapy has transformed clinical practice, but this benefit depends on identifying ATTR-CM before advanced irreversible myocardial dysfunction develops [4-8].

The intersection between ATTR-CM and AS is clinically compelling because both disorders are age-related, both may present with dyspnea, syncope, exertional intolerance, left-ventricular hypertrophy, atrial arrhythmia, conduction disease and HFpEF, and both may be misattributed to “normal aging” or hypertension. In an elderly patient with severe AS, the hypertrophied, stiff ventricle may be interpreted as a pressure-overload response, while amyloid infiltration remains unsuspected. Conversely, amyloid-related low stroke volume may modify the echocardiographic phenotype of AS, contributing to paradoxical low-flow low-gradient presentations and diagnostic uncertainty.

Nuclear cardiology is central to this field. The landmark non-biopsy diagnostic work by Gillmore et al. established that grade 2 or 3 cardiac uptake on bone-avid tracer scintigraphy, when combined with the absence of a monoclonal protein, can diagnose ATTR-CM with very high specificity and positive predictive value [6]. Subsequent expert consensus documents from cardiac imaging societies clarified acquisition, interpretation, reporting, and the need for SPECT or SPECT/CT confirmation of myocardial rather than blood-pool uptake [7,8]. The original visual scoring system introduced with DPD scintigraphy remains the foundation for clinical interpretation [9].

The aim of this review is to synthesize evidence on the prevalence of ATTR-CM in AS, emphasize the dual-pathology phenotype in elderly patients, and provide an academically grounded approach to nuclear diagnosis. The intended practical output is a diagnostic framework for clinicians evaluating severe AS—particularly TAVI candidates—in whom ATTR-CM may be clinically silent, prognostically relevant, and therapeutically actionable.

## MATERIALS AND METHODS

This manuscript was designed as an extended narrative review with structured search methodology rather than a formal systematic review or meta-analysis. The objective was to integrate evidence across epidemiology, cardiology, nuclear medicine, multimodality imaging, and therapeutics, while allowing contextual discussion of implementation and clinical decision-making.

A targeted literature search was conceptualized for PubMed/MEDLINE, Scopus, Google Scholar, and major society documents available up to 9 May 2026. Search concepts included: “transthyretin cardiac amyloidosis”, “ATTR-CM”, “ATTRwt”, “ATTRv”, “aortic stenosis”, “severe aortic stenosis”, “low-flow low-gradient aortic stenosis”, “TAVI”, “TAVR”, “SAVR”, “99mTc-PYP”, “99mTc-DPD”, “99mTc-HMDP”, “bone scintigraphy”, “Perugini score”, “heart-to-contralateral lung ratio”, “SPECT/CT”, and “RAISE score”. Reference lists of key reviews, consensus documents, and pivotal cohort studies were also considered.

Eligible material included peer-reviewed cohort studies, systematic reviews, meta-analyses, case-based reviews with substantial literature synthesis, nuclear cardiology consensus statements, cardiology guidelines, and major therapeutic trials relevant to ATTR-CM. Studies were prioritized when they included elderly AS populations, TAVI/TAVR candidates, explicit nuclear diagnostic protocols, or outcomes after valve replacement. Non-cardiac amyloidosis literature was included only when it supported systemic-recognition concepts relevant to cardiac diagnosis, as in digestive amyloidosis trends.

## RESULTS AND DISCUSSION

### 1. Conceptual background: why ATTR-CM and AS coexist?

*1.1 Aortic stenosis as an aging-related pressure-overload disease*

Calcific AS is an active fibro-calcific process involving endothelial injury, lipid infiltration, inflammation, valvular interstitial-cell activation, extracellular matrix remodeling, and progressive leaflet mineralization. Its clinical expression is not determined solely by valvular area or gradient. The LV response—concentric hypertrophy, fibrosis, diastolic dysfunction, impaired longitudinal mechanics, pulmonary hypertension, and reduced flow reserve—modulates symptoms and outcomes. Contemporary valve guidelines therefore distinguish high-gradient severe AS, classical low-flow low-gradient AS with reduced EF, and low-flow low-gradient AS with preserved EF [2,3].

Low-flow low-gradient AS is especially relevant to amyloidosis because both conditions can converge on a small, stiff, hypertrophied ventricle with low stroke volume despite preserved EF. In such patients, the calculated valve area may suggest severe AS while the mean gradient is lower than expected. ATTR-CM can contribute to this phenotype by reducing longitudinal shortening, impairing diastolic filling, and increasing myocardial stiffness.

*1.2 ATTR-CM as an infiltrative and systemic disorder*

ATTR amyloidosis results from misfolding of transthyretin, a tetrameric transport protein mainly synthesized by the liver. Because hepatic function is central to transthyretin production, liver diseases and systemic inflammatory conditions may influence hepatic protein homeostasis and should be considered in the broader context of transthyretin-related disorders [10]. Tetramer dissociation is the rate-limiting step in amyloidogenesis; misfolded monomers aggregate into oligomers and fibrils that deposit in the extracellular matrix. In ATTRwt, the transthyretin sequence is normal, and the disease is strongly age-associated, classically affecting older men but increasingly recognized in women. In ATTRv, a pathogenic TTR variant destabilizes the tetramer and produces hereditary disease with variable cardiac, neurologic, autonomic, and gastrointestinal phenotypes [4,5,11-14].

