# Advances in Clinical and Experimental Medicine

MONTHLY ISSN 1899-5276 (PRINT) ISSN 2451-2680 (ONLINE) advances.umw.edu.pl

2023, Vol. 32, No. 10 (October)

Impact Factor (IF) – 2.1
Ministry of Science and Higher Education – 140 pts
Index Copernicus (ICV) – 168.52 pts

Wroclaw Medical University

Advances
in Clinical and Experimental
Medicine

# Advances
# in Clinical and Experimental Medicine

ISSN 1899-5276 (PRINT) ISSN 2451-2680 (ONLINE) advances.umw.edu.pl

**MONTHLY 2023**
**Vol. 32, No. 10**
**(October)**

Advances in Clinical and Experimental Medicine (*Adv Clin Exp Med*) publishes high-quality original articles, research-in-progress, research letters and systematic reviews and meta-analyses of recognized scientists that deal with all clinical and experimental medicine.

**Editorial Office**
ul. Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 12 05
E-mail: redakcja@umw.edu.pl

**Publisher**
Wroclaw Medical University
Wybrzeże L. Pasteura 1
50-367 Wrocław, Poland

Online edition is the original version of the journal

## Editorial Policy

Advances in Clinical and Experimental Medicine (Adv Clin Exp Med) is an independent multidisciplinary forum for exchange of scientific and clinical information, publishing original research and news encompassing all aspects of medicine, including molecular biology, biochemistry, genetics, biotechnology and other areas. During the review process, the Editorial Board conforms to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" approved by the International Committee of Medical Journal Editors (www.ICMJE.org). The journal publishes (in English only) original papers and reviews. Short works considered original, novel and significant are given priority. Experimental studies must include a statement that the experimental protocol and informed consent procedure were in compliance with the Helsinki Convention and were approved by an ethics committee.

For all subscription-related queries please contact our Editorial Office: redakcja@umw.edu.pl
For more information visit the journal's website: advances.umw.edu.pl

Pursuant to the ordinance of the Rector of Wroclaw Medical University No. 12/XVI R/2023, from February 1, 2023, authors are required to pay a fee for each manuscript accepted for publication in the journal Advances in Clinical and Experimental Medicine. The fee amounts to 990 EUR for original papers and meta-analyses, 700 EUR for reviews, and 350 EUR for research-in-progress (RIP) papers and research letters.

Advances in Clinical and Experimental Medicine has received financial support from the resources of Ministry of Science and Higher Education within the "Social Responsibility of Science – Support for Academic Publishing" project based on agreement No. RCN/SP/0584/2021.

Ministry of Education and Science
Republic of Poland

Czasopismo Advances in Clinical and Experimental Medicine korzysta ze wsparcia finansowego ze środków Ministerstwa Edukacji i Nauki w ramach programu „Społeczna Odpowiedzialność Nauki – Rozwój Czasopism Naukowych" na podstawie umowy nr RCN/SP/0584/2021.

Ministerstwo
Edukacji i Nauki

Indexed in: MEDLINE, Science Citation Index Expanded, Journal Citation Reports/Science Edition, Scopus, EMBASE/Excerpta Medica, Ulrich's™ International Periodicals Directory, Index Copernicus

Typographic design: Piotr Gil, Monika Kolęda
DTP: Wydawnictwo UMW
Cover: Monika Kolęda
Printing and binding: Drukarnia I-BiS Bierońscy Sp.k.

# Advances
## in Clinical and Experimental Medicine

MONTHLY 2023, Vol. 32, No. 10 (October)

ISSN 1899-5276 (PRINT)
ISSN 2451-2680 (ONLINE)
advances.umw.edu.pl


# Contents

## Research letters

# Donanemab: Not two without a third

Markku Kurkinen[A–F]

NeuroActiva, Inc., San Jose, USA

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1085–1087

**Address for correspondence**
Markku Kurkinen
E-mail: markku@genetics.wayne.edu

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
I thank Alberto Espay, Kate Swaffer and Nadeem Toodayan for their interest, comments and support. Special thanks to Risto Kurkinen for the graphic abstract.

Received on July 26, 2023
Reviewed on September 10, 2023
Accepted on September 21, 2023

Published online on October 13, 2023

## Abstract

Recently, the U.S. Food and Drug Administration (FDA) approved 2 anti-amyloid monoclonal antibodies, aducanumab (June 7, 2021) and lecanemab (July 6, 2023), for the treatment of Alzheimer's disease (AD) patients, and will most likely also approve a 3rd one, donanemab, soon. While these antibodies have been shown to significantly reduce amyloid in the brain, there is little, if any, evidence that they provide clinically meaningful benefit for AD patients by slowing cognitive decline. I have said it before, and I say it again: the reported benefits of anti-amyloid antibodies observed in clinical trials are erroneous and based on misinterpretation of data and a trivial miscalculation. For example, Sims et al. (2023) reported in a phase III clinical trial that donanemab treatment of early symptomatic AD patients with amyloid and tau pathology provided 35% and 36% slowing of clinical progression and cognitive decline, respectively, as measured using the Integrated Alzheimer's Disease Rating Scale (iADRS) and Clinical Dementia Rating-Sum of Boxes (CDR-SB) psychometric tests. Here, in this editorial, I show that 2.5% and 9.6% would be better estimates for less cognitive impairment with donanemab treatment.

**Keywords:** clinical trial, Alzheimer, amyloid, donanemab

**Cite as**
Kurkinen M. Donanemab: Not two without a third. *Adv Clin Exp Med*. 2023;32(10):1085–1087. doi:10.17219/acem/172673

**DOI**
10.17219/acem/172673

## Introduction

The hypothesis that amyloid-beta (Aβ) peptides and amyloid formation in the brain drive the development and progression of Alzheimer's disease (AD) is stronger than ever and back in the spotlight of AD research and clinical trials, as exemplified by the recent U.S. Food and Drug Administration (FDA) approval of the anti-Aβ monoclonal antibodies, aducanumab and lecanemab, for the treatment of patients with AD.[1–3] However, these "transformative treatments that redefine AD therapeutics,"[4] as Jeff Cummings put it, are not supported by any experimental evidence to show that anti-Aβ antibodies slow AD progression and reduce cognitive decline. While they do reduce amyloid in the brain, they can also cause serious health problems due to brain bleeding and swelling.[5–9]

What could be the reason for this apparent resurrection of the amyloid hypothesis? For one thing, it cannot be the reported benefits of aducanumab or lecanemab observed in clinical trials.[10,11] For example, as I have argued previously, the clinical benefit of lecanemab treatment is 9.6% less cognitive decline compared to placebo, while the claimed 27% benefit is based on misinterpretation of data and a trivial miscalculation.[1,2] Another example of misinterpretation of AD anti-amyloid therapy data, the topic of this editorial, is provided by the clinical trial of donanemab.[3,12]

## Donanemab

Donanemab is an anti-Aβ monoclonal antibody that binds to pyroglutamate-modified Aβ peptide found only in amyloid plaques.[13] Sims et al. (2023) reported that donanemab slowed disease progression by 35.1% compared to placebo in a phase III clinical trial of 1736 participants with early symptomatic AD and amyloid and tau pathology.[3] The findings were estimated using the Integrated Alzheimer's Disease Rating Scale (iADRS), which measures cognition and function on a 144-point scale, with lower scores indicating worse performance. Here, from baseline to 76 weeks, iADRS score change was −6.02 in the donanemab group and −9.27 in the placebo group, a difference of 3.25, which the authors interpreted as a 35.1% (3.25/9.27) slowing of cognitive decline, or clinical progression, as the authors called it.[3] This is a mistake. A score of 9.27 is not a score of cognition (the 'amount' of cognition) in the placebo group but is a change in cognition score calculated from the iADRS scores at baseline and 76 weeks. In other words, the 35.1% calculation disregarded the final iADRS scores at the conclusion of the 76-week trial to the effect that they would be irrelevant to cognition.

Obviously, it is not the score change but the final score that matters in cognition, which was 101.31 in the donanemab group and 98.88 in the placebo group.[3] These are the scores that must be used when calculating the effect of donanemab treatment on cognition. Accordingly, I suggest the 101.31 − 98.88 = 2.43 difference, or 2.46% (2.43/98.88), is a better estimate for less AD progression and cognitive impairment with donanemab compared to placebo. Even in a trial as large as 1736 study participants, 2.46% is not a statistically significant difference. I also suggest, lest we forget, that the people living with AD, their family members and friends have the final say on donanemab, whether it provides 35% or 2.5% slower clinical progression. These numbers are different enough to make a difference. Subjective, yes, but that is what matters most in real life.[14,15]

Sims et al. (2023) also measured donanemab's clinical benefit using other psychometric tests, such as the Clinical Dementia Rating-Sum of Boxes (CDR-SB), an 18-point scale, with higher scores indicating worse performance. They reported that donanemab slowed disease progression by 36%. Here, the CDR-SB score change from baseline to 76 weeks was 1.20 in the donanemab group and 1.88 in the placebo group, a difference of −0.68, or 36% (0.68/1.88) of slowing. Similar to the 35.1% benefit on iADRS, 36% is erroneous and due to a misunderstanding of the difference between cognition and change in cognition when calculating the clinical benefit of donanemab. The final score at the study conclusion was 4.64 with donanemab and 5.13 with placebo, so I suggest the 5.13 − 4.64 = 0.49 difference, or 9.55% (0.49/5.13), is a better estimate for less AD progression and cognitive impairment with donanemab compared to placebo.

## Conclusions

Answers come and go, but the question remains: If amyloid drives AD, why have anti-amyloid therapies not yet slowed cognitive decline?[16] But if amyloid does not drive AD, what is the question then? Therefore, in the spirit of "prevention is the only cure," we have to do much more research on preventive therapies and increase public awareness of the role of healthy lifestyles in delaying the onset of AD. It's about time. It's about the human mind.[17–19]

### ORCID iDs

Markku Kurkinen https://orcid.org/0000-0001-5723-5298

### References

1. Kurkinen M, Fułek M, Fułek K, Beszłej JA, Kurpas D, Leszek J. The amyloid cascade hypothesis in Alzheimer's disease: Should we change our thinking? *Biomolecules*. 2023;13(3):453. doi:10.3390/biom13030453
2. Kurkinen M. Lecanemab (Leqembi) is not the right drug for patients with Alzheimer's disease. *Adv Clin Exp Med*. 2023;32(9):943–947. doi:10.17219/acem/171379
3. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: The TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330(6):512–527. doi:10.1001/jama.2023.13239
4. Cummings J. Anti-amyloid monoclonal antibodies are transformative treatments that redefine Alzheimer's disease therapeutics. *Drugs*. 2023;83(7):569–576. doi:10.1007/s40265-023-01858-9

5. Kurkinen M. Alzheimer's trials: A cul-de-sac with no end in sight. *Adv Clin Exp Med*. 2021;30(7):653–654. doi:10.17219/acem/139501
6. Asher S, Priefer R. Alzheimer's disease failed clinical trials. *Life Sci*. 2022;306:120861. doi:10.1016/j.lfs.2022.120861
7. Plascencia-Villa G, Perry G. Lessons from antiamyloid-β immunotherapies in Alzheimer's disease. *Handb Clin Neurol*. 2023;193:267–291. doi:10.1016/B978-0-323-85555-6.00019-9
8. Castellani RJ, Shanes ED, McCord M, et al. Neuropathology of anti-amyloid-β immunotherapy: A case report. *J Alzheimers Dis*. 2023; 93(2):803–813. doi:10.3233/JAD-221305
9. Hampel H, Elhage A, Cho M, Apostolova LG, Nicoll JAR, Atri A. Amyloid-related imaging abnormalities (ARIA): Radiological, biological and clinical characteristics [published online as ahead of print on June 6, 2023]. *Brain*. 2023. doi:10.1093/brain/awad188
10. Cummings J, Aisen P, Lemere C, Atri A, Sabbagh M, Salloway S. Aducanumab produced a clinically meaningful benefit in association with amyloid lowering. *Alzheimers Res Ther*. 2021;13(1):98. doi:10.1186/s13195-021-00838-z
11. Van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2023;388(1):9–21. doi:10.1056/NEJMoa2212948
12. Rashad A, Rasool A, Shaheryar M, et al. Donanemab for Alzheimer's disease: A systematic review of clinical trials. *Healthcare*. 2022;11(1):32. doi:10.3390/healthcare11010032
13. Donanemab. Alzforum: Networking for a Cure. 2023. http://www.alzforum.org/therapeutics/donanemab. Accessed July 20, 2023.
14. Lansdall CJ, McDougall F, Butler LM, et al. Establishing clinically meaningful change on outcome assessments frequently used in trials of mild cognitive impairment due to Alzheimer's disease. *J Prev Alzheimers Dis*. 2023;10(1):9–18. doi:10.14283/jpad.2022.102
15. Petersen RC, Aisen PS, Andrews JS, et al. Expectations and clinical meaningfulness of randomized controlled trials. *Alzheimers Dement*. 2023;19(6):2730–2736. doi:10.1002/alz.12959
16. Haass C, Selkoe D. If amyloid drives Alzheimer disease, why have anti-amyloid therapies not yet slowed cognitive decline? *PLoS Biol*. 2022;20(7):e3001694. doi:10.1371/journal.pbio.3001694
17. Kurkinen M. The amyloid hypothesis is too good to be true. *Alzheimers Dement Cogn Neurol*. 2017;1(1):1–9. doi:10.15761/ADCN.1000106
18. Frisoni GB, Altomare D, Ribaldi F, et al. Dementia prevention in memory clinics: Recommendations from the European task force for brain health services. *Lancet Reg Health Eur*. 2023;26:100576. doi:10.1016/j.lanepe.2022.100576
19. Jia J, Zhao T, Liu Z, et al. Association between healthy lifestyle and memory decline in older adults: 10 year, population based, prospective cohort study. *BMJ*. 2023;380:e072691. doi:10.1136/bmj-2022-072691

# Efficacy and safety of negative allosteric modulators of 5-hydroxytryptamine 2A receptors in the treatment of Alzheimer's disease psychosis: A systematic review and meta-analysis

Yuxiao Chen[1,A], Jie Sun[2,A,B], Zhen Yang[3,C,D], Hongyan Bi[1,E,F]

[1] Department of Rehabilitation, Shandong University of Traditional Chinese Medicine Affiliated Hospital, Jinan, China
[2] Department of Neurology, Liaocheng Veterans Hospital, China
[3] Department of Movement Sciences, Faculty of Medicine, Katholieke Universiteit Leuven, Belgium

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1089–1098

**Address for correspondence**
Hongyan Bi
E-mail: Hy__bi@163.com

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on September 16, 2022
Reviewed on November 18, 2022
Accepted on February 12, 2023

Published online on May 11, 2023

## Abstract

**Background.** Psychosis is a very common feature of Alzheimer's disease (AD) that can emerge as the neurodegenerative disease progresses. The 5-hydroxytryptamine (5-HT2A) receptors are located postsynaptically to serotonergic neurons in the frontal cortex and mediate both excitatory and inhibitory neurotransmissions. However, the effectiveness and tolerance of negative modulators of 5-HT2A receptors in Alzheimer's disease psychosis (ADP) are uncertain.

**Objectives.** To detect the negative modulators of the 5-HT2A receptor as a cure for ADP.

**Materials and methods.** The primary outcome indicator was the total Neuropsychiatric Inventory (NPI) score. Other prognostic indicators included Mini-Mental State Examination (MMSE), the Katz Index of Independence in Activities of Daily Living (KATZ), the discontinuation rate, and adverse events.