Although the present review focuses on cardiac disease, systemic clues matter. Bilateral carpal tunnel syndrome, lumbar spinal stenosis, biceps tendon rupture, peripheral or autonomic neuropathy, unexplained weight loss, and digestive symptoms may precede cardiac diagnosis. Bucurica et al. reviewed digestive amyloidosis and underscored the importance of recognizing multiorgan manifestations, biopsy confirmation with Congo red staining, and protein typing when gastrointestinal disease is suspected [13]. In elderly AS, such extracardiac clues can shift the clinical probability toward ATTR-CM and justify nuclear evaluation.

*1.3 ATTRwt versus ATTRv in the AS population*

Most dual AS-ATTR cohorts identify predominantly ATTRwt, which is biologically plausible because both degenerative AS and ATTRwt are common in later life. Nevertheless, ATTRv cannot be excluded by age, sex, ethnicity, or cardiac phenotype alone. After a non-biopsy diagnosis of ATTR-CM, current consensus statements recommend TTR genetic testing to distinguish ATTRwt from ATTRv, because the result affects family counseling, cascade testing, and awareness of neurologic or mixed phenotypes [4,5].

For review framing, ATTRwt should be treated as the principal subtype expected in elderly severe AS and TAVI referral pathways, while ATTRv should be discussed as a mandatory differential subtype once ATTR-CM is confirmed. This distinction also avoids a common diagnostic error: a positive bone-avid tracer scan identifies ATTR-CM only after AL amyloidosis has been excluded and does not, by itself, determine whether the patient has wild-type or variant TTR disease [4-8].

*1.4 Epidemiology and prevalence of dual AS–ATTR-CM*

The reported prevalence of ATTR-CM among patients with AS varies by population, age threshold, AS severity, referral setting, tracer, imaging protocol, and diagnostic definition. The highest-yield populations are older patients with severe AS referred for TAVI/TAVR, because advanced age enriches for ATTRwt and TAVI pathways often include systematic imaging, biomarker, and frailty assessment. Surgical cohorts and unselected AS cohorts are more heterogeneous.

Early pathologic and imaging work suggested that occult ATTR may be present in a substantial minority of older AS patients. Treibel et al. identified occult transthyretin amyloid in patients undergoing SAVR and reported adverse prognostic associations, supporting the concept that amyloid infiltration may be clinically relevant rather than merely incidental [15]. Castaño et al. then prospectively evaluated elderly patients with severe calcific AS undergoing TAVR using PYP scintigraphy and found ATTR-CM in 16%, with associations including low-flow low-gradient physiology and reduced longitudinal systolic velocity [16].

Scully et al. evaluated patients referred for TAVI using DPD scintigraphy and reported dual AS-amyloid pathology in approximately 13%, with higher biomarkers and structural/functional differences; importantly, TAVI was still associated with improved outcome compared with medical management in the dual-pathology group [17]. Nitsche et al. expanded this concept in severe AS cohorts by examining both AL and ATTR and by proposing structured screening possibilities. In the JACC cohort, DPD uptake compatible with ATTR-CM was present in a clinically meaningful minority of patients, and the RAISE score was introduced to identify patients with increased likelihood of concomitant amyloid [18,19].

Narrative and systematic reviews have since consolidated these observations. Ternacle et al. estimated that cardiac amyloidosis may affect up to approximately 15% of AS patients and a higher proportion of those with low-flow low-gradient AS, emphasizing that coexistence can contribute to treatment futility and adverse outcomes if unrecognized [20]. Ho et al. pooled 21 studies and reported an overall cardiac amyloidosis prevalence of 14.4% among AS patients, while noting higher mortality in the dual-pathology group [21]. Jaiswal et al. summarized the state of the art and described coexistence in the approximate 4% to 16% range among older AS/TAVI populations [22]. More recent meta-analyses specifically addressing TAVR/TAVI populations continue to support a nontrivial prevalence and the need for standardized diagnostic pathways [23-25].

Adam et al. provide a clinically useful bridge between case-based recognition and literature synthesis. Their report of severe AS with ATTRwt amyloidosis in an aging patient illustrates how AS may mask amyloid-related restrictive cardiomyopathy, and how low-flow

low-gradient physiology, ventricular hypertrophy, biomarker elevation, and multimodality imaging should prompt ATTR evaluation [14].

Taken together, the literature supports the following practical statement: ATTR-CM is not rare among elderly patients with severe AS, particularly those referred for TAVI, and its prevalence is sufficiently high to justify systematic suspicion and selective or protocolized screening in enriched subgroups. However, prevalence should not be quoted without describing diagnostic criteria. Studies that include Perugini grade 1 uptake, do not perform SPECT confirmation, or do not fully exclude AL may overestimate or misclassify definite ATTR-CM.

**Table 1:** Representative literature on ATTR-CM/cardiac amyloidosis in aortic stenosis.

| Study | Population / diagnostic approach | Key finding | Relevance to this review |
|---|---|---|---|
| **Treibel et al., 2016 [15]** | SAVR-oriented severe calcific AS cohort; histology and imaging-oriented assessment. | Occult transthyretin amyloid was identified in a subset and associated with adverse prognosis. | Established that amyloid may be clinically relevant in surgically treated AS, not merely an incidental autopsy finding. |
| **Castaño et al., 2017 [16]** | Elderly severe calcific AS patients undergoing TAVR; PYP scintigraphy with monoclonal-protein assessment. | ATTR-CM identified in 16%; associated with low-flow low-gradient AS and lower tissue Doppler systolic velocity. | Key prospective TAVR study supporting nuclear screening in enriched AS populations. |
| **Scully et al., 2020 [17]** | Patients aged ≥75 years referred for TAVI; DPD scintigraphy and clinical outcomes. | Dual AS-amyloid pathology in approximately 13%; biomarkers higher; TAVI remained beneficial compared with medical management. | Important for clinical decision-making: ATTR-CM should not automatically preclude TAVI. |
| **Nitsche et al., 2020 [18]** | Severe AS cohort examining AL and ATTR diagnostic pathways. | Emphasized screening possibilities, monoclonal workup, and outcome analysis. | Highlights that AL must be considered and excluded in AS patients with suspected amyloid. |
| **Nitsche et al., 2021 [19]** | 407 severe AS patients screened with DPD; introduced RAISE score. | Grade 2/3 uptake in 7.9%; one-year mortality higher in AS-CA; TAVR improved survival and should not be withheld. | Provides a pragmatic screening score and outcome-oriented evidence. |
| **Ternacle et al., 2019 [20]** | JACC review focused on AS and cardiac amyloidosis. | Estimated clinically meaningful coexistence, especially in low-flow low-gradient AS. | Frames dual pathology as a contributor to heart failure and treatment futility. |
| **Ho et al., 2022 [21]** | Systematic review/meta-analysis of 21 studies. | Pooled cardiac amyloidosis prevalence in AS: 14.4%; dual pathology associated with higher mortality. | Quantifies burden and supports systematic suspicion. |
| **Jaiswal et al., 2023 [22]** | State-of-the-art review. | Coexistence has been reported at 4–16% in older AS/TAVR populations. | Useful contemporary synthesis of imaging and management. |
| **Fatima et al., 2024; Cannata et al., 2022; Jaiswal et al., 2023 [23-25]** | Meta-analyses/systematic reviews focused on TAVR/TAVI outcomes. | Suggest altered procedural and post-procedural risk profiles, including conduction disease and heart-failure outcomes, with heterogeneous mortality findings. | Supports the need for standardized diagnosis and prospective outcome studies. |
| **Adam et al., 2020 [14]** | Case report and literature review of severe AS with ATTRwt. | Illustrates diagnostic vigilance in aging and the overlap between AS and ATTRwt phenotypes. | Clinically anchors the present review question. |

*1.5 Clinical phenotype: when to suspect ATTR-CM in AS*

The diagnostic challenge is that AS already explains many findings that also occur in ATTR-CM. Therefore, suspicion should be triggered not by a single sign but by disproportion, clustering, or trajectory. Severe concentric LVH in a patient with only modest hypertension, small LV cavity, restrictive filling, right-ventricular thickening, biatrial enlargement, pericardial effusion, impaired longitudinal strain, or unexpectedly high NT-proBNP and troponin should not be automatically attributed to valvular pressure overload.

Low-flow low-gradient AS is a particularly important clinical phenotype. In ATTR-CM, stroke volume can be reduced by small ventricular cavity size, impaired diastolic filling, atrial dysfunction, and longitudinal systolic impairment despite preserved EF. This may produce discordant AS measurements and may lower gradients despite severe leaflet obstruction. Castaño et al. and later cohorts found enrichment of amyloid in low-flow low-gradient presentations [16,19,20].

Electrocardiography can provide additional clues. Classical low QRS voltage is not universally present in ATTR-CM, and AS-related LVH may further obscure voltage-wall thickness discordance. Nevertheless, pseudo-infarct pattern, conduction delay, atrioventricular block, bundle-branch block, atrial fibrillation, and pacemaker requirement are important red flags. The RAISE score incorporated remodeling, age, injury, systemic clues, and electrical disturbances to identify AS patients more likely to have concomitant amyloid [19].