**Results.** Compared to placebo, 5-HT2A inverse agonists significantly reduced the NPI total score, the KATZ and the MMSE score. The pooled odds ratio (OR) was 1.64 (95% confidence interval (95% CI): 1.01–2.65) and the heterogeneity variance was estimated at $Tau^2 = 0.52$ with an $I^2$ value of 90%, a $\chi^2$ value of 111.31, $p = 0.04$, and z-value of 2.01. The risk difference (RD) between the 5-HT2A receptor negative modulators and placebo groups was 0.12 (95% CI: 0.00–0.24) and the heterogeneity was estimated at $Tau^2 = 0.03$, $\chi^2$ value of 127.23, degrees of freedom (df) value of 9, $I^2$ value of 93%, z-value of 1.92, and p-value of 0.01 (<0.05).

**Conclusions.** Our results suggest that negative modulators of 5-HT2A receptors are beneficial and well-tolerated in the treatment of ADP.

**Key words:** mirtazapine, trazodone, pimavanserin, Alzheimer's disease psychosis (ADP), 5-hydroxytryptamine (5-HT2A) receptor

**Cite as**
Chen Y, Sun J, Yang Z, Bi H. Efficacy and safety of negative allosteric modulators of 5-hydroxytryptamine 2A receptors in the treatment of Alzheimer's disease psychosis: A systematic review and meta-analysis. *Adv Clin Exp Med*. 2023;32(10):1089–1098. doi:10.17219/acem/161159

**DOI**
10.17219/acem/161159

## Introduction

One of the most common illnesses affecting older people is dementia, which poses a significant challenge to global public healthcare. Nearly 47 million people worldwide were living with dementia in 2015 and, according to data from the World Alzheimer Report 2015, that number is expected to nearly double every 20 years.[1,2] A specific type of dementia, Alzheimer's disease (AD), accounts for 50–70% of all dementia cases.[3,4] While AD is often thought of as a memory illness, behavioral and cognitive symptoms of dementia, including psychotic symptoms, are practically ubiquitous. During the course of the disease, more than half of affected people will suffer psychotic symptoms,[5,6] which frequently leads to the increased need of care, patient stress, institutionalization, and a reduction in quality of life.[7–10]

The presence of delusions, delusional misidentification and hallucinations characterizes psychosis. Although this appears to be comparable to functional psychoses like schizophrenia, the psychotic experience of dementia patients is considerably distinct, manifesting largely as delusions and hallucinations. Approximately 35% of affected people have simple delusions; frequent symptoms include stealing, victimization, adultery, desertion, or the belief that a deceased relative is still alive.[11,12] Other misidentification-related delusions in AD include the conviction that one's house is not their own, that a member of the family is somebody else, a copy or an impostor (Capgras delusion), or that some uknown person is residing in their home (phantom hostage delusion).[13] Misinterpreting television images, mirror images or captured images for actual people or objects is a more typical symptom. With a median incidence of 23% in studies, illusions are one of the most prevalent psychotic symptoms in AD.[14]

Antipsychotics are perhaps the most commonly prescribed medicines for treating Alzheimer's disease psychosis (ADP). However, their effectiveness is limited at best, and the risk of major side effects remains a concern.[15] For example, risperidone and olanzapine, while used to reduce aggression in AD, have both been linked to significant detrimental cerebrovascular occurrences (such as stroke) and extrapyramidal adverse effects[16]; although quetiapine is frequently used, 2 meta-analyses showed no effect of quetiapine on neuropsychiatric symptoms (NPS).[17,18] Furthermore, all antipsychotics come with a warning label from the U.S. Food and Drug Administration (FDA) about increased mortality risk in Alzheimer's patients (FDA, 2005).[19] For all these reasons, treatment of ADP requires safer and more effective medication.

The ideal therapeutic approach requires a better understanding of the underlying neurobiological mechanisms. The pathological processes underlying AD are thought to be influenced by changes in the serotonergic neurotransmitter system. Studies showing deficiencies in serotonergic neurotransmission and a loss of dopamine-producing neurons and serotonin receptors in AD provide evidence for the role of serotonin in AD.[20–23] The 5-hydroxytryptaminergic system has long been implicated in the etiology of psychiatric disorders. Previous research suggests that excessive 5-hydroxytryptamine receptor 2A (5-HT2A receptor) activation may be disrupted, generating apical dendritic ion channel malfunction and dementia-related psychosis (DRP) clinical symptoms.[24] Furthermore, a postmortem analysis of cortical nerve cells obtained from AD patients revealed that Aβ plaques disrupt peri-somatic contact of 5-HT2A receptor-expressing parvalbumin-positive interneurons with γ-aminobutyric acid (GABA)ergic pyramidal neurons,[25] potentially promoting hyperactivity of neurons in contact with plaques.

Negative allosteric modulators of 5-HT2A receptors have been considered a potential treatment strategy for ADP, and these compounds, called negative allosteric modulators, have different inhibitory potencies ranging from almost neutral antagonists to full inverse agonists.[26] Notably, 5-HT2A receptors also have a powerful effect on cognitive activity in AD and, as an antipsychotic, 5-HT2A receptor inverse agonists and antagonists improve cognitive impairment. Thus, suppressing 5-HT2A receptor activity with selective 5-HT2A inverse agonists or 5-HT2A receptor inverse agonists might potentially provide a novel treatment strategy for ADP. Through a meta-analysis of all relevant randomized controlled trials (RCTs), we examined whether a negative regulation of 5-HT2A receptors promotes ADP.

Differences in results may be explained by the small sample sizes. A meta-analysis is a statistical method that uses both weighted and combined statistics to compute a meaningful statistic in aggregate and then uses the combined estimated statistics to perform tests and assessments.[27,28] By combining the results of many comparable individual studies, meta-analysis increases the strength of the argument for preliminary conclusions and the strength of analytical assessment of effects. By meta-analyzing all relevant RCTs, we examined whether negative regulation of 5-HT2A receptors alleviates ADP.

## Objectives

The study aimed to perform the first meta-analysis of randomized, double-blind, placebo-controlled trials investigating negative modulators of the 5-HT2A receptor as a cure for ADP.

## Materials and methods

This study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[29] We conducted a systematic literature search based on the PICO strategy (Patients: AD; Intervention: 5-HT2A receptor negative modulator; Control: placebo;

Outcome: Neuropsychiatric Inventory (NPI) total score (primary outcome)). Other prognostic indicators included Mini-Mental State Examination (MMSE), the Katz Index of Independence in Activities of Daily Living (KATZ), rate of discontinuation, and adverse events.

## Criteria for inclusion, search strategy, data extraction, and outcome measurement

Blinded and unblinded RCTs of 5-HT2A receptor-negative modulators (including 5-HT2A receptor antagonists and 5-HT2A receptor inverse agonists) in patients with ADP were included. Such drug combinations are essential to advance the development of antineuropsychiatric drugs as they treat the disease while avoiding many of the side effects caused by nonselective receptor interactions. To determine all potentially relevant research, searched 5 different databases: PubMed, MEDLINE, Cochrane Library, Embase, and Web of Science. We considered all studies published up to 6 January 2022, without language restrictions. The following categories were included in the search: "serotonin 2A antagonist" OR "5-HT2A antagonist" OR "negative allosteric modulators of 5-HT2A receptors" OR "5-HT2A receptor antagonists" OR "5-HT2A receptor inverse agonists" OR "ritanserin" OR "ketanserin" OR "mirtazapine" OR "nefazodone" OR "cyproheptadine" OR "trazodone" OR "eplivanserin" OR "pimavanserin" OR "volinanserin". For other applicable studies, we looked through the reference lists of pertinent papers.

The first 3 authors thoroughly examined the patient requirements in the reviewed studies. If the required data were missing, we contacted the author for more information. As a final step, the first 3 authors reviewed, extracted and entered the data into ReviewManager (RevMan) 5.3 (Cochrane, London, UK) independently. The methodological quality of the included articles was evaluated using Cochrane Risk Of Bias criteria (https://training.cochrane.org/online-learning/core-software/revman) We utilized the Cochrane statistical tool, Review Manager 5 (RevMan 2008; Cochrane), for quantitative tests.

## Data synthesis and statistical analyses

Each reported outcome measure was assessed in the 10 included studies. The primary outcome indicator of efficacy is the change in ADP symptoms measured using the NPI.[30] Secondary outcome indicators were changes in memory cognition and daily living ability measured using the MMSE[31] and the KATZ.[32] These outcomes were analyzed in terms of odds ratio (OR) and risk difference (RD).

RevMan v. 5.3 software was used for data analysis. Referring to the Cochrane Handbook for Systematic Evaluation 5.1.0,[33] RD was employed as the impact indicator for dichotomous variables, and it served as the effect indicator for continuous variables. The effect indicators for the continuous variables were OR and RD. The estimates and 95% confidence intervals (95% CIs) are provided for each effect. Heterogeneity was determined with the $\chi^2$ test and the $I^2$ test, and random effects model was used for the meta-analysis. The value of $p < 0.05$ was considered statistically significant.

# Results

## General information on the inclusion of the study

The database search identified a total of 375 papers, of which 270 were duplicates. After reading the article titles and abstracts, 45 studies were excluded due to being non-RCTs, as well as to study population, interventions and outcome indicators not meeting the inclusion criteria; also, more duplicates were eliminated. During the full-text screening, we excluded a further 65 studies due to being review articles and to inconsistencies in the statistical methods used. Ultimately, 10 RCTs were analyzed to evaluate inverse activation of the 5-HT2A binding site in AD (Fig. 1).[34–43]

The characteristics of the trials included in this study are shown in Table 1. Ten RCTs included 1986 patients with ADP. Two studies lasted 6 weeks, 2 studies lasted 2 weeks, 2 studies lasted 12 weeks, and there was 1 study lasting for each of the following periods: 13, 8, 24, and 26 weeks. The number of patients examined in the analyzed studies varied from 24 to 219 in total. The patients were, on average, 71 years old. The pharmaceutical industry supported 4 studies. Two studies were conducted in Brazil, 3 in the USA and the remaining 5 in the UK. To assess the methodological quality of all studies, we used the Cochrane Risk of Bias criteria (Fig. 2,3). All 10 studies used a randomization method and accounted for the specific method of randomization. The methodological quality of the included studies was high and the risk of bias was low with the Begg's test p-value equal to 0.376 for the OR and 0.473 for the risk ratio (RR) (Fig. 4).

## Results of the meta-analysis

Among the 10 included studies, 2 assessed each of the outcomes, with the gain in psychiatric symptoms measured with the total NPI score as the main indicator of efficacy. The MMSE scale was used for the examination of intellectual status and the KATZ was used for the examination of daily functioning. The ORs and RRs for the 10 included studies are summarized in Table 2.

## Results of the meta-analysis for primary and secondary outcomes

The current study has a pooled OR of 1.64 (95% CI: 1.01–2.65) with heterogeneity estimated at $Tau^2 = 0.52$, $\chi^2$ value

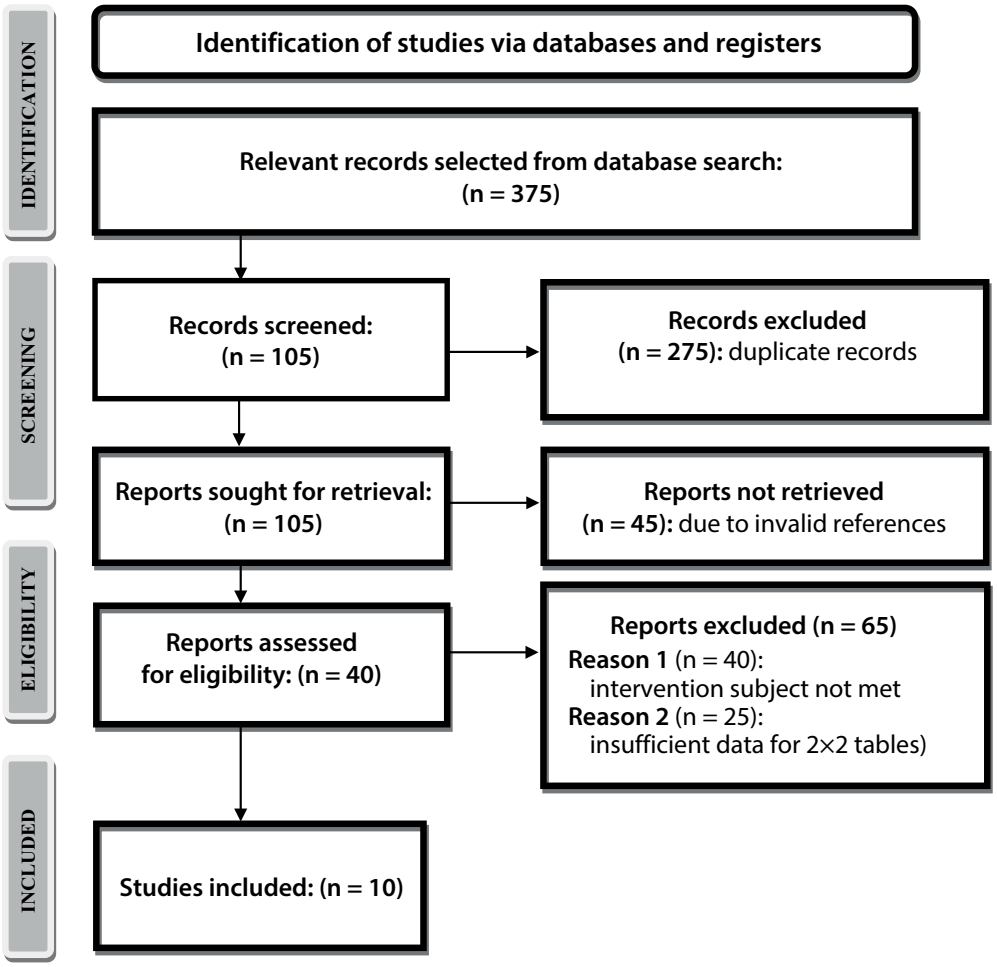



Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of literature screening

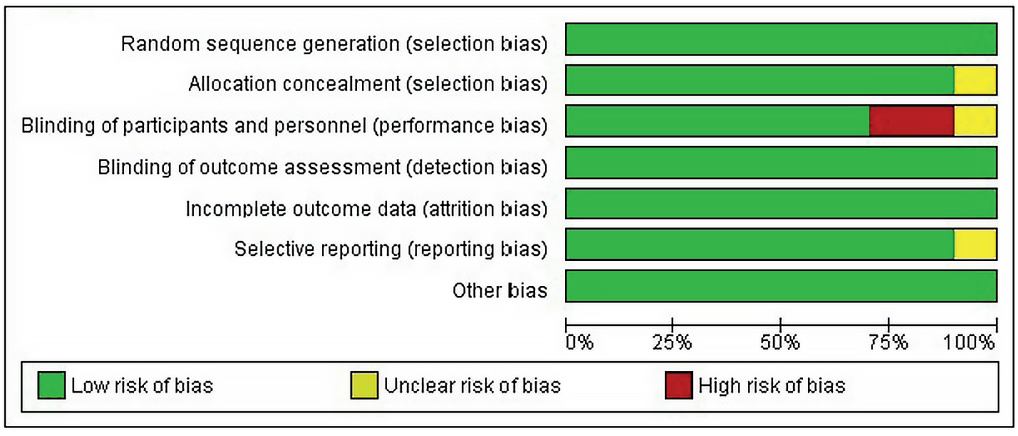



Fig. 2. Risk of bias graph

of 111.31, degrees of freedom (df) of 9, $I^2$ of 92%, z-value of 2.01, and p-value of 0.04 (<0.05) (Fig. 5). The pooled OR greater than 1 indicates that the positive outcomes are more likely and, therefore, that 5-HT2A receptor negative allosteric modulators are more effective in the treatment of ADP compared to placebo. Additionally, compared to placebo, 5-HT2A receptor antagonists significantly reduced the total NPI score, the MMSE score and the KATZ. The RD between the 5-HT2A receptor negative modulators and placebo groups was 0.12 (95% CI: 0.00–0.24) with heterogeneity of $Tau^2 = 0.03$, $\chi^2$ value of 127.23, df value of 9, $I^2$ value of 93%, z-value of 1.92, and p-value of 0.01 (<0.05). The pooled RD value of less than 1 indicates that an adverse outcome is less probable with 5-HT2A receptor negative allosteric modulators and that such modality is safe, when compared to placebo, for the treatment of ADP (Fig. 6). There were almost no statistically significant differences between the groups in the occurrence of side effects such as agitation, falls, aggression, anemia, urinary tract infection, contusion, blood urea increased, peripheral edema, cellulitis, anxiety, behavioral and psychiatric symptoms, blood potassium increase, and gastrointestinal issues.