Extracardiac features may be decisive. Bilateral carpal tunnel syndrome, lumbar spinal stenosis, biceps tendon rupture, neuropathy, autonomic symptoms, unexplained gastrointestinal dysmotility, proteinuria, or a history of monoclonal gammopathy should be actively sought. Bucurica et al. emphasize that gastrointestinal amyloidosis may present with nonspecific symptoms and requires pathologic and protein-typing confirmation [13]. In the AS clinic, such clues help identify patients who should undergo amyloid evaluation rather than routine valve-only assessment.

**Table 2:** Red flags for ATTR-CM or systemic amyloidosis in patients with aortic stenosis.

| Domain | Clinical finding | Diagnostic significance |
|---|---|---|
| **Demographic** | Age >75–80 years; male sex; frailty or disproportionate functional decline. | ATTRwt and degenerative AS are both enriched in advanced age. |
| **AS phenotype** | Paradoxical low-flow low-gradient AS; low stroke-volume index; discordant valve area/gradient. | Amyloid-related restrictive physiology can reduce flow and mask the severity of the gradient. |
| **Echocardiography** | Disproportionate LV wall thickness; small LV cavity; severe diastolic dysfunction; biatrial enlargement; RV thickening; small pericardial effusion. | Suggest infiltrative disease beyond pressure overload. |
| **Strain imaging** | Reduced global longitudinal strain with relative apical sparing; severe longitudinal impairment despite preserved EF. | Supports amyloid suspicion, although AS can reduce specificity. |
| **ECG/conduction** | Low voltage or voltage-mass discordance; pseudo-infarct pattern; AV block; bundle-branch block; atrial fibrillation; pacemaker. | Electrical disease is common in ATTR-CM and may worsen after TAVI. |
| **Biomarkers** | NT-proBNP or troponin elevation disproportionate to AS severity, renal function, or symptoms. | Reflects myocardial injury/stress and may aid risk stratification. |
| **Extracardiac ATTR clues** | Bilateral carpal tunnel syndrome, lumbar spinal stenosis, biceps tendon rupture, peripheral/autonomic neuropathy. | May precede cardiac diagnosis by years. |
| **Systemic amyloidosis clues** | Unexplained proteinuria, macroglossia, purpura, gastrointestinal dysmotility, weight loss, monoclonal gammopathy. | Raises concern for AL or systemic amyloid and mandates hematologic evaluation. |

## 2. Nuclear diagnosis of ATTR-CM in the AS patient

### *2.1 Rationale for bone-avid tracer scintigraphy*

Bone-avid radiotracer scintigraphy has become the cornerstone of non-invasive ATTR-CM diagnosis. The clinically used tracers are 99mTc-PYP, 99mTc-DPD, and 99mTc-HMDP. Although their exact molecular binding to amyloid is not fully reducible to one mechanism, they localize to ATTR cardiac deposits sufficiently reliably to support diagnosis when interpreted in the correct clinical and laboratory context [6-11]. Recent reviews also emphasize that nuclear medicine in cardiac amyloidosis includes validated bisphosphonate scintigraphy for ATTR-CM and emerging amyloid-specific PET methods for disease-burden assessment and follow-up [26].

This is particularly valuable in AS. Endomyocardial biopsy is definitive but invasive and not always feasible in frail elderly patients already undergoing valve assessment. Cardiovascular magnetic resonance can strongly suggest amyloidosis through diffuse late gadolinium enhancement, abnormal gadolinium kinetics, elevated native T1, and extracellular volume expansion, but it cannot reliably type amyloid and may be limited by renal dysfunction, arrhythmia, implanted devices, or access. Scintigraphy, in contrast, can non-invasively establish ATTR-CM when paired with a negative monoclonal-protein evaluation [4-8,27].

### *2.2 Perugini visual scoring and the definition of a positive scan*

The Perugini visual score remains the most widely used semiquantitative approach. Grade 0 indicates absent myocardial uptake with normal bone uptake. Grade 1 indicates myocardial uptake less than bone uptake. Grade 2 indicates myocardial uptake equal to bone uptake. Grade 3 indicates myocardial uptake greater than bone uptake, often with reduced or absent skeletal visualization due to intense cardiac localization [9].

For ATTR-CM diagnosis, grade 2 or 3 myocardial uptake is considered strongly positive only after AL amyloidosis has been excluded. Gillmore et al. showed that radionuclide scintigraphy has excellent sensitivity for ATTR-CM, but specificity becomes effectively diagnostic when grade 2/3 uptake is combined with absence of a monoclonal protein [6]. Grade 1 uptake is not sufficient for a non-biopsy ATTR diagnosis. It may reflect early ATTR, AL amyloid, blood-pool artifact, rib/valvular activity, or technical factors; such cases require careful SPECT review, repeat imaging, CMR, or tissue diagnosis depending on clinical probability [4-8,12].

*2.3 Mandatory exclusion of AL amyloidosis*

The most important safety principle is that a positive bone-avid tracer scan does not exclude AL amyloidosis. AL can occasionally show cardiac uptake, and missing AL has major consequences because it is a hematologic emergency requiring urgent therapy. Therefore, every suspected cardiac amyloidosis patient should undergo serum free light-chain assay, serum immunofixation, and urine immunofixation. Serum protein electrophoresis alone is insufficient. If a monoclonal protein is present, ATTR-CM cannot be diagnosed non-invasively on scintigraphy alone; tissue biopsy with amyloid typing is generally required [4-8].

This point is especially relevant in elderly AS because monoclonal gammopathy of undetermined significance is common with age. A patient may have AS, ATTRwt, and an unrelated monoclonal gammopathy, or may have AL amyloidosis with AS. The diagnostic algorithm must therefore be designed to avoid both underdiagnosis and misclassification.

*2.4 Why SPECT or SPECT/CT is essential*

Planar images are useful for global visual assessment and heart-to-contralateral quantification, but they are insufficient as the sole basis for diagnosis. SPECT or SPECT/CT confirms whether activity is truly myocardial rather than intravascular blood pool, overlying rib, sternal, valvular, annular, or pulmonary activity. In severe AS, annular and valvular calcification are common, and blood-pool retention may be increased in heart failure or renal impairment. These issues increase the importance of tomographic confirmation [7,8,12].

Stan et al. demonstrated that implementation gaps remain clinically relevant. In their survey of Romanian nuclear medicine physicians, incidental diffuse cardiac uptake was frequently encountered, but awareness, interpretation, tracer selection, and multidisciplinary communication were variable [12]. Although the survey is regional, its message is general: diagnostic accuracy depends not only on tracer availability but also on standardized acquisition, correct interpretation, reporting language, and referral pathways.

*2.5 Quantitative indices: H/CL ratio and their limitations*

The H/CL ratio has been widely used, especially in PYP imaging, as a semi-quantitative adjunct. Thresholds differ by acquisition timing and local protocols, and the ratio can be affected by blood-pool activity, body habitus, contralateral-lung region placement, pleural or pulmonary disease, and extracardiac overlap. Expert statements therefore recommend that H/CL should support but not replace visual SPECT-confirmed diagnosis [7,8]. A high H/CL ratio without definite myocardial uptake on SPECT should not be reported as diagnostic ATTR-CM.

*2.6 Suggested scan interpretation in AS*

For AS patients, reports should explicitly state the tracer used, injected activity, acquisition timing, planar and SPECT/SPECT-CT findings, visual grade, whether uptake is myocardial or blood pool, whether H/CL ratio was measured, and whether findings are compatible with ATTR-CM only in the context of negative monoclonal-protein testing. Suggested reporting categories are: negative for ATTR-CM, equivocal, positive for ATTR-CM if AL excluded, or positive cardiac uptake with monoclonal-protein/biopsy correlation required.

*2.7 False-positive scintigraphic results: technical pitfalls, non-amyloid myocardial injury and recent myocardial infarction*

Although grade 2 or 3 myocardial uptake on bone-avid tracer scintigraphy, in the absence of a monoclonal protein, has very high diagnostic specificity for ATTR-CM, false-positive results can occur when acquisition, reconstruction, localization, clinical context, or amyloid typing requirements are not rigorously respected [7,8,28,29]. This point is particularly important in elderly AS patients, in whom renal dysfunction, heart failure with delayed blood-pool clearance, valvular or annular calcification, previous coronary disease, rib lesions, and complex thoracic anatomy may coexist and may complicate planar interpretation.

The most frequent interpretative pitfall is mistaking persistent intracavitary or vascular blood-pool activity for true myocardial tracer uptake. This problem is most relevant for early imaging and for planar-only interpretation, and it is magnified when myocardial uptake is assessed without the characteristic tomographic distribution of tracer within the ventricular walls. In a large clinical experience with 99mTc-PYP, Poterucha et al. showed that reliance on planar grade 2 findings can generate false-positive interpretations when SPECT is used as the reference standard, reinforcing the need for tomographic confirmation of myocardial retention [29]. Accordingly, SPECT or preferably SPECT/CT should be considered mandatory when cardiac uptake is suspected, because it distinguishes myocardial uptake from blood pool, ribs, sternum, valvular/annular calcification, and extracardiac activity [7,8,28].

**Table 3:** Nuclear diagnosis checklist for suspected ATTR-CM in aortic stenosis.

| Step | Recommended approach | Clinical rationale |
|---|---|---|
| **Tracer** | 99mTc-PYP, 99mTc-DPD, or 99mTc-HMDP. | Use validated bone-avid tracers; do not extrapolate diagnostic criteria to non-validated tracers. |
| **Acquisition** | Planar plus SPECT; SPECT/CT where available. | Tomography is necessary to confirm myocardial uptake and avoid blood-pool/overlap artifacts. |
| **Visual score** | Perugini grade 0–3. | Grade 2/3 is strongly positive; grade 1 is equivocal and not diagnostic alone. |
| **Laboratory exclusion of AL** | Serum free light chains, serum immunofixation, urine immunofixation. | Mandatory before making a non-biopsy ATTR-CM diagnosis. |
| **H/CL ratio** | Optional adjunct, especially for PYP. | Helpful for reproducibility but must not override SPECT-confirmed visual interpretation. |
| **ATTRwt vs ATTRv** | TTR genetic testing after ATTR-CM diagnosis. | Determines hereditary status, family counseling, and systemic phenotype surveillance. |
| **AS-specific pitfalls** | Valvular/annular calcification, blood-pool retention, renal dysfunction, heart failure, rib uptake. | May mimic or obscure myocardial uptake; SPECT/CT improves localization. |
| **Communication** | The report should recommend a hematology/genetics/cardiology correlation as appropriate. | Prevents false reassurance, missed AL, and incomplete ATTR workup. |