**Table 1.** General characteristics of included trial

| Study ID and year | Total number of patients | Inclusion criteria | MMSE (baseline) | Duration [weeks] | Age [years] (M ±SD) | Drug, male sex [%] | Drug, dose | Number of patients in different groups | Outcomes |
|---|---|---|---|---|---|---|---|---|---|
| Ballard et al.[34] 2018 | 57 | patients with psychotic symptoms; a score ≥4 on either hallucination (frequency × severity) or delusions (frequency × severity) on the NPI-NH psychosis subscale or a total combined score ≥6 (hallucinations + delusions) | 10.3 ±5.4/9.8 ±5.0 | 6 | mirtazapine: 84.9 ±7.4 placebo: 85.9 ±5.3 | mirtazapine: 18.5 placebo: 16.7 | mirtazapine (34 mg) or placebo | mirtazapine: 27 placebo: 30 | mirtazapine = placebo: NPI-NH-H+D, CMAI-SF, ADCS- CGIC, ADCS-ADL |
| Banerjee et al.[35] 2011 | 219 | co-existing depression (≥4 weeks) | 18.2 ±7.4/17.6 ±6.0 | 13 | mirtazapine: 79 ±8.4 placebo: 79 ±8.8 | mirtazapine: 29 placebo: 36 | mirtazapine (150 mg) or placebo | mirtazapine: 117 placebo: 102 | mirtazapine = placebo: CSDD |
| Scoralick et al.[36] 2017 | 24 | AD patients with SDs | 12.0 ±6.7 | 2 | mirtazapine: 83.4 ±9.1 placebo: 80.8 ±5.4 | mirtazapine: 25 placebo: 25 | mirtazapine (15 mg) or placebo | mirtazapine: 16 placebo: 8 | mirtazapine = placebo: MMSE, KATZ |
| Camargos et al.[37] 2014 | 36 | AD patients with SDs, CDR: 2–3 | 11.2 ±6.2 | 2 | trazodone: 81.5 ±9 placebo: 80.5 ±5.5 | trazodone: 46.7 placebo: 20 | tradozone (50 mg) or placebo | trazodone: 15 placebo: 15 | trazodone = placebo: KATZ, MMSE |
| Banerjee et al.[38] 2021 | 204 | AD patients with SDs, CDR: 2–3 | 13.4 ±8.1/16.1 ±6.7 | 12 | mirtazapine: 82.2 ±7.8 placebo: 82.8 ±7.7 | mirtazapine: 25 placebo: 42 | mirtazapine (45 mg) | mirtazapine: 104 placebo: 100 | mirtazapine = placebo: CAMI, MMSE, NPI total score, NPI agitation/aggression subscore, NPI depression, anxiety, and irritability subscore |
| Ballard et al.[39] 2018 | 181 | age ≥50; the Jeste and Finke criteria for psychosis of AD; hallucinations or delusions domains of the NPI-NH ≥ 4 or (hallucinations + delusions) ≥6 | 10.3 ±5.4/9.8 ±5.0 | 6 | pimavanserin: 85.6 ±7 placebo: 86.1 ±6 | pimavanserin: 18 placebo: 20 | pimavanserin (17 mg) or placebo | pimavanserin: 97 placebo: 84 | pimavanserin = placebo: NPI- NH-H+D, NPI-NH total score, NPI-NH-A/A subscore, ADCS– ADL, CMAI-SF |
| Wilkinson et al.[40] 2014 | 278 | AD patients | 11.3 ±5.8 | 24 | idalopirdine: 80 ±2.11 placebo: 78 ±1.5 | idalopirdine: 50 placebo: 45 | idalopirdine (90 mg) or placebo | idalopirdine: 140 placebo: 120 | idalopirdine =placebo: ADAS-cog |
| Fullerton et al.[41] 2018 | 342 | AD patients | 10.78 ±2.8 | 12 | donepezil: 79 ±2.11 placebo: 78 ±2.11 | donepezil: 175 placebo: 80 | donepezil (5–10 mg) or placebo | donepezil: 190 placebo: 143 | donepezil = placebo: AD MMSE score |
| Nirogi et al.[42] 2022 | 537 | AD patients | 11.1 ±2.5 | 26 | masupirdine: 81 ±2.41 placebo: 80 ±1.81 | masupirdine: 241 placebo: 158 | masupirdine (50 mg) or placebo | masupirdine: 355 placebo: 122 | masupirdine = placebo: AD MMSE score, ADAS-cog 11 |
| Umbricht et al.[43] 2014 | 108 | AD patients with schizophrenia | 10.7 ±4.5 | 8 | RG3487: 82.16 ±1.31 placebo: 80.22 ±1.29 | RG3487: 40 placebo: 35 | mirtazapine (15 mg) or placebo | RG3487: 54 placebo: 53 | RG3487 = placebo: MMSE, MCCB composite t-scores |

AD – Alzheimer's disease; NPI-NH – Neuropsychiatric Inventory – Nursing Home Version; M ±SD – mean ± standard deviation; SDs – sleep disorders; CDR – Clinical Dementia Rating; MMSE – Mini-Mental State Examination; KATZ – Katz Index of Independence in Activities of Daily Living; ADAS-cog – Alzheimer's Disease Assessment Scale-cognitive subscale; CSDD – Cornell Scale for Depression in Dementia; NPI-NH-H+D –Neuropsychiatric Inventory Nursing Home Version–Hallucinations+Delusions; NPI-NH-A/A – Neuropsychiatric Inventory Nursing Home Version-Agitation/Aggression; ADCS-ADL –Alzheimer's Disease Cooperative Study-ADL instrument; CMAI-SF – Cohen–Mansfield Agitation Inventory-short form; ADCS-CGIC – Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; CMAI – Cohen–Mansfield Agitation Inventory; MCCB – MATRICS Consensus Cognitive Battery.

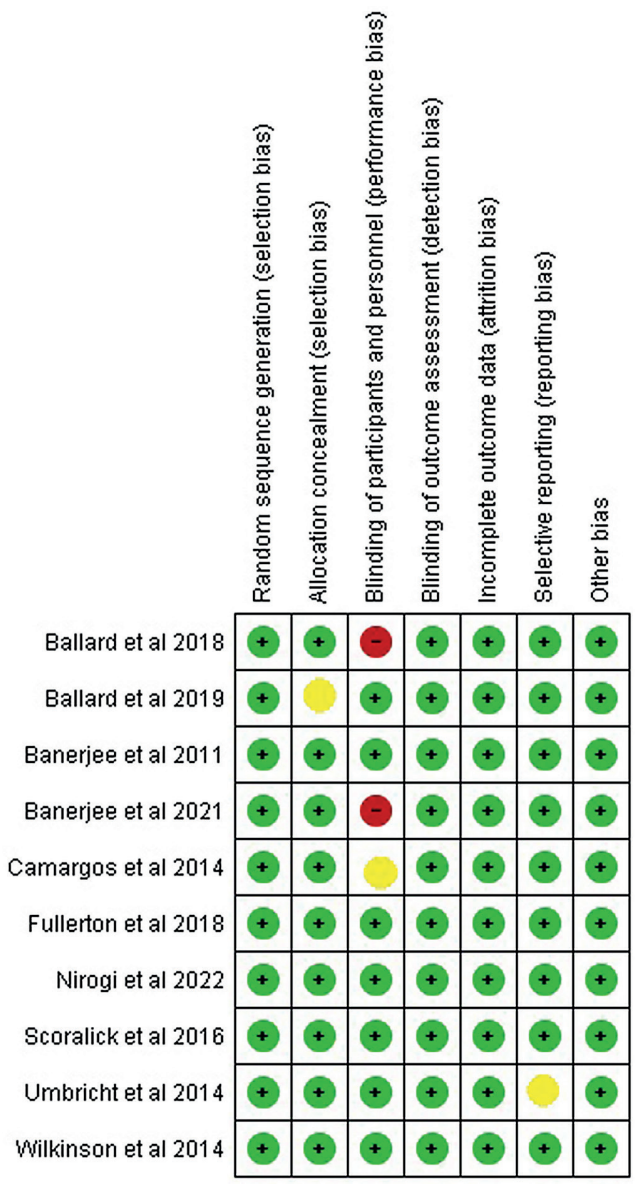



Fig. 3. Risk of bias summary

## Discussion

To the best of our knowledge, this is the first comprehensive meta-analysis of RCTs on the efficacy and tolerability of 5-HT2A receptor-negative modulators for the treatment of ADP. Of all the available 5-HT2A receptor-negative modulators, mirtazapine, trazodone, RG3487, donepezil, idalopirdine, masupirdine, and pimavanserin were studied

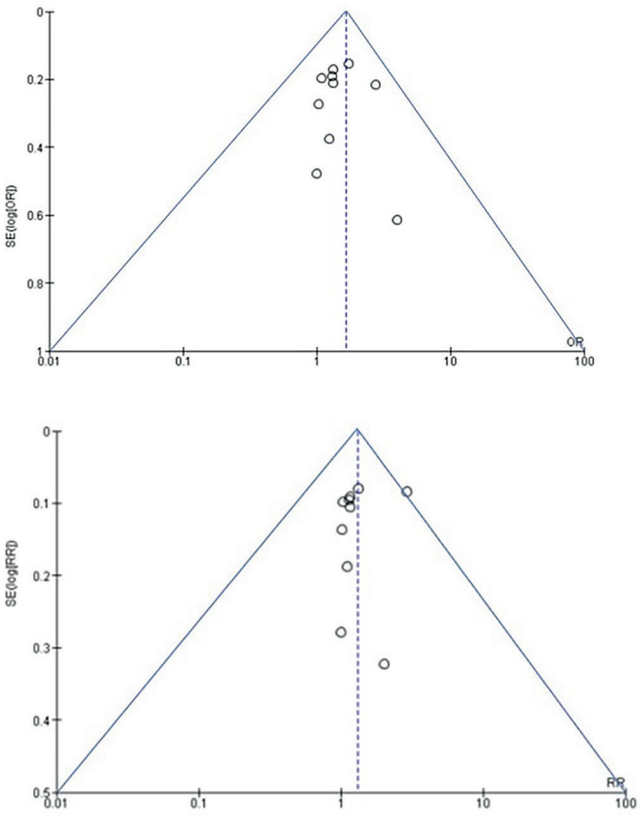



Fig. 4. Funnel plot for publication bias

SE – standard error; OR – odds ratio; RR – risk ratio.

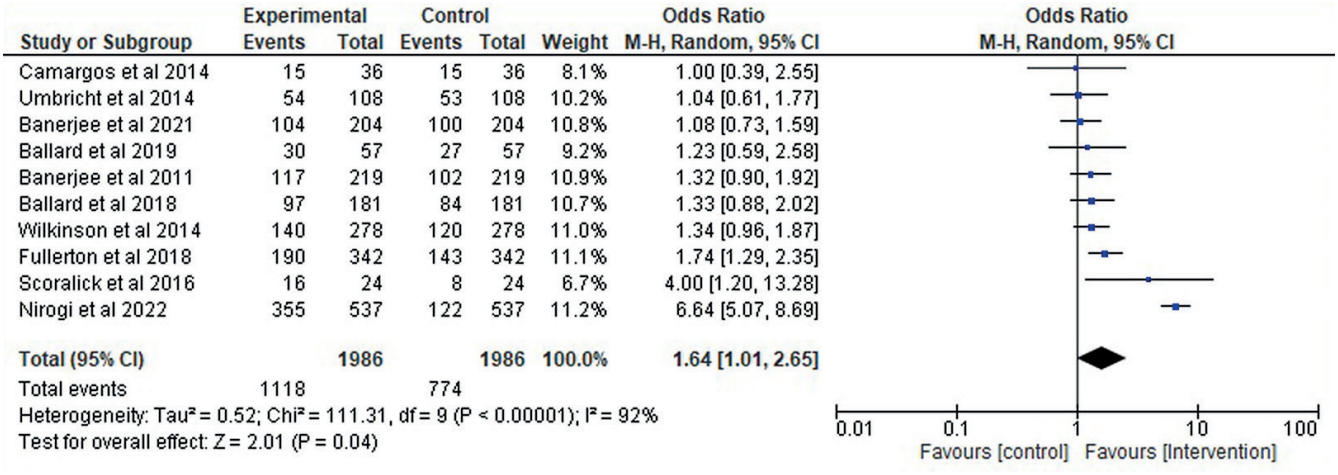



Fig. 5. Forest plot for odds ratio

95% CI – 95% confidence interval; df – degrees of freedom.

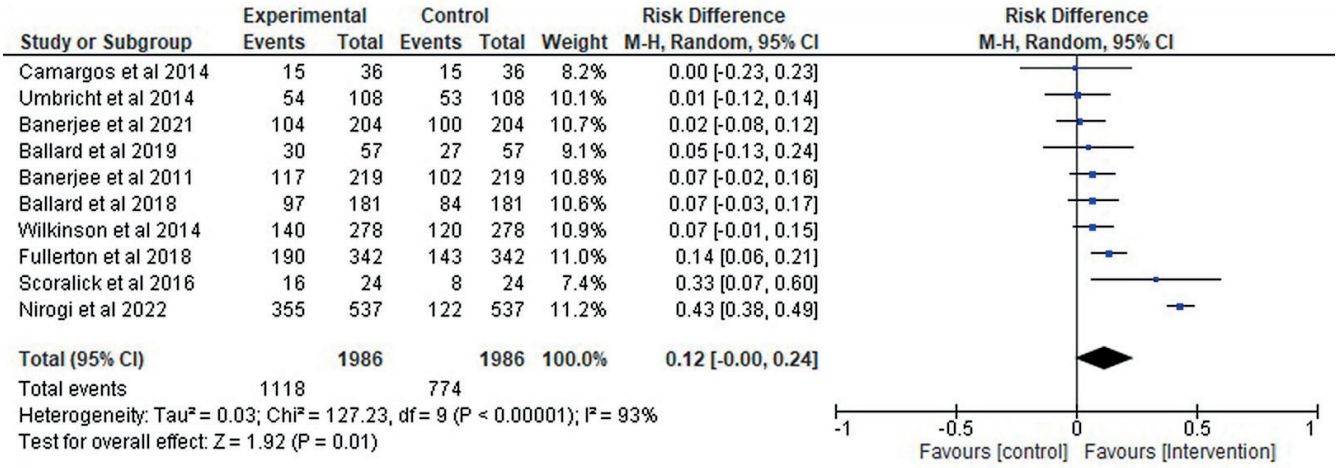



Fig. 6. Forest plot of risk difference

95% CI – 95% confidence interval; df – degrees of freedom.

Table 2. Statistical summary of the included studies

| Study ID and year | Odds ratio (95% CI) | Risk difference (95% CI) |
|---|---|---|
| Ballard et al.[34] 2018 | 1.23 (0.59–2.58) | 0.05 (–0.13–0.24) |
| Banerjee et al.[35] 2011 | 1.32 (0.90–1.92) | 0.07 (–0.02–0.16) |
| Scoralick et al.[36] 2017 | 4.00 (1.20–13.28) | 0.33 (0.07–0.60) |
| Camargos et al.[37] 2014 | 1.00 (0.39–2.55) | 0.00 (–0.23–0.23) |
| Banerjee et al.[38] 2021 | 1.08 (0.73–1.59) | 0.02 (–0.08–0.12) |
| Ballard et al.[39] 2018 | 1.33 (0.88–2.02) | 0.07 (–0.03–0.17) |
| Wilkinson et al.[40] 2014 | 1.34 (1.96–1.87) | 0.07 (–0.01–0.15) |
| Fullerton et al.[41] 2018 | 1.74 (1.29–2.35) | 0.14 (0.06–0.21) |
| Nirogi et al.[42] 2022 | 6.64 (5.07–8.69) | 0.43 (0.38–0.49) |
| Umbricht et al.[43] 2014 | 1.04 (0.61–1.77) | 0.01 (–0.12–0.14) |

95% CI – 95% confidence interval.

in this meta-analysis. The main finding of our study was that the 5-HT2A receptor-negative modulators improved the overall NPI scores of ADP patients more than the placebo. There were notable variations in MMSE score, KATZ and incidence of adverse events between the 2 groups. Although the effect size of the benefit for psychiatric symptoms was small, overall, this study adds to the evidence base suggesting a potential benefit from this type of medication for people with ADP. Pimavanserin has a different mechanism of action and a different safety profile compared to the other 6 drugs, including a beneficial effect among AD patients who exhibited more severe psychotic symptoms and, thus, providing a potentially unique advantage in this population. However, future longer and larger trials are needed to study the efficacy and safety of this class of drugs in treatment of ADP.

The total NPI score was the primary outcome indicator of our study. Compared to the placebo, the improved total NPI score in the treatment group of ADP patients suggests that the negative allosteric modulators of 5-HT2A receptors studied here, namely mirtazapine, trazodone, RG3487, donepezil, idalopirdine, masupirdine, and pimavanserin, may alleviate NPS in AD patients. Their mechanism of action may lie in the fact that, first, 5-HT2A receptors play a role in the secretion of amyloid precursor proteins, which may promote the formation of amyloid plaques in AD, such that the negative regulation of this receptor could influence the etiological basis of AD.[44–48] Second, several genetic polymorphisms associated with AD symptoms (such as hallucinations and delusions, which are the most common NPS) have been discovered that link the uptake of 5-HT2A receptors to the therapeutic management of psychiatric serotonin disorders.[44] Finally, the cerebral availability of the serotonin precursor plasma tryptophan level decreases along with the activation of the immune system, which is believed to be upregulated in AD.[49,50] However, between the 2 types of negative modulators of 5-HT2A, the efficacy of mirtazapine for the treatment of ADP is likely to be marginal. Participants of the pimavanserin study had more severe psychotic symptoms and worse cognitive dysfunction than those in the mirtazapine study. This also suggests that the effect of pimavanserin was significantly higher in the prespecified subgroup with more severe psychotic symptoms.

More notably, the 2 RCTs reported in detail the effects of pimavanserin on NPI subdomain scores, although the reported subdomains were not the same. One study showed a beneficial effect of pimavanserin on irritability/instability in the NPI subdomain, while the other showed a significant effect of pimavanserin on alleviating hallucinations and delusions in the Neuropsychiatric Inventory – Nursing

Home Version (NPI-NH) subdomain. Inverse agonists/antagonists of dopaminergic, muscarinic, histaminergic, and adrenergic receptors showed a minimal affinity for these receptors.[51] The discovery that most antipsychotic drugs work by antagonizing the 5-HT2A receptor led to the development of pimavanserin.[52] In the future, it is reasonable to assume that pimavanserin will be of greater value in the treatment of ADP. Furthermore, as the psychiatric symptomatology of ADP patients is diverse, future studies could examine the relationship between different symptomatology and drug efficacy, rather than just limiting it to a few symptomatologies of NPI.

Cognitive impairment is an important determinant of functional impairment in AD. In addition, several studies have shown that people with AD are more likely to experience NPS.[53,54] Cummings described the importance of appropriate interventions for psychiatric symptoms and hypothesized that reducing these symptoms might improve cognitive function in AD.[55] The MMSE is one of the most common scales used for evaluating cognitive dysfunction[56]; thus, we used this tool to investigate the effects of negative allosteric modulators of 5-HT2A receptors on the cognitive function of patients with ADP. In our study, negative allosteric modulators of 5-HT2A receptors did not affect cognitive function in patients with ADP compared to placebo. Cognitive function was only assessed using MMSE in the current studies related to negative allosteric modulators of 5-HT2A receptors, so we were unable to analyze this variable more closely. In addition, baseline factors may have an impact on the effect size of these drugs in the AD population. Therefore, future studies might attempt to explore the response of subgroups of participants with different MMSE baseline scores to these types of drugs and refine the ideal target group for ADP treatment.

The psychotic symptoms associated with AD can lead to excessive functional dependence. However, there are cognitive and behavioral predictors of progression rates in AD.[57,58] Longitudinal studies have demonstrated that psychotic symptoms were associated with a decline in the ability to execute tasks of daily living.[59–62] Tran et al. showed that, over time, the higher the frequency of psychotic symptoms resulted in a more rapid decline of the activity of daily living (ADL).[63,64] In light of previous studies, we chose the KATZ as an assessment tool for ADL and wanted to explore whether changes in psychiatric symptoms caused by this drug might affect the presentation of ADL. However, the KATZ did not reveal any significant differences between the 2 groups, i.e., the effect of these drugs on improving psychiatric symptoms does not add to their benefit in treating AD patients' ability to perform ADL. Although such medications do not improve ADL in patients with ADP, a better understanding of the variables associated with functional deterioration may guide the combination of medications or the development of new medications to promote independence and improve the quality of life in people with ADP and their caregivers.

Patients with AD should be cautious when taking antipsychotics, so we evaluated safety of this class of medication. Our findings showed no difference in the total number of adverse events between the 2 groups, with the most common adverse events being falls, urinary tract infections and agitation, suggesting that this medication may be a safe treatment for ADP. Additional studies are still needed to further elucidate the long-term safety of negative allosteric modulators of 5-HT2A receptors in patients with ADP and to assess tolerability related to aspects not included in this study.

To the best of our knowledge, this is the first complete meta-analysis of RCTs on the effect and tolerance of negative modulators of the 5-HT2A receptor for the treatment of ADP. We included 7 widely used receptor inverse agonists (mirtazapine, trazodone, RG3487, donepezil, idalopirdine, masupirdine, and pimavanserin). In addition to mirtazapine, trazodone and pimavanserin for patients with ADP, we discovered no RCTs on other 5-HT2A receptor-negative modulators. The primary finding of this study was that 5-HT2A receptor-negative modulators enhanced the total NPI score for individuals with AD much more than placebo. There was no significant difference in the MMSE score and the KATZ for patients compared to placebo, i.e., we did not observe a negative modulator effect on ADL and cognitive function. In addition, there were no differences in individual adverse events between the 5-HT2A receptor-negative modulators group and the placebo group. Our findings suggest that this new class of antipsychotics is effective in managing ADP and has an acceptable safety and tolerability profile.

Two RCTs reported the effects of pimavanserin on NPI subdomain scores, but the subdomains measured were not the same, so only descriptive analyses were presented. Pimavanserin treatment was shown to be beneficial for irritability/unsteadiness, but not for other NPI domains; another study showed significant efficacy for pimavanserin in improving the NPI-NH subdomains of hallucinations and delusions. Together, this evidence suggests that pimavanserin may have greater potential efficacy in improving psychiatric symptoms than other 5-HT2A receptor antagonists. In addition, the effects of other 5-HT2A receptor antagonists on the NPI subdomain will need to be reported in future trials. To better confirm the efficacy of 5-HT2A receptor antagonists on ADP, we suggest that future investigators rate NPS individually (e.g., the most common symptoms of hallucinations and delusions) or in multiple subdomains.

## Limitations

The limited number of included studies resulted in a funnel plot analysis being unable to detect publication bias. In addition, although the NPI-total effect is statistically

robust, the lack of pooled analysis of sub-domain results may diminish its clinical significance. The lack of overall ADL and MMSE benefits may be the result of scores from different patients with different clinical courses in various trials. Finally, AD is a progressive neurodegenerative disease, which may also affect the results when all data are finally pooled.

## Conclusions

Our findings show that negative allosteric modulators of 5-HT2A receptors (mirtazapine, trazodone, RG3487, donepezil, idalopirdine, masupirdine, and pimavanserin) have some benefits over placebo in the treatment of ADP. According to studies evaluating the drug, the most promising treatment for ADP seems to be pimavanserin. However, psychosis relief should be balanced against possible neurologic, metabolic and cardiovascular adverse effects. It is reasonable to consider negative allosteric modulators of 5-HT2A receptors as a possible treatment option given their safety and tolerability profiles. It is difficult, however, to draw any conclusions regarding the added value of using negative allosteric modulators of 5-HT2A receptors or other types of atypical antipsychotics. These drugs need to be evaluated for their efficacy and tolerability in ADP in greater detail. By doing so, we will be able to provide better guidance on how to use the medications in patients with APD.

### ORCID iDs

Yuxiao Chen https://orcid.org/0000-0002-5423-588X
Jie Sun https://orcid.org/0009-0002-6202-8693
Zhen Yang https://orcid.org/0000-0002-6851-5874
Hongyan Bi https://orcid.org/0000-0002-6032-8759

### References

1. Desale SE, Chidambaram H, Chinnathambi S. G-protein coupled receptor, PI3K and Rho signaling pathways regulate the cascades of Tau and amyloid-β in Alzheimer's disease. *Mol Biomed*. 2021;2(1):17. doi:10.1186/s43556-021-00036-1
2. Wang EJ, Wu MY, Lu JH. Ferulic acid in animal models of Alzheimer's disease: A systematic review of preclinical studies. *Cells*. 2021;10(10): 2653. doi:10.3390/cells10102653
3. Du Z, Li Y, Li J, Zhou C, Li F, Yang X. Physical activity can improve cognition in patients with Alzheimer's disease: A systematic review and meta-analysis of randomized controlled trials. *Clin Interv Aging*. 2018;13:1593–1603. doi:10.2147/CIA.S169565
4. Blennow K, Zetterberg H. Biomarkers for Alzheimer's disease: Current status and prospects for the future. *J Intern Med*. 2018;284(6): 643–663. doi:10.1111/joim.12816
5. Haupt M, Kurz A, Jänner M. A 2-year follow-up of behavioural and psychological symptoms in Alzheimer's disease. *Dement Geriatr Cogn Disord*. 2000;11(3):147–152. doi:10.1159/000017228
6. Kales HC, Gitlin LN, Lyketsos CG. Assessment and management of behavioral and psychological symptoms of dementia. *BMJ*. 2015; 350:h369. doi:10.1136/bmj.h369
7. Mason VL. Alzheimer's Association International Conference on Alzheimer's Disease 2015 (AAIC 2015) (July 18–23, 2015 – Washington, D.C., USA). *Drugs Today*. 2015;51(7):447–452. doi:10.1358/dot.2015.51.7.2375989
8. Qiu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer's disease: Occurrence, determinants, and strategies toward intervention. *Dialogues Clin Neurosci*. 2009;11(2):111–128. doi:10.31887/DCNS.2009.11.2/cqiu
9. Wolf MU, Goldberg Y, Freedman M. Aggression and agitation in dementia. *Continuum (Minneap Minn)*. 2018;24(3):783–803. doi:10.1212/CON.0000000000000605
10. Kong EH. Agitation in dementia: Concept clarification. *J Adv Nurs*. 2005;52(5):526–536. doi:10.1111/j.1365-2648.2005.03613.x
11. Scarmeas N, Brandt J, Albert M, et al. Delusions and hallucinations are associated with worse outcome in Alzheimer disease. *Arch Neurol*. 2005;62(10):1601–1608. doi:10.1001/archneur.62.10.1601
12. Ropacki SA, Jeste DV. Epidemiology of and risk factors for psychosis of Alzheimer's disease: A review of 55 studies published from 1990 to 2003. *Am J Psychiatry*. 2005;162(11):2022–2030. doi:10.1176/appi.ajp.162.11.2022
13. Tariot P, Mack J, Patterson M, et al. The behavior rating scale for dementia of the Consortium to Establish a Registry for Alzheimer's Disease. The Behavioral Pathology Committee of the Consortium to Establish a Registry for Alzheimer's Disease. *Am J Psychiatry*. 1995; 152(9):1349–1357. doi:10.1176/ajp.152.9.1349
14. Rubin EH, Drevets WC, Burke WJ. The nature of psychotic symptoms in senile dementia of the Alzheimer type. *J Geriatr Psychiatry Neurol*. 1988;1(1):16–20. doi:10.1177/089198878800100104
15. Ballard CG, Waite J, Birks J. Atypical antipsychotics for aggression and psychosis in Alzheimer's disease. *Cochrane Database Syst Rev*. 2006;12(12):CD013304. doi:10.1002/14651858.CD003476.pub2
16. Creese B, Da Silva MV, Johar I, Ballard C. The modern role of antipsychotics for the treatment of agitation and psychosis in Alzheimer's disease. *Exp Rev Neurother*. 2018;18(6):461–467. doi:10.1080/14737175.2018.1476140
17. Battle CE, Abdul-Rahim AH, Shenkin SD, Hewitt J, Quinn TJ. Cholinesterase inhibitors for vascular dementia and other vascular cognitive impairments: A network meta-analysis. *Cochrane Database Syst Rev*. 2021;2(2):CD013306. doi:10.1002/14651858.CD013306.pub2
18. Bui Q. Antidepressants for agitation and psychosis in patients with dementia. *Am Fam Physician*. 2012;85(1):20–22. PMID:22230303.
19. Shah S, Reichman WE. Treatment of Alzheimer's disease across the spectrum of severity. *Clin Interv Aging*. 2006;1(2):131–142. doi:10.2147/ciia.2006.1.2.131
20. Micheli D, Bonvicini C, Rocchi A, et al. No evidence for allelic association of serotonin 2A receptor and transporter gene polymorphisms with depression in Alzheimer disease. *J Alzheimers Dis*. 2006;10(4): 371–378. doi:10.3233/JAD-2006-10405
21. Cross AJ, Crow TJ, Ferrier IN, Johnson JA, Bloom SR, Corsellis JAN. Serotonin receptor changes in dementia of the Alzheimer type. *J Neurochem*. 1984;43(6):1574–1581. doi:10.1111/j.1471-4159.1984.tb06081.x
22. Lai MK, Tsang SW, Alder JT, et al. Loss of serotonin 5-HT2A receptors in the postmortem temporal cortex correlates with rate of cognitive decline in Alzheimer's disease. *Psychopharmacology*. 2005; 179(3):673–677. doi:10.1007/s00213-004-2077-2
23. Polesskaya OO, Sokolov BP. Differential expression of the C and T alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. *J Neurosci Res*. 2002;67(6):812–822. doi:10.1002/jnr.10173
24. Cummings JL, Devanand DP, Stahl SM. Dementia-related psychosis and the potential role for pimavanserin. *CNS Spectr*. 2022;27(1):7–15. doi:10.1017/S1092852920001765
25. Van Steenwinckel J, Noghero A, Thibault K, Brisorgueil MJ, Fischer J, Conrath M. The 5-HT2A receptor is mainly expressed in nociceptive sensory neurons in rat lumbar dorsal root ganglia. *Neuroscience*. 2009;161(3):838–846. doi:10.1016/j.neuroscience.2009.03.087
26. Lu J, Zhang C, Lv J, et al. Antiallergic drug desloratadine as a selective antagonist of $5HT_{2A}$ receptor ameliorates pathology of Alzheimer's disease model mice by improving microglial dysfunction. *Aging Cell*. 2021;20(1):e13286. doi:10.1111/acel.13286
27. Cohn LD, Becker BJ. How meta-analysis increases statistical power. *Psychol Methods*. 2003;8(3):243–253. doi:10.1037/1082-989X.8.3.243
28. Cipriani A, Koesters M, Furukawa TA, et al. Duloxetine versus other anti-depressive agents for depression. *Cochrane Database Syst Rev*. 2012;10:CD006533. doi:10.1002/14651858.CD006533.pub2