Technical and general nuclear-medicine artifacts should also be considered before assigning a positive ATTR-CM diagnosis. Excess free pertechnetate, suboptimal radiochemical preparation, premature acquisition, patient motion, attenuation, extracardiac overlap, reconstruction artifacts, and SPECT/CT misregistration may alter visual interpretation or create apparent focal or diffuse activity in the cardiac region. Mititelu et al. provide a practical nuclear cardiology framework for recognizing artifacts arising from patient-related, equipment-related, and technique-related factors, including motion, attenuation, extracardiac activity, and acquisition and reconstruction problems. Their emphasis on raw-data review, correct positioning, artifact recognition, attenuation or overlap assessment, and systematic quality control is directly relevant to ATTR scintigraphy, because the same interpretative discipline helps prevent false-positive myocardial localization and false-negative examinations in bone-avid tracer imaging [30].

Several biological or disease-related causes of false-positive cardiac uptake have been described. AL amyloidosis remains the most clinically dangerous mimic, because it may occasionally show moderate or intense bone-avid tracer uptake and cannot be excluded by scintigraphy alone; this is why serum free light chains, serum immunofixation and urine immunofixation are mandatory before any non-biopsy ATTR-CM diagnosis [4,6-8,27]. Other reported causes include hypertrophic cardiomyopathy, hydroxychloroquine- or chloroquine-induced cardiotoxicity, myocarditis, post-radiation myocardial injury, doxorubicin-induced cardiotoxicity, myocardial calcification, and focal uptake related to rib or skeletal lesions projecting over the heart [28,31,32]. In AS patients, hypertrophic remodeling, coronary artery disease, and valvular/annular calcification are frequent background abnormalities, so a positive scan should be interpreted only after confirming that uptake is truly myocardial and that the clinical pattern is not better explained by another process.

Recent myocardial infarction deserves specific emphasis. 99mTc-PYP was originally introduced in the 1970s for radionuclide imaging of acute myocardial infarction, because myocardial necrosis is associated with calcium influx and formation of intramyocardial calcium complexes with affinity for phosphate-based bone tracers [28,33]. Therefore, a patient scanned shortly after an acute infarct may show intense myocardial tracer uptake that can mimic ATTR-CM. In contrast to the diffuse or circumferential ventricular-wall uptake expected in typical ATTR-CM, post-infarction uptake is often focal or regional and follows a coronary-territory distribution. Khor et al. illustrate this pitfall with a patient scanned 10 days after left anterior descending artery infarction, in whom intense septal uptake represented recent infarction rather than amyloid; they also note that PYP uptake after infarction may become less prominent after approximately two weeks but can occasionally persist for months [28]. Makivic et al. similarly described acute myocardial infarction with reperfusion-related myocardial edema as a differential diagnosis for cardiac ATTR, emphasizing that scintigraphic findings should be interpreted alongside clinical history, ECG, biomarkers, coronary anatomy, and CMR when appropriate [34].

In practical terms, a recent acute coronary syndrome, revascularization, a marked troponin rise, a new regional wall-motion abnormality, or a CMR pattern consistent with infarction should preclude an automatic ATTR-CM label. In such cases, the report should describe the distribution of uptake, state that recent myocardial injury is a potential cause of false positivity, and recommend delayed repeat scintigraphy, CMR correlation, or biopsy with amyloid typing if diagnostic uncertainty persists. This approach is especially relevant before TAVI/TAVR, because elderly AS patients often have coexisting coronary artery disease and may undergo amyloid evaluation during the same period as coronary assessment or acute decompensation.

*2.8 Multimodality imaging and integrated diagnostic probability*

Nuclear imaging should not be interpreted in isolation. Echocardiography remains the first-line imaging modality in AS and is indispensable for assessing valve severity, gradients, valve area, stroke volume index, LV wall thickness, diastolic function, pulmonary pressures, and coexisting valve disease. The challenge is that AS produces LVH and diastolic dysfunction even without amyloid. Thus, echo should be used to identify disproportionate features and to stratify amyloid probability rather than to "rule out" ATTR-CM.

Speckle-tracking strain can be useful. Relative apical sparing is a classic amyloid clue, described as a base-to-apex gradient of longitudinal dysfunction [35]. However, severe AS itself alters strain, and apical sparing is not universally present in dual pathology. In Castaño et al., conventional amyloid strain patterns were less discriminative than other functional parameters in the TAVR cohort [16]. Therefore, strain should be interpreted alongside age, AS phenotype, biomarkers, ECG, and extracardiac clues.

CMR adds tissue characterization. Diffuse subendocardial or transmural late gadolinium enhancement, high native T1, abnormal myocardial nulling, and elevated extracellular volume support cardiac amyloidosis. CMR is also useful when scintigraphy is equivocal, monoclonal testing is abnormal, or multiple cardiomyopathic processes coexist. Nevertheless, CMR cannot reliably distinguish ATTR from AL, and nuclear plus hematologic testing remains decisive for non-biopsy ATTR typing [4-8].

Biomarkers are particularly relevant in dual disease. NT-proBNP and troponin may be elevated in severe AS, ATTR-CM, renal dysfunction, and atrial fibrillation, so specificity is limited. However, disproportionate elevation should raise suspicion for infiltrative myocardial disease and may refine risk stratification. Nitsche et al. incorporated biomarkers into screening logic, and systematic reviews have associated dual pathology with higher biomarker burden and worse outcomes [19,21].

**Figure 1:** Practical diagnostic algorithm for ATTR-CM screening in elderly patients with severe AS or TAVI evaluation.

| |
|---|
| **Step 1 — AS population: severe AS, especially age ≥75 years, TAVI/TAVR referral, paradoxical low-flow low-gradient AS, or unexplained HFpEF phenotype.** |
| ↓ |
| **Step 2 — Search for ATTR red flags: disproportionate LV wall thickness, restrictive filling, high NT-proBNP/troponin, conduction disease, AF/pacemaker, carpal tunnel syndrome, lumbar stenosis, biceps tendon rupture, neuropathy or systemic amyloid clues.** |
| ↓ |
| **Step 3 — Perform parallel tests: bone-avid tracer scintigraphy with SPECT/SPECT-CT plus serum free light chains, serum immunofixation and urine immunofixation.** |
| ↓ |
| **Step 4 — Interpret: Perugini grade 2/3 myocardial uptake + no monoclonal protein = non-biopsy ATTR-CM diagnosis; grade 1, absent uptake with high suspicion, or any monoclonal abnormality = specialist review, CMR and/or biopsy with amyloid typing.** |

*2.9 Proposed screening and diagnostic pathway*

There is no universal mandate to perform bone scintigraphy in every AS patient. A balanced approach is to screen patients in whom the pre-test probability is meaningfully enriched. Reasonable candidates include: patients aged 75 years or older with severe AS referred for TAVI; patients with paradoxical low-flow low-gradient AS; patients with disproportionate LVH or restrictive physiology; patients with conduction disease or atrial fibrillation out of proportion to AS; patients with high biomarkers; and patients with extracardiac ATTR clues.

The pathway should be operationally simple. First, identify red flags during the valve-clinic evaluation. Second, order both nuclear scintigraphy and monoclonal-protein testing rather than staging them weeks apart. Third, interpret scintigraphy with SPECT/SPECT/CT confirmation and report visual grade. Fourth, if grade 2/3 myocardial uptake is present and AL workup is negative, diagnose ATTR-CM and perform TTR genetic testing. Fifth, if uptake is grade 1 or monoclonal testing is abnormal, proceed to specialist evaluation, CMR and/or biopsy with amyloid typing. Sixth, discuss valve intervention and ATTR-specific therapy in a multidisciplinary team.

**3. Prognostic and therapeutic implications**

*3.1 Does ATTR-CM change the decision to perform TAVI?*

The presence of ATTR-CM should not be interpreted as an automatic contraindication to TAVI. Observational data suggest that patients with dual AS-ATTR may have higher heart-failure burden, more conduction disease, and worse long-term prognosis, but valve intervention can still improve survival and symptoms compared with conservative management in appropriately selected patients [17,19,21-25]. A useful clinical interpretation is that TAVI treats afterload obstruction but not infiltrative cardiomyopathy; therefore, residual dyspnea, biomarker elevation, diastolic dysfunction, and arrhythmic risk may persist after technically successful valve replacement.

This distinction matters for patient counseling. In isolated severe AS, symptomatic improvement after TAVI may be dramatic. In dual pathology, improvement may occur but be incomplete. Recognizing ATTR-CM pre-procedurally helps avoid unrealistic expectations, guides post-TAVI monitoring, and may explain persistent HFpEF symptoms that would otherwise be misattributed to prosthesis dysfunction or deconditioning.

*3.2 Conduction disease and probability of pacemaker indications*

Both AS and ATTR-CM are associated with conduction abnormalities. TAVI itself carries risk of new conduction disturbance and permanent pacemaker implantation. Dual pathology may therefore identify a subgroup requiring closer rhythm surveillance. Existing meta-analyses suggest higher rates of conduction-related outcomes in cardiac amyloidosis patients undergoing valve intervention, although estimates vary by study design and diagnostic criteria [21,23-25]. Pre-existing PR prolongation, bundle-branch block, atrioventricular block, or pacemaker history should be integrated into procedural planning.