29. Moher D, Liberati A, Tetzlaff J, Altman DG; The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med*. 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097
30. Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The neuropsychiatric inventory: Comprehensive assessment of psychopathology in dementia. *Neurology*. 1994;44(12):2308–2314. doi:10.1212/WNL.44.12.2308
31. Jia X, Wang Z, Huang F, et al. A comparison of the Mini-Mental State Examination (MMSE) with the Montreal Cognitive Assessment (MoCA) for mild cognitive impairment screening in Chinese middle-aged and older population: A cross-sectional study. *BMC Psychiatry*. 2021;21(1):485. doi:10.1186/s12888-021-03495-6
32. Ciesla JR, Shi L, Stoskopf CH, Samuels ME. Reliability of Katz's Activities of Daily Living Scale when used in telephone interviews. *Eval Health Prof*. 1993;16(2):190–203. doi:10.1177/016327879301600204
33. Tawfik GM, Dila KAS, Mohamed MYF, et al. A step by step guide for conducting a systematic review and meta-analysis with simulation data. *Trop Med Health*. 2019;47:46. doi:10.1186/s41182-019-0165-6
34. Ballard C, Youakim JM, Coate B, Stankovic S. Pimavanserin in Alzheimer's disease psychosis: Efficacy in patients with more pronounced psychotic symptoms. *J Prev Alzheimers Dis*. 2018;6(1):27–33. doi:10.14283/jpad.2018.30
35. Banerjee S, Hellier J, Dewey M, et al. Sertraline or mirtazapine for depression in dementia (HTA-SADD): A randomised, multicentre, double-blind, placebo-controlled trial. *Lancet*. 2011;378(9789):403–411. doi:10.1016/S0140-6736(11)60830-1
36. Scoralick FM, Louzada LL, Quintas JL, Naves JOS, Camargos EF, Nóbrega OT. Mirtazapine does not improve sleep disorders in Alzheimer's disease: Results from a double-blind, placebo-controlled pilot study. *Psychogeriatrics*. 2017;17(2):89–96. doi:10.1111/psyg.12191
37. Camargos EF, Louzada LL, Quintas JL, Naves JOS, Louzada FM, Nóbrega OT. Trazodone improves sleep parameters in Alzheimer disease patients: A randomized, double-blind, and placebo-controlled study. *Am J Geriatr Psychiatry*. 2014;22(12):1565–1574. doi:10.1016/j.jagp.2013.12.174
38. Banerjee S, High J, Stirling S, et al. Study of mirtazapine for agitated behaviours in dementia (SYMBAD): A randomised, double-blind, placebo-controlled trial. *Lancet*. 2021;398(10310):1487–1497. doi:10.1016/S0140-6736(21)01210-1
39. Ballard C, Banister C, Khan Z, et al. Evaluation of the safety, tolerability, and efficacy of pimavanserin versus placebo in patients with Alzheimer's disease psychosis: A phase 2, randomised, placebo-controlled, double-blind study. *Lancet Neurol*. 2018;17(3):213–222. doi:10.1016/S1474-4422(18)30039-5
40. Wilkinson D, Windfeld K, Colding-Jørgensen E. Safety and efficacy of idalopirdine, a 5-HT 6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): A randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*. 2014;13(11):1092–1099. doi:10.1016/S1474-4422(14)70198-X
41. Fullerton T, Binneman B, David W, et al. A phase 2 clinical trial of PF-05212377 (SAM-760) in subjects with mild to moderate Alzheimer's disease with existing neuropsychiatric symptoms on a stable daily dose of donepezil. *Alzheimers Res Ther*. 2018;10(1):38. doi:10.1186/s13195-018-0368-9
42. Nirogi R, Ieni J, Goyal VK, et al. Effect of masupirdine (SUVN-502) on cognition in patients with moderate Alzheimer's disease: A randomized, double-blind, phase 2, proof-of-concept study. *Alzheimers Dement (N Y)*. 2022;8(1):e12307. doi:10.1002/trc2.12307
43. Umbricht D, Keefe RS, Murray S, et al. A randomized, placebo-controlled study investigating the nicotinic α7 agonist, RG3487, for cognitive deficits in schizophrenia. *Neuropsychopharmacology*. 2014;39(7):1568–1577. doi:10.1038/npp.2014.17
44. Lee RKK, Knapp S, Wurtman RJ. Prostaglandin $E_2$ stimulates amyloid precursor protein gene expression: Inhibition by immunosuppressants. *J Neurosci*. 1999;19(3):940–947. doi:10.1523/JNEUROSCI.19-03-00940.1999
45. Lee RKK, Wurtman RJ. Regulation of APP synthesis and secretion by neuroimmunophilin ligands and cyclooxygenase inhibitors. *Ann N Y Acad Sci*. 2006;920(1):261–268. doi:10.1111/j.1749-6632.2000.tb06934.x
46. Nitsch RM, Deng M, Growdon JH, Wurtman RJ. Serotonin 5-HT2a and 5-HT2c receptors stimulate amyloid precursor protein ectodomain secretion. *J Biol Chem*. 1996;271(8):4188–4194. doi:10.1074/jbc.271.8.4188
47. Nitsch RM, Wurtman RJ, Growdon JH. Regulation of APP processing potential for the therapeutical reduction of brain amyloid burden. *Ann N Y Acad Sci*. 1996;777(1):175–182. doi:10.1111/j.1749-6632.1996.tb34416.x
48. Holmes C. 5-HT2A and 5-HT2C receptor polymorphisms and psychopathology in late onset Alzheimer's disease. *Hum Mol Genet*. 1998;7(9):1507–1509. doi:10.1093/hmg/7.9.1507
49. Akiyama H. Inflammation and Alzheimer's disease. *Neurobiol Aging*. 2000;21(3):383–421. doi:10.1016/S0197-4580(00)00124-X
50. Bonaccorso S, Lin A, Song C, et al. Serotonin-immune interactions in elderly volunteers and in patients with Alzheimer's disease (DAT): Lower plasma tryptophan availability to the brain in the elderly and increased serum interleukin-6 in DAT. *Aging Clin Exp Res*. 1998;10(4):316–323. doi:10.1007/BF03339794
51. Vanover KE, Weiner DM, Makhay M, et al. Pharmacological and behavioral profile of N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N′-(4-(2-methylpropyloxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel 5-hydroxytryptamine(2A) receptor inverse agonist. *J Pharmacol Exp Ther*. 2006;317(2):910–918. doi:10.1124/jpet.105.097006
52. Hacksell U, Burstein ES, McFarland K, Mills RG, Williams H. On the discovery and development of pimavanserin: A novel drug candidate for Parkinson's psychosis. *Neurochem Res*. 2014;39(10):2008–2017. doi:10.1007/s11064-014-1293-3
53. Mortimer JA, Ebbitt B, Jun SP, Finch MD. Predictors of cognitive and functional progression in patients with probable Alzheimer's disease. *Neurology*. 1992;42(9):1689–1689. doi:10.1212/WNL.42.9.1689
54. Wilson RS. Hallucinations, delusions, and cognitive decline in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2000;69(2):172–177. doi:10.1136/jnnp.69.2.172
55. Cummings J. New approaches to symptomatic treatments for Alzheimer's disease. *Mol Neurodegeneration*. 2021;16(1):2. doi:10.1186/s13024-021-00424-9
56. Yoelin AB, Saunders NW. Score disparity between the MMSE and the SLUMS. *Am J Alzheimers Dis Other Demen*. 2017;32(5):282–288. doi:10.1177/1533317517705222
57. Buccione I, Perri R, Carlesimo GA, et al. Cognitive and behavioural predictors of progression rates in Alzheimer's disease. *Eur J Neurol*. 2007;14(4):440–446. doi:10.1111/j.1468-1331.2007.01693.x
58. Negovanska V, Hergueta T, Guichart-Gomez E, Dubois B, Sarazin M, Bungener C. Bénéfice d'un programme cognitivo-comportemental et pluridisciplinaire de prise en charge de la maladie d'Alzheimer sur l'anxiété du conjoint : Étude française ELMMA. *Rev Neurol*. 2011;167(2):114–122. doi:10.1016/j.neurol.2010.07.002
59. Bidzan M, Bidzan L. Neurobehavioral manifestation in early period of Alzheimer disease and vascular dementia [in Polish]. *Psychiatr Pol*. 2014;48(2):319–330. PMID:25016769.
60. Lopez OL, Becker JT, Sweet RA, et al. Psychiatric symptoms vary with the severity of dementia in probable Alzheimer's disease. *J Neuropsychiatry Clin Neurosci*. 2003;15(3):346–353. doi:10.1176/jnp.15.3.346
61. de Oliveira FF, de Almeida SS, Chen ES, Smith MC, Naffah-Mazzacoratti M da G, Bertolucci PHF. Lifetime risk factors for functional and cognitive outcomes in patients with Alzheimer's disease. *J Alzheimers Dis*. 2018;65(4):1283–1299. doi:10.3233/JAD-180303
62. Stern Y, Albert M, Brandt J, et al. Utility of extrapyramidal signs and psychosis as predictors of cognitive and functional decline, nursing home admission, and death in Alzheimer's disease: Prospective analyses from the Predictors Study. *Neurology*. 1994;44(12):2300–2307. doi:10.1212/WNL.44.12.2300
63. Tran M, Bédard M, Dubois S, Weaver B, Molloy DW. The influences of psychotic symptoms on the activities of daily living of individuals with Alzheimer disease: A longitudinal analysis. *Aging Ment Health*. 2013;17(6):738–747. doi:10.1080/13607863.2013.770446
64. Tran M, Bédard M, Molloy DW, Dubois S, Lever JA. Associations between psychotic symptoms and dependence in activities of daily living among older adults with Alzheimer's disease. *Int Psychogeriatr*. 2003;15(2):171–179. doi:10.1017/S1041610203008858

# Meta-analysis of outcomes related to the quality of life after orthodontic-surgical treatment

Xiangyu Li[A–F], Weiying Zheng[A–F], Xi Zhang[A–F], Yuwen Zhang[A–F]

Department of Dentistry, Jiaxing Second Hospital, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1099–1111

**Address for correspondence**
Yuwen Zhang
E-mail: Zhangyuwen_zyw@outlook.com

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on October 19, 2022
Reviewed on December 20, 2022
Accepted on February 21, 2023

Published online on April 19, 2023

## Abstract

Orthognathic surgery causes functional and aesthetic changes, which could affect patients' quality of life (QOL). The current analysis assessed the impact of orthodontic-surgical treatment on the parameters affecting the QOL using different scoring systems. The criteria for inclusion were studies written in various languages that compared the effects of the intervention on patients' QOL before surgery and at various periods after surgery (3 weeks to several months), which resulted in including 19 studies into this meta-analysis. The outcomes of these studies underwent random-effect modeling to calculate the mean difference (MD) and 95% confidence intervals (95% CIs) of the impact of different surgical techniques on clinical parameters, and publication bias was analyzed with Begg's test. According to the total score of the Orthognathic Quality of Life Questionnaire (OQLQ), surgery significantly affected patients' QOL after 2 months or less ($p = 0.049$), up to 6 months ($p < 0.001$), and when comparing 2 months or less with up to 6 months (2–6 months) ($p < 0.001$). In addition, the total Oral Health Impact Profile-14 (OHIP-14) score showed a significant difference in the QOL after 6 months ($p = 0.003$) and up to 12 months ($p = 0.002$) after surgery. Therefore, orthodontic-surgical treatment significantly improves patients' QOL after surgery compared to before surgery.

**Key words:** quality of life, OHIP-14, oral function, orthodontic surgery, OQLQ

**Cite as**
Li X, Zheng W, Zhang X, Zhang Y. Meta-analysis of outcomes related to the quality of life after orthodontic-surgical treatment. *Adv Clin Exp Med*. 2023;32(10):1099–1111. doi:10.17219/acem/161490

**DOI**
10.17219/acem/161490

# Introduction

Orthodontic surgery consisting of a combination of orthognathic surgery and orthodontic treatment is the optimal method for correcting dentofacial deformities,[1–3] and results in the harmonization of the facial skeletal structure and anatomical–functional adjustments by rearranging the maxillary bones.[4] Therefore, such treatment addresses biological, functional and aesthetic considerations.[5] The treatment consists of several phases, including the initial planning, orthodontic preparation, orthognathic surgical repositioning of the facial skeleton, postsurgical orthodontic refinement, and the containment phase that follows the removal of the fixed orthodontic appliance.[3,6]

The World Health Organization (WHO) defines the quality of life (QOL) as "the individual's perception of his or her position in life in the context of the culture and value system in which he lives and in relation to his goals, expectations, standards, and concerns."[7] However, this is a broad and comprehensive concept that is affected in a complex way by physical health, psychological state, social relationships, and environment.[7] The assessment of QOL is a rapidly expanding research area in the fields of medicine and dentistry,[8] which have widely investigated the impact of various conditions on patients.[9] Orthognathic surgery, by altering facial aesthetics, can affect personal attitudes, social attitudes and behaviors as it involves changes in the patients' functional and aesthetic aspects.[10,11] These alterations may be associated with patient-reported changes in QOL levels.[9]

Dental problems can affect several factors, such as personal characteristics, personality traits[12,13] and QOL.[14–17] For instance, malocclusion detrimentally affects QOL, social interaction, interpersonal relationships, and psychological health.[14] In addition, malocclusion had an increased detrimental effect on social wellbeing.[15] Furthermore, one study found a correlation between skeletal malocclusion and myofascial pain, major depression, and chronic pain.[16] Moreover, those with malocclusion may experience low self-esteem and social stigma.[17]

The term "body image" refers to a multi-faceted concept encompassing how a person perceives and conceptualizes their physical form.[18] Dissatisfaction with personal physical appearance often stems from an inaccurate self-perception of physical characteristics. Among the validated numerical questionnaires assessing QOL is the Orthognathic Quality of Life Questionnaire (OQLQ) and Oral Health Impact Profile-14 (OHIP-14). The OQLQ has 22 questions on a 5-point Likert scale ranging from "does not bother me at all" (score 0) to "bothers me a lot" (score 4). The possible total range of points is 0–88, with a lower score preferable.[19] Meanwhile, the responses for the OHIP-14 questionnaire use a 5-point Likert scale including 0 – never, 1 – hardly ever, 2 – occasionally, 3 – fairly often, and 4 – very often/every day. Higher OHIP-14 scores indicate a worse QOL, and lower scores indicate a better QOL.[20]

# Objectives

The current study aimed to assess the impact of orthodontic surgery on a patient's QOL compared to pre-surgery using numerical scores from validated questionnaires (OHIP-14 and OQLQ).

# Materials and methods

## Study design

This meta-analysis of clinical trials was included in the epidemiological declaration and followed a predetermined study design. Data collection and analysis encompassed the following databases: OVID, PubMed, Cochrane Library, Embase, and Google Scholar.

## Data pooling

Studying the consequences and outcomes of surgery required the analysis of clinical investigations concentrating on assessing the influence of orthodontic surgery on QOL indicators using validated numerical scoring systems to compare post-surgery and pre-surgery scores. Only human-related studies were included, regardless of language and sample size. However, noninterventional research, such as reviews, editorials and letters, was excluded. Figure 1 depicts the entire study identification process.

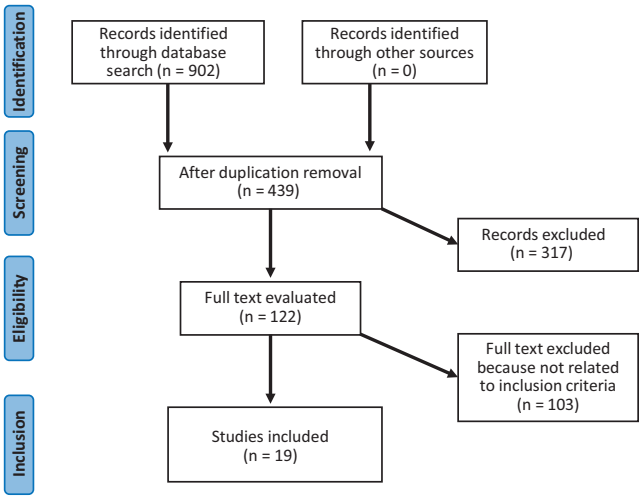



Fig. 1. Flowchart of the study

## Eligibility and inclusion criteria

A summary was created by analyzing the impact of orthodontic surgery treatments on postoperative outcomes and QOL scores compared to preoperative scores.

Only studies describing the impact of interventions on the parameters related to oral function, social impact,

physical pain, and awareness, and those which used validated questionnaires were included in the sensitivity analysis.

The following criteria had to be met for an article to be considered for inclusion in the meta-analysis:

1. Studies in which patients with dentofacial abnormalities underwent orthodontic-surgical treatment;
2. Studies that used validated questionnaires to assess the QOL of patients before and after surgery or compared such patients to a control group free from any dentofacial deformity;
3. No restrictions were placed on the type of malocclusion, gender, age, ethnicity, study language, or publication date;
4. The intervention regimen compared postoperative outcomes with preoperative QOL parameters using different scoring systems.

The exclusion criteria were:

1. Studies that failed to use a numerical score to assess QOL based on different parameters;
2. Studies on subjects suffering from syndromes, such as dentofacial malformations or orofacial neoplasia, and those with cleft palates or exclusively affected on the lip or palate;
3. Studies on patients who did not undergo orthognathic surgery;
4. Studies not reporting QOL-related parameters after surgery compared to before surgery.

## Identification

The Population, Intervention, Comparison, Outcome, and Study Design (PICOS) principle was used to outline a protocol of search strategies for: P (population) – orthodontic surgery subjects; I (intervention/exposure) – orthodontic surgery; and C (comparison) – various QOL parameters scored numerically using questionnaires (OHIP-14 and OQLQ). Oral function, social, body and psychological aspects, and awareness-related features are all study outcomes. Research design refers to whether or not clinical investigations were random.

A thorough search of the Cochrane Library, PubMed, OVID, Embase, and Google Scholar databases up until July 2022 was conducted using the keywords and related terms provided in Table 1. The titles and abstracts of all publications collated into a reference managing program were reviewed. Any research that did not establish a connection between orthodontic surgeries and their impact on quality of life was excluded from the study. Two authors (XL and XZ) served as reviewers to find relevant papers.

## Screening

The following criteria were used to narrow down the data: study-related features in a standard format, the surname of the first author, the duration of the study, the year of publication, the country of the study, the design of the study, the population type recruited, the total number of subjects, demographic information, clinical and treatment characteristics, the information source, and the outcome. Each study was checked for bias, and the methodological quality of the chosen studies was rated by 2 authors (WZ and YZ) in a blinded manner.

Table 1. Search strategy for each database

| Database | Search strategy |
|---|---|
| Cochrane Library | #1 "orthodontic": ti,ab,kw OR "surgery": ti,ab,kw (word variations have been searched)<br>#2 "quality of life": ti,ab,kw OR "oral function": ti,ab,kw (word variations have been searched)<br>#3 #1 AND #2 |
| PubMed | #1 "orthodontic" [MeSH terms] OR "surgery" [all fields]<br>#2 "quality of life" [MeSH terms] OR "oral function" [all fields]<br>#3 #1 AND #2 |
| OVID | #1 "orthodontic" [all fields] OR "surgery" [all fields]<br>#2 "quality of life" [all fields] OR "oral function" [all fields]<br>#3 #1 AND #2 |
| Google Scholar | #1 "orthodontic" OR "surgery"<br>#2 "quality of life" OR "oral function"<br>#3 #1 AND #2 |
| Embase | #1 "orthodontic"/exp OR "surgery"<br>#2 "quality of life"/exp OR "oral function"<br>#3 #1 AND #2 |

MeSH – medical subject headings; ti,ab,kw – terms in either title or abstract or keyword fields; exp – exploded indexing term.

## Statistical analyses

Data analysis employed Jamovi 2.3 software (https://www.jamovi.org/download.html).[21] The analysis utilized the standardized mean difference (MD) to measure outcomes by subtracting preoperative values from postoperative values and subtracting scores at 2 months from scores at 6 months post-surgery. Lower values indicated better QOL for both scoring systems. A random-effects model was fitted to the data, and the constrained maximum likelihood estimator calculated the level of heterogeneity ($\tau^2$). In addition to the $\tau^2$ estimate, the Q-test for heterogeneity and the $I^2$ statistic are provided. Any level of heterogeneity identified (i.e., $\tau^2 > 0$ regardless of the Q-test findings) led to the calculation of a prediction interval for the true outcomes. Studentized residuals and Cook's distances allowed to determine whether the studies may be outliers and their importance within the model. Studies with a studentized residual greater than $100 \times (1 - 0.05/(2 \times \text{kth}))$ percentile of a standard normal distribution were regarded as potential outliers (using the Bonferroni correction with two-sided $\alpha = 0.05$ for included studies). Studies considered influential had a Cook's distance greater than the median plus 6 times the interquartile range (IQR) of Cook's distances. Utilizing the standard error of the observed results as a predictor, the rank correlation test and the regression test examined the possibility of publication bias.

# Results

After reviewing 902 relevant studies, 19 studies published between 2008 and 2022 fit the inclusion criteria.[7,22–39] Table 2 summarizes the review findings and includes study characteristics such as year, country, the total number of subjects, and the scoring system used.

## Orthognathic Quality of Life Questionnaire

The analysis of studies assessing QOL with the OQLQ score involved 20 different subgroup analyses using 5 score parameters, with each parameter analyzed for 4 different comparisons. Most studies showed a significant impact of the intervention on QOL by demonstrating lower postoperative OQLQ scores compared to preoperative scores, and lower scores after 6 months compared to 2 months. However, social aspects (up to 12 months), facial aesthetics (up to 12 months), oral function (2 months or less and up to 12 months), awareness (2 months or less and up to 12 months), and the total score (up to 12 months) were not significantly impacted by surgery, as shown in Table 3 and Fig. 2–4.

## Oral Health Impact Profile-14

The evaluation of studies assessing QOL using the OHIP-14 score included the analysis of 16 different subgroups using 8 score parameters, with each parameter compared at 2 recovery periods, as shown in Table 3. All 16 subgroup analyses showed significantly lower OHIP-14 scores postsurgery and at 6 and 12 months, for all parameters (Fig. 5,6).

The heterogeneity of different analyses is expressed in Table 3 as $\tau^2$, $I^2$, Q-test, and Cook's distances, the latter of which estimates the influence of a data point and indicates the number of studies that could be considered overly influential. Most models showed significant heterogeneity for both scoring systems (OHIP-14 and OQLQ), while only 5 analyses showed nonsignificant heterogeneity (Table 3). Variability of $I^2$ values ranged from 7% to 99%.

## Risk of bias

The risk of bias assessment used Egger's regression and Begg's test, as shown in Table 3. All parameters related to the OQLQ score, including social aspects, facial aesthetics, oral function, awareness, and the total score, and analyzed for differences at 2 months or less, 2–6 months, and up to 12 months showed nonsignificant publication

**Table 2.** Characteristics of the included studies

| Study | Year | Country | Total number of subjects | Scoring system |
|---|---|---|---|---|
| Göelzer et al.[7] | 2014 | Brazil | 74 | OHIP-14 score |
| Eslamipour et al.[20] | 2017 | Iran | 43 | OQLQ score |
| Alanko et al.[22] | 2017 | Finland | 60 | OQLQ score |
| Antoun et al.[23] | 2015 | New Zealand | 29 | OHIP-14 score |
| Baherimoghaddam et al.[24] | 2016 | Iran | 75 | OHIP-14 score |
| Chaurasia et al.[25] | 2018 | Nepal | 14 | OQLQ score<br>OHIP-14 score |
| Choi et al.[26] | 2010 | Hong Kong | 32 | OQLQ score<br>OHIP-14 score |
| Tabaie et al.[27] | 2022 | Iran | 90 | OHIP-14 score |
| Geramy et al.[28] | 2019 | Iran | 29 | OHIP-14 score |
| Ilic et al.[29] | 2022 | Bosna | 40 | OHIP-14 score |
| Kavin et al.[30] | 2012 | India | 14 | OQLQ score |
| Kiyak[31] | 2008 | USA | 197 | OQLQ score |
| Lancaster et al.[32] | 2020 | USA | 71 | OQLQ score |
| Lee et al.[33] | 2008 | Hong Kong | 36 | OQLQ score<br>OHIP-14 score |
| Murphy et al.[34] | 2011 | Ireland | 52 | OQLQ score |
| Rezaei et al.[35] | 2019 | Iran | 112 | OQLQ score |
| Silva et al.[36] | 2016 | Sweden | 50 | OQLQ score<br>OHIP-14 score |
| Soh et al.[37] | 2015 | India | 66 | OQLQ score |
| Sun et al.[38] | 2018 | China | 85 | OQLQ score<br>OHIP-14 score |

OQLQ – Orthognathic Quality of Life Questionnaire; OHIP-14 – Oral Health Impact Profile-14.

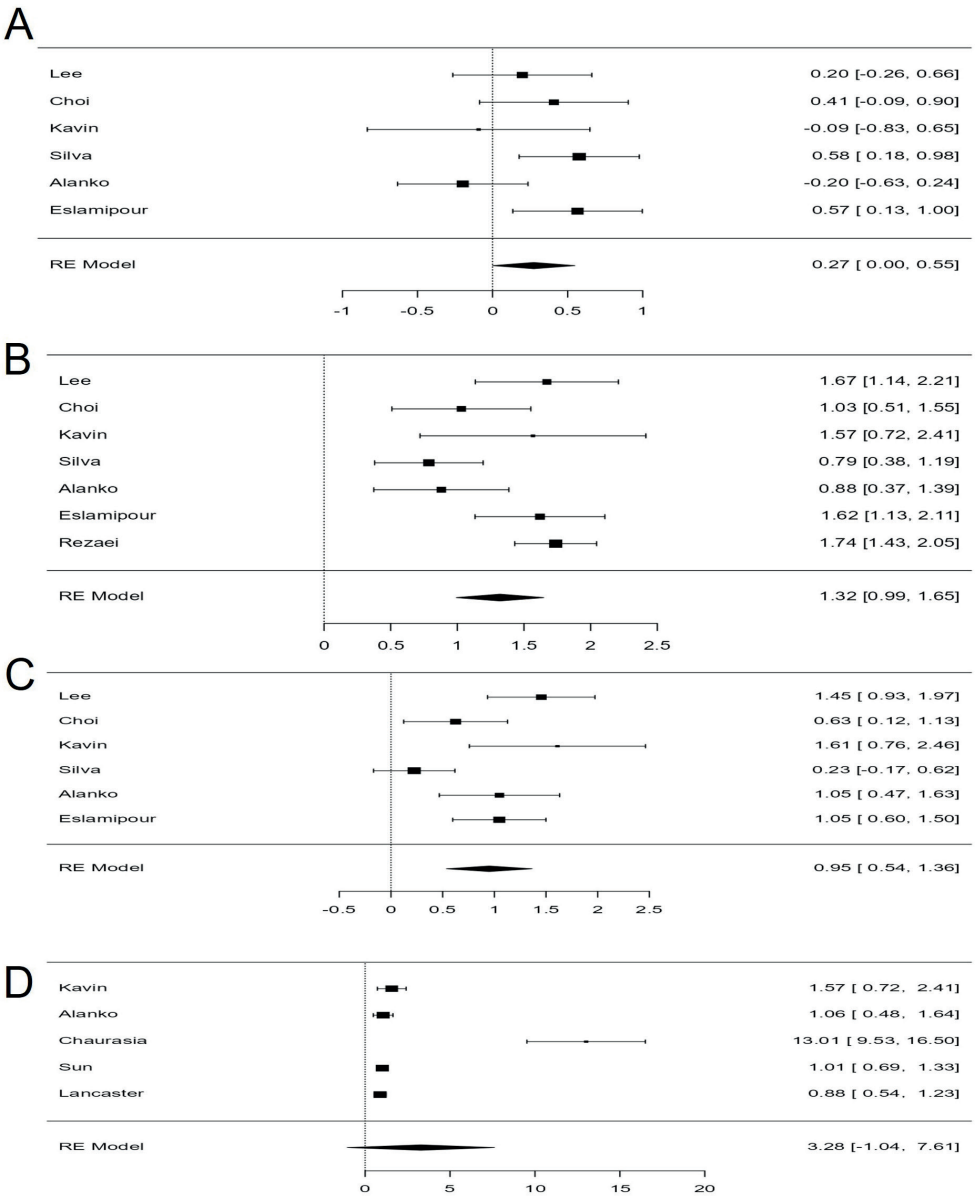



Fig. 2. Forest plot showing the impact of orthodontic-surgical treatment on quality of life according to Orthognathic Quality of Life Questionnaire (OQLQ) total score before surgery and after surgery at 2 months or less (A), 2–6 months (B), comparing 2 months with 6 months (C), and up to 12 months (D)

RE – random effects.

bias ranging from p = 0.06 to p = 0.99. On the other hand, the analysis of 8 parameters related to OHIP-14 score and analyzed at up to 6 and 12 months post-surgery showed different results, with significant publication bias in 12 parameters (p < 0.05). This included functional limitation (up to 12 months), physical pain (up to 6 and 12 months), psychological discomfort (up to 6 months), physical disability (up to 12 months), psychological disability (up to 6 months), social disability (up to 6 months), handicap (up to 6 months), and the total OHIP-14 score itself (up to 6 and 12 months). The analysis of other OHIP-14-related parameters showed nonsignificant publication bias, as shown in Table 3.

## Discussion

The current meta-analysis included 19 studies assessing the impact of orthodontic-surgical treatment on QOL using the OQLQ and OHIP-14 scoring systems. According to the total OQLQ score, patients' QOL significantly improved after surgery within 2 months or less (p = 0.049), up to 6 months (p < 0.001), and when comparing 2 months or less with 2–6 months (p < 0.001). In addition, the OHIP-14 scores showed a significant difference in QOL post-surgery at 6 months (p = 0.003) and up to 12 months (p = 0.002).