*3.3 Disease-modifying ATTR therapy*

The therapeutic landscape of ATTR-CM has changed rapidly. Tafamidis, a transthyretin stabilizer, reduced all-cause mortality and cardiovascular-related hospitalization in ATTR-CM in ATTR-ACT, establishing disease-modifying therapy as a practical reason to diagnose ATTR-CM [36]. Acoramidis, another TTR stabilizer, improved clinical outcomes in ATTR-CM in the ATTRibute-CM trial [37]. Vutrisiran, an RNA interference therapeutic that reduces hepatic TTR production, improved outcomes in ATTR-CM in the HELIOS-B trial, further expanding the therapeutic rationale for early detection [38]. Stanciu et al. summarize this shift from molecular pathogenesis to radionuclide diagnosis and pharmacologic intervention [11].

In AS patients, evidence from trials specific to dual AS-ATTR is limited. However, once ATTR-CM is diagnosed, treatment decisions should be guided by ATTR-CM stage, functional status, renal function, comorbidities, drug availability, and goals of care. Valve intervention and ATTR therapy should be viewed as complementary rather than mutually exclusive in suitable candidates.

*3.4 Multidisciplinary care*

Optimal care requires coordination among valve cardiology, heart failure, nuclear medicine, hematology, genetics, geriatric medicine, imaging specialists, and, when needed, neurology or gastroenterology. Stan et al. demonstrate that nuclear medicine physicians may encounter incidental cardiac uptake without always triggering appropriate referral pathways [12]. This underscores the need for standardized report language and institutional protocols that connect nuclear findings to hematologic exclusion of AL, genetic testing, and cardiology follow-up.

*3.5 Research gaps and future directions*

First, prevalence estimates require harmonization. Future studies should distinguish definite ATTR-CM from equivocal grade 1 uptake, document SPECT/SPECT-CT confirmation, and report monoclonal protein testing. Without these elements, pooled prevalence estimates risk mixing true ATTR-CM with technical or biologic uncertainty.

Second, prospective outcome studies are needed to determine which AS patients benefit most from screening. Universal scintigraphy in all AS patients may not be cost-effective, whereas risk-based pathways using age, LFLG phenotype, biomarkers, conduction disease, strain, and extracardiac clues may be feasible. The RAISE score is a useful step in this direction, but external validation in diverse populations remains important [19].

Third, the impact of ATTR-specific therapy after TAVI is not established. Trials of tafamidis, acoramidis, and vutrisiran were not designed specifically for dual AS-ATTR populations. Whether early nuclear diagnosis before valve intervention improves post-TAVI quality of life, hospitalization, rhythm outcomes, or survival remains an important research question [36-38].

Fourth, implementation science deserves attention. Even when evidence supports nuclear diagnosis, real-world practice depends on tracer availability, acquisition protocols, SPECT/CT access, reporting templates, clinician awareness, and multidisciplinary referral. The survey by Stan et al. provides an instructive example of how awareness gaps can limit the diagnostic value of available imaging technology [12].

Finally, sex-specific and phenotype-specific issues require further study. ATTRwt has historically been considered predominantly male, but women may be underdiagnosed because wall-thickness thresholds, ECG voltage expectations, and frailty phenotypes differ. Similarly, ATTRv may be missed in elderly patients if genetic testing is omitted after a positive scan.

**CONCLUSION**

ATTR-CM, especially ATTRwt, is a clinically meaningful and likely underdiagnosed comorbidity among elderly patients with severe AS, particularly those referred for TAVI/TAVR. Its presence can mimic, amplify, or modify the AS phenotype by contributing to low-flow low-gradient physiology, HFpEF, disproportionate LV thickening, conduction disease, biomarker elevation, and persistent symptoms after valve replacement.

Nuclear diagnosis is central to modern recognition. Bone-avid tracer scintigraphy with 99mTc-PYP, 99mTc-DPD or 99mTc-HMDP should be interpreted using SPECT/SPECT/CT-confirmed myocardial uptake and Perugini visual grading. A non-biopsy diagnosis of ATTR-CM requires grade 2/3 myocardial uptake and mandatory exclusion of AL amyloidosis by serum free light chains, serum immunofixation and urine immunofixation. TTR genetic testing should follow to distinguish ATTRwt from ATTRv.

The practical message for the AS clinic is not to screen indiscriminately but to think systematically. In elderly severe AS, especially with low-flow low-gradient physiology, disproportionate myocardial disease, conduction abnormalities, high biomarkers, or extracardiac ATTR clues, nuclear evaluation can transform an apparently valve-only disease into a dual diagnosis with implications for prognosis, valve strategy, disease-modifying therapy, genetics, and follow-up.

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***Authors' contribution***

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