Quality of life is a vital indicator at any stage of surgical orthodontic treatment and is crucial when addressing the patient's mental health.[24] Functional constraints, decreased masticatory efficiency, discomfort, edema, sensorineural abnormalities, and morbidities specific to the surgical process all contribute to a significant drop in QOL in the postoperative period, as reported by Choi et al.[26] In contrast, research by Lee et al. demonstrated that 6 weeks after surgery, patients experienced a considerable improvement in QOL associated

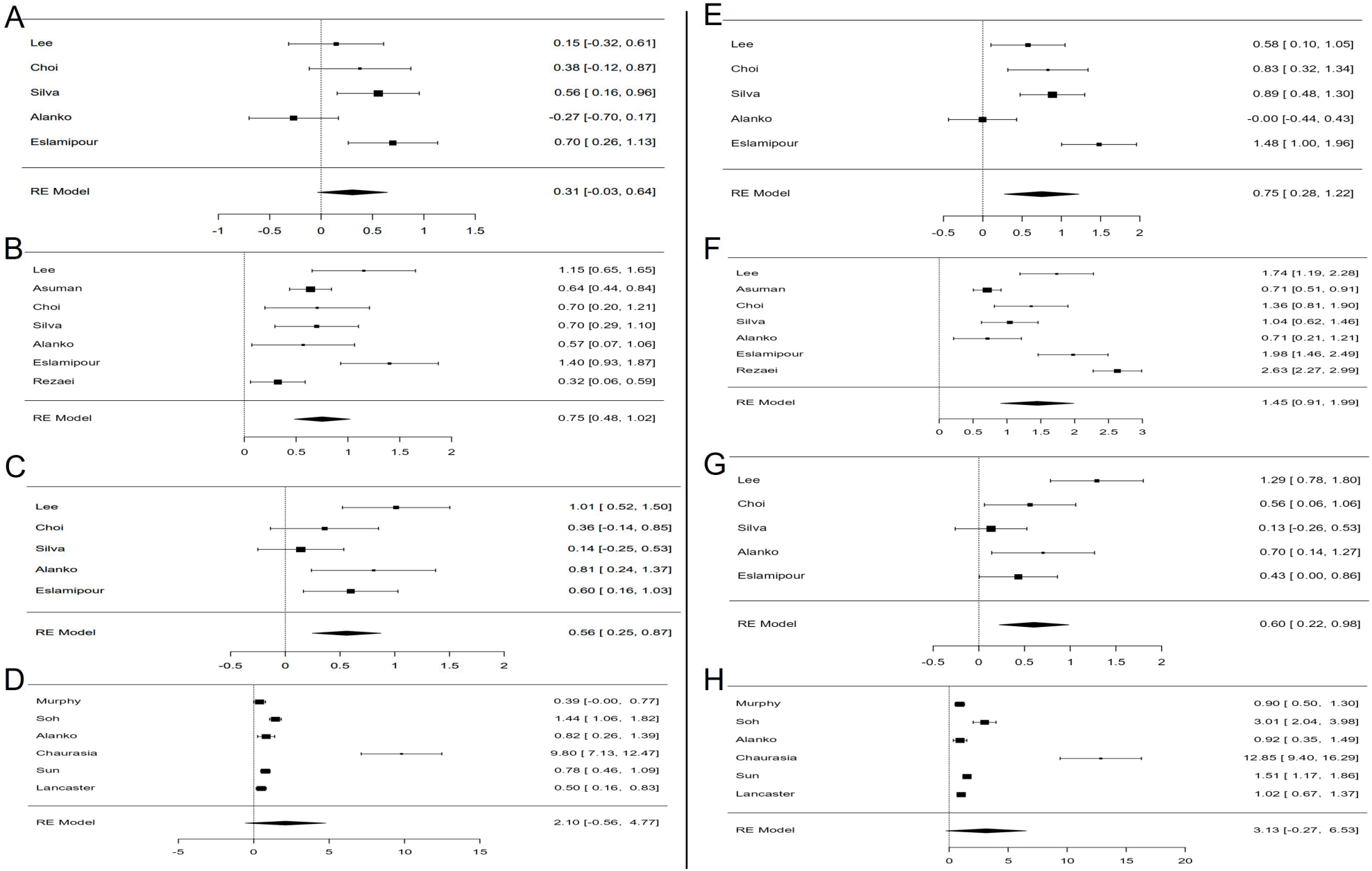



**Fig. 3.** Forest plot showing the impact of orthodontic-surgical treatment on quality of life according to Orthognathic Quality of Life Questionnaire (OQLQ) score for social aspects before surgery and after surgery at 2 months or less (A), 2–6 months (B), comparing 2 months with 6 months (C), and up to 12 months (D). The impact of surgery on facial aesthetics at 2 months or less (E), 2–6 months (F), comparing 2 months with 6 months (G), and up to 12 months (H) is presented

RE – random effects.

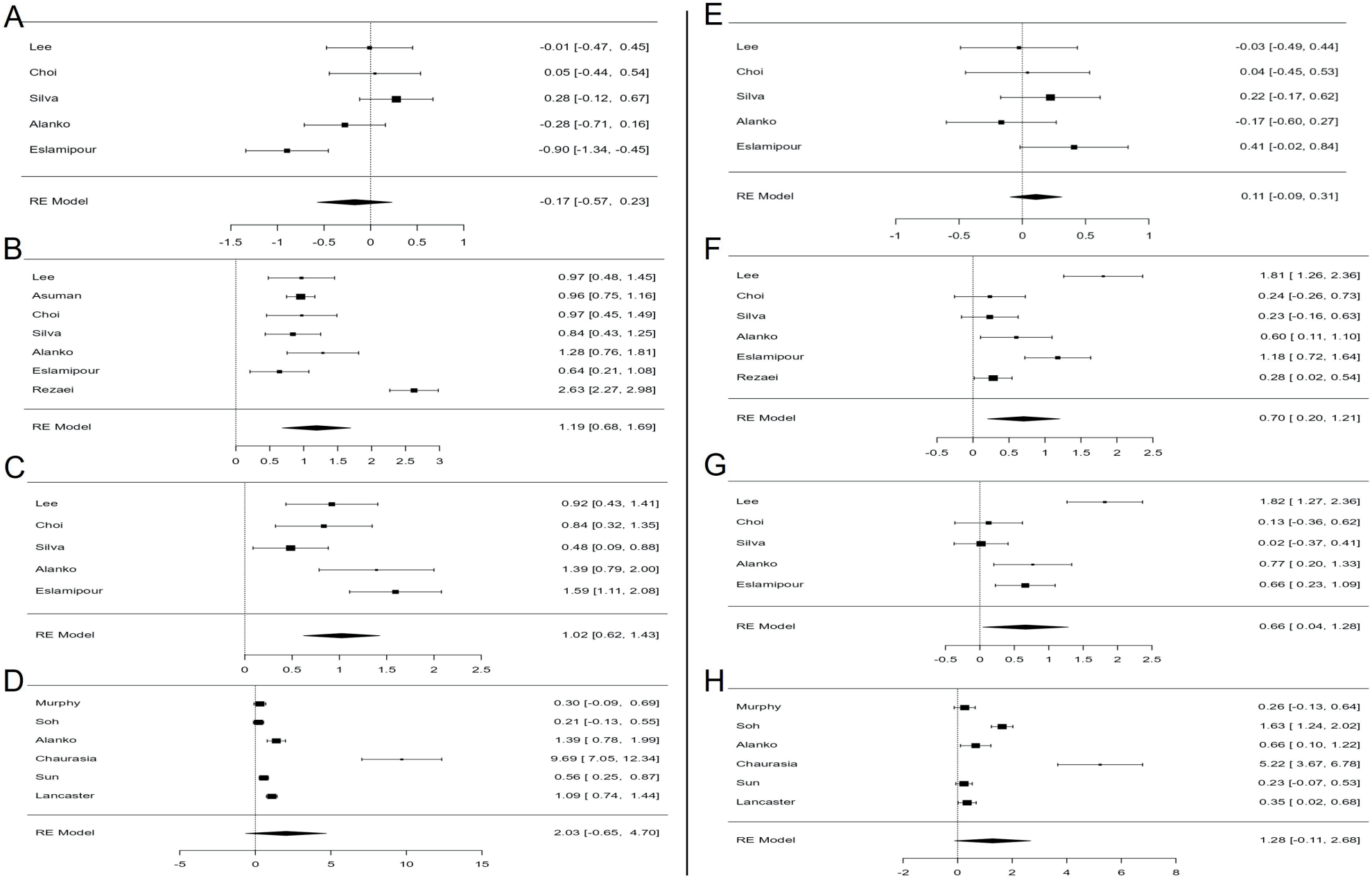



**Fig. 4.** Forest plot showing the impact of orthodontic-surgical treatment on quality of life according to Orthognathic Quality of Life Questionnaire (OQLQ) score for oral function 2 months or less after surgery (A), 2–6 months (B), comparing 2 months with 6 months (C), and up to 12 months (D). The impact of surgery on awareness after 2 months or less (E), 2–6 months (F), comparing 2 months with 6 months (G), and up to 12 months (H) is presented

RE – random effects.

Table 3. Results of statistical analyses, heterogeneity and publication bias assessment

| Score | Element | Comparison | Estimate | Lower limit | Upper limit | p-value | $\tau^2$ | $I^2$ [%] | Q-test | Cook's distances (number of studies could be considered overly influential) | Egger's regression | Begg and Mazumdar rank correlation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OQLQ Score | social aspects | 2 months or less | 0.3 | −0.03 | 0.64 | 0.07 | 0.1 | 66 | 11.8* | none | 0.76 | 0.99 |
| | | 2–6 months | 0.75 | 0.48 | 1.02 | <0.001 | 0.09 | 73 | 19.7* | none | 0.13 | 0.56 |
| | | 2 months or less compared to 2–6 months | 0.56 | 0.25 | 0.87 | <0.001 | 0.07 | 55 | 8.94 | none | 0.12 | 0.48 |
| | | up to 12 months | 2.1 | −0.56 | 4.77 | 0.122 | 10.79 | 99 | 61.9* | none | 0.1 | 0.47 |
| | facial aesthetics | 2 months or less | 0.75 | 0.28 | 1.22 | 0.002 | 0.24 | 81 | 21.6* | none | 0.55 | 0.82 |
| | | 2–6 months | 1.45 | 0.91 | 1.98 | <0.001 | 0.48 | 92 | 100.6* | none | 0.65 | 0.77 |
| | | 2 months or less compared to 2–6 months | 0.6 | 0.22 | 0.98 | 0.002 | 0.13 | 69 | 13.1* | none | 0.07 | 0.08 |
| | | up to 12 months | 3.13 | −0.269 | 6.53 | 0.07 | 17.5 | 99 | 64.2* | 1 | 0.1 | 0.27 |
| | oral function | 2 months or less | −0.170 | −0.570 | 0.23 | 0.4 | 0.16 | 75 | 16.7* | none | 0.79 | 0.99 |
| | | 2–6 months | 1.19 | 0.68 | 1.69 | <0.001 | 0.41 | 91 | 77.3* | 1 | 0.74 | 0.24 |
| | | 2 months or less compared to 2–6 months | 1.02 | 0.62 | 1.43 | <0.001 | 0.15 | 70 | 14.2* | none | 0.14 | 0.82 |
| | | up to 12 months | 2.03 | −0.65 | 4.7 | 0.137 | 10.9 | 99 | 66.5* | 1 | 0.1 | 0.27 |
| | awareness | 2 months or less | 0.108 | −0.095 | 0.31 | 0.30 | 0.003 | 7 | 4.1 | none | 0.41 | 0.82 |
| | | 2–6 months | 0.705 | 0.203 | 1.207 | 0.006 | 0.34 | 88 | 35.6* | 1 | 0.13 | 0.06 |
| | | 2 months or less compared to 2–6 months | 0.664 | 0.044 | 1.283 | 0.036 | 0.44 | 88 | 31.2* | none | 0.12 | 0.23 |
| | | up to 12 months | 1.28 | −0.11 | 2.68 | 0.07 | 2.9 | 98 | 71.3* | 1 | 0.051 | 0.06 |
| | total score | 2 months or less | 0.274 | 0.001 | 0.547 | 0.049 | 0.06 | 49 | 9.8 | none | 0.35 | 0.27 |
| | | 2–6 months | 1.32 | 0.993 | 1.647 | <0.001 | 0.13 | 68 | 21.1* | none | 0.94 | 0.99 |
| | | 2 months or less compared to 2–6 months | 0.951 | 0.538 | 1.365 | <0.001 | 0.19 | 73 | 19.9* | none | 0.052 | 0.27 |
| | | up to 12 months | 3.28 | −1.044 | 7.612 | 0.14 | 23.7 | 99 | 47.6* | 1 | 0.2 | 0.083 |

with cosmetic facial elements.[33] The current study, which obtained data through meta-analytical estimation of QOL in patients 6 weeks after surgery using the OQLQ questionnaire, corroborates these findings by demonstrating that QOL for social aspects, functional aspects, awareness, and the total score did not change from the initial to final stages (without any treatment). The results also demonstrated that facial aesthetics significantly improved 6 weeks post-surgery compared to pre-treatment levels. If the OQLQ questionnaire is directly related to dentofacial experiences, these results make sense, given that orthodontic-surgical treatment corrects the dentofacial abnormality. Compared to questionnaires that take a more generalized approach to oral

**Table 3.** Results of statistical analysis, heterogeneity and publication bias assessment – cont.

| Score | Element | Comparison | Estimate | Lower limit | Upper limit | p-value | $\tau^2$ | $I^2$ [%] | Q-test | Cook's distances (number of studies could be considered overly influential) | Egger's regression | Begg and Mazumdar rank correlation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OHIP-14 | functional limitation | up to 6 months | 0.662 | 0.425 | 0.900 | <0.001 | 0.02 | 28 | 6.9 | none | 0.052 | 0.06 |
| | | up to 12 months | 1.13 | 0.316 | 1.954 | 0.007 | 1.1 | 96 | 74.8* | none | 0.001 | 0.01 |
| | physical pain | up to 6 months | 0.785 | 0.487 | 1.084 | <0.001 | 0.07 | 53 | 10.8 | none | 0.002 | 0.003 |
| | | up to 12 months | 1 | 0.363 | 1.639 | 0.002 | 0.7 | 93 | 47.5* | none | 0.001 | 0.03 |
| | psychological discomfort | up to 6 months | 1.64 | 0.609 | 2.664 | 0.002 | 1.5 | 95 | 61.7* | 1 | 0.001 | 0.003 |
| | | up to 12 months | 2.16 | 0.803 | 3.523 | 0.002 | 3.2 | 98 | 141.4* | none | 0.1 | 0.14 |
| | physical disability | up to 6 months | 0.704 | 0.376 | 1.032 | <0.001 | 0.1 | 61 | 13.6* | none | 0.052 | 0.06 |
| | | up to 12 months | 1.01 | 0.098 | 1.928 | 0.030 | 1.4 | 97 | 49.9* | 1 | 0.01 | 0.03 |
| | psychological disability | up to 6 months | 1.57 | 0.591 | 2.559 | 0.002 | 1.4 | 94 | 64.9* | none | 0.01 | 0.02 |
| | | up to 12 months | 1.80 | 0.636 | 2.971 | 0.002 | 2.4 | 97 | 1111.8* | none | 0.2 | 0.07 |
| | social disability | up to 6 months | 0.979 | 0.496 | 1.463 | <0.001 | 0.3 | 81 | 24.8* | none | 0.01 | 0.02 |
| | | up to 12 months | 0.904 | 0.357 | 1.452 | 0.001 | 0.5 | 91 | 64.7* | none | 0.61 | 0.38 |
| | handicap | up to 6 months | 1.12 | 0.686 | 1.548 | <0.001 | 0.2 | 75 | 20.2* | none | 0.01 | 0.02 |
| | | up to 12 months | 0.963 | 0.475 | 1.451 | <0.001 | 0.4 | 88 | 54.5* | none | 0.6 | 0.56 |
| | total OHIP-14 score | up to 6 months | 2.16 | 0.730 | 3.584 | 0.003 | 3.1 | 97 | 105.9* | 1 | 0.002 | 0.003 |
| | | up to 12 months | 2.66 | 0.983 | 4.337 | 0.002 | 5.6 | 98 | 147.8* | none | 0.01 | 0.03 |

OQLQ – Orthognathic Quality of Life Questionnaire; OHIP-14 – Oral Health Impact Profile-14; *significant heterogeneity.

health, the level of specificity increases the sensitivity with which QOL changes can be detected, even in their earliest stages.[33]

Many studies show a link between dentofacial abnormalities and psychological issues,[40–42] and when comparing patients with other types of malocclusion,[40] those with class III malocclusion are more likely to experience feelings of insecurity, depression and psychological stress. Such issues arise due to the difficulty of hiding the skeletal discrepancy caused by the protrusion of the jaw and the concavity of the facial profile, both of which are viewed as unattractive. Patients with class III malocclusion have orthognathic surgery around 4 years earlier than patients with other abnormalities,[40] suggesting that psychological factors play a role in the decision to undertake surgical treatment. Variations in emotional behavior, depression intensity and self-esteem are linked to gender, and there are more noticeable alterations to the female facial profile.[22,40,43,44] Moreover, as shown by Bortoluzzi et al., QOL has a greater effect on females and elicits diverse responses from patients of different ages who suffer from dentofacial deformities.[22,45]

Bias regarding the type of deformities and the number of patients between groups may influence the results due to the cross-sectional design of research and the inclusion of multiple types of malocclusion in the same sample.[46] Confounding factors such as gender, age, marital status, and type of malocclusion were taken into account in the review and data analysis in just 12 of the included studies. This could help account for the relatively high $I^2$ value found in this meta-analysis. However, the direction of the impact in the individual results demonstrated the same effect tendency, even in meta-analyses that reported considerable heterogeneity.

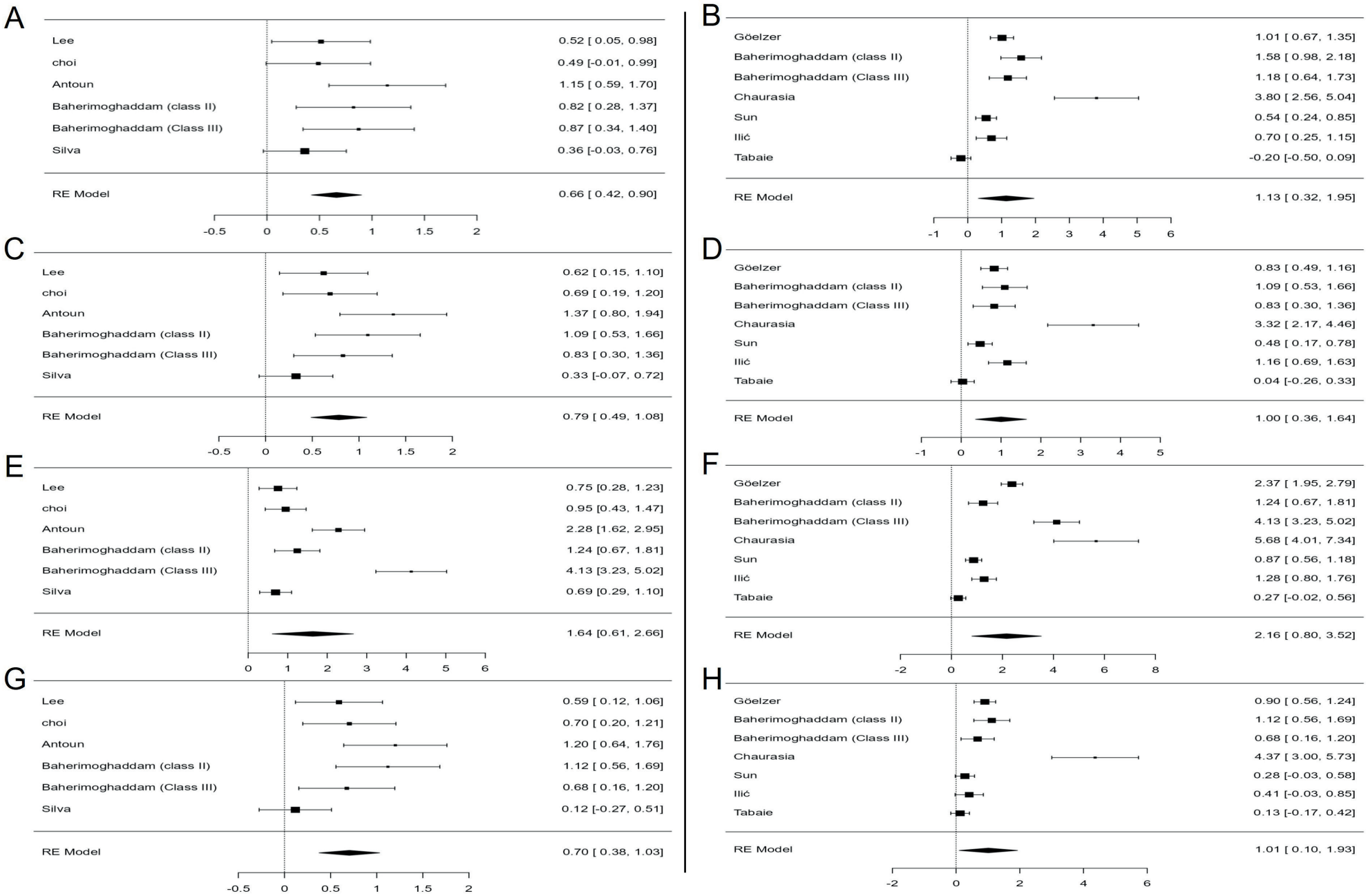



**Fig. 5.** Forest plot showing the impact of orthodontic-surgical treatment on quality of life according to Oral Health Impact Profile-14 (OHIP-14) score for functional limitation within 6 months (A) and 12 months (B), physical pain within 6 months (C) and 12 months (D), psychological discomfort within 6 months (E) and 12 months (F), and physical disability within 6 months (G) and 12 months (H)

RE – random effects.

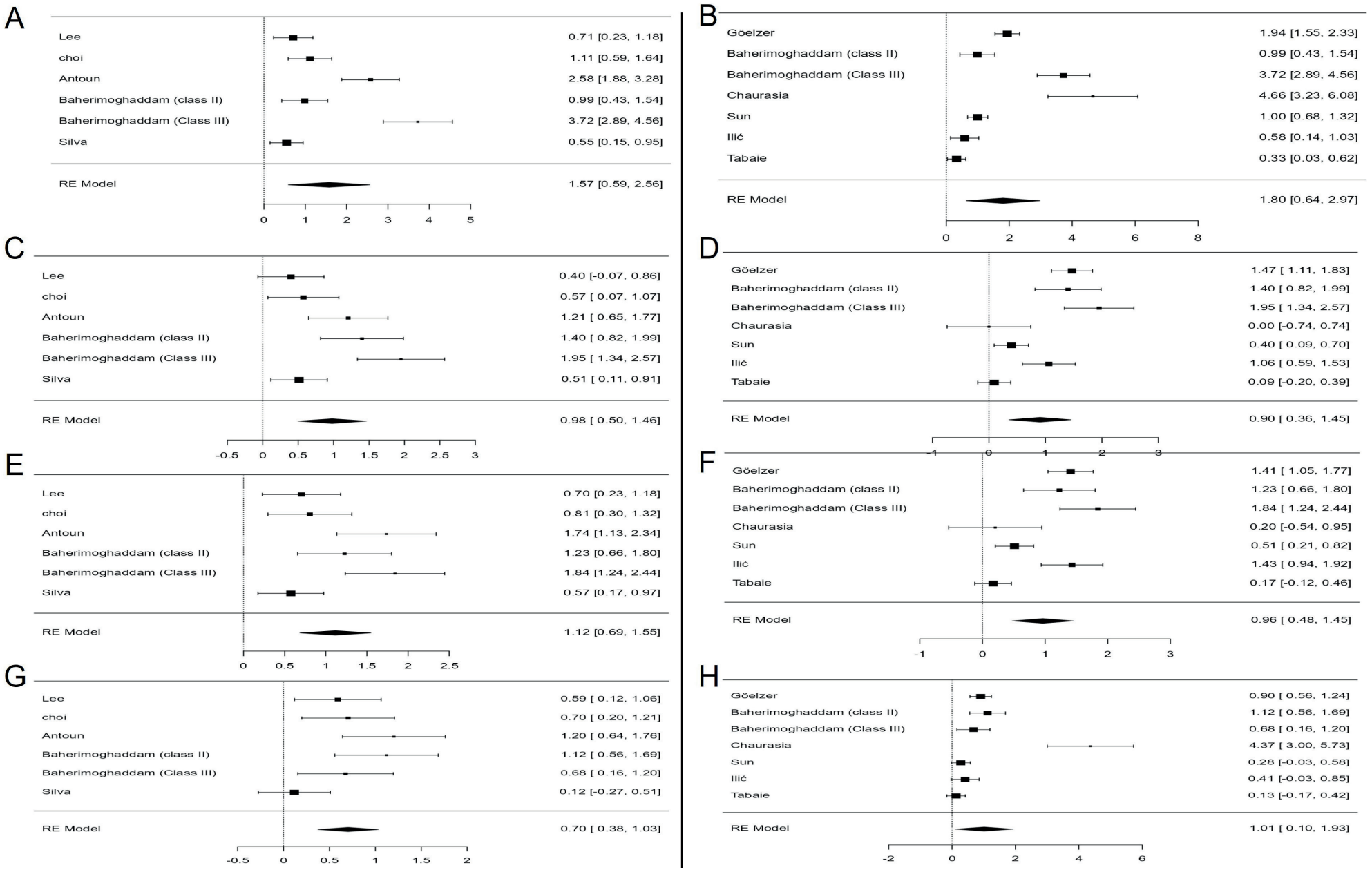



**Fig. 6.** Forest plot showing the impact of orthodontic-surgical treatment on quality of life according to Oral Health Impact Profile-14 (OHIP-14) score for psychological disability within 6 months (A) and 12 months (B), social disability within 6 months (C) and 12 months (D), handicap within 6 months (E) and 12 months (F), and overall score within 6 months (G) and 12 months (H)

RE – random effects.

## Limitations

Age, gender, type of malocclusion, and patient expectations of orthodontic-surgical treatment are confounding factors that are intrinsic to the issue and may alter the estimations if not controlled by adequate sampling and randomization techniques. Since most of the studies were uncontrolled and observational, they could not control the exposure factor or use random allocation methods. Also, some studies had a small sample size of less than 20 subjects. In addition, not all studies measured the outcomes at the same time after surgery, and many studies were excluded due to not stating the exact timing of the results.

# Conclusions

Orthodontic-surgical treatment resulted in a significant enhancement in patient QOL after surgery compared to before surgery. The OHIP-14 demonstrated significant improvement in all elements covered by this questionnaire, while the OQLQ showed varied findings that depended on the time after surgery. However, future multicenter clinical studies are required to make more definitive conclusions.


### ORCID iDs

Xiangyu Li https://orcid.org/0009-0003-1337-6955
Weiying Zheng https://orcid.org/0009-0008-8697-1081
Xi Zhang https://orcid.org/0009-0002-8530-7900
Yuwen Zhang https://orcid.org/0009-0005-0347-2489


### References


1. Denadai R, Chou PY, Su YY, et al. Facial appearance and psychosocial features in orthognathic surgery: A FACE-Q- and 3D facial image-based comparative study of patient-, clinician-, and lay-observer-reported outcomes. *J Clin Med*. 2019;8(6):909. doi:10.3390/jcm8060909
2. Zamboni R, de Moura FRR, Brew MC, et al. Impacts of orthognathic surgery on patient satisfaction, overall quality of life, and oral health-related quality of life: A systematic literature review. *Int J Dent*. 2019;2019:2864216. doi:10.1155/2019/2864216
3. Palomares NB, Celeste RK, Miguel JAM. Impact of orthosurgical treatment phases on oral health-related quality of life. *Am J Orthod Dentofacial Orthop*. 2016;149(2):171–181. doi:10.1016/j.ajodo.2015.07.032
4. Su YY, Denadai R, Ho CT, Lai BR, Lo LJ. Measuring patient-reported outcomes in orthognathic surgery: Linguistic and psychometric validation of the Mandarin Chinese version of FACE-Q instrument. *Biomed J*. 2020;43(1):62–73. doi:10.1016/j.bj.2019.05.011
5. Larsson P, Bondemark L, Häggman-Henrikson B. The impact of orofacial appearance on oral health-related quality of life: A systematic review. *J Oral Rehabil*. 2021;48(3):271–281. doi:10.1111/joor.12965
6. Asada K, Motoyoshi M, Tamura T, Nakajima A, Mayahara K, Shimizu N. Satisfaction with orthognathic surgery of skeletal class III patients. *Am J Orthod Dentofacial Orthop*. 2015;148(5):827–837. doi:10.1016/j.ajodo.2015.05.021
7. Göelzer JG, Becker OE, Haas OL Jr, et al. Assessing change in quality of life using the Oral Health Impact Profile (OHIP) in patients with different dentofacial deformities undergoing orthognathic surgery: A before and after comparison. *Int J Oral Maxillofac Surg*. 2014;43(11):1352–1359. doi:10.1016/j.ijom.2014.06.015
8. Bock JJ, Odemar F, Fuhrmann RAW. Assessment of quality of life in patients undergoing orthognathic surgery. *J Orofac Orthop*. 2009;70(5):407–419. doi:10.1007/s00056-009-9903-4
9. Al-Ahmad HT, Al-Sa'di WS, Al-Omari IK, Al-Bitar ZB. Condition-specific quality of life in Jordanian patients with dentofacial deformities: A comparison of generic and disease-specific measures. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol*. 2009;107(1):49–55. doi:10.1016/j.tripleo.2008.05.040
10. Al-Bitar ZB, Al-Ahmad HT. Anxiety and post-traumatic stress symptoms in orthognathic surgery patients. *Eur J Orthod*. 2017;39(1):92–97. doi:10.1093/ejo/cjw029
11. Flanary CM, Barnwell GM, VanSickels JE, Littlefield JH, Rugh AL. Impact of orthognathic surgery on normal and abnormal personality dimensions: A 2-year follow-up study of 61 patients. *Am J Orthod Dentofacial Orthop*. 1990;98(4):313–322. doi:10.1016/S0889-5406(05)81488-X
12. Newton JT, Prabhu N, Robinson PG. The impact of dental appearance on the appraisal of personal characteristics. *Int J Prosthodont*. 2003;16(4):429–434. PMID:12956500.
13. Sinko K, Jagsch R, Benes B, Millesi G, Fischmeister F, Ewers R. Facial aesthetics and the assignment of personality traits before and after orthognathic surgery. *Int J Oral Maxillofac Surg*. 2012;41(4):469–476. doi:10.1016/j.ijom.2011.10.026
14. de Paula DF Jr, Santos NCM, da Silva ÉT, Nunes MF, Leles CR. Psychosocial impact of dental esthetics on quality of life in adolescents. *Angle Orthod*. 2009;79(6):1188–1193. doi:10.2319/082608-452R.1
15. Bittencourt JM, Martins LP, Bendo CB, Vale MP, Paiva SM. Negative effect of malocclusion on the emotional and social well-being of Brazilian adolescents: A population-based study. *Eur J Orthod*. 2017;39(6):628–633. doi:10.1093/ejo/cjx020
16. Sebastiani AM, Meger MN, Bergamashi I, et al. Depression and pain in individuals with skeletal malocclusion. *Int J Oral Maxillofac Surg*. 2019;48:261. doi:10.1016/j.ijom.2019.03.797
17. Fernandes MJ, Ruta DA, Ogden GR, Pitts NB, Ogston SA. Assessing oral health-related quality of life in general dental practice in Scotland: Validation of the OHIP-14. *Commun Dent Oral Epidemiol*. 2006;34(1):53–62. doi:10.1111/j.1600-0528.2006.00254.x
18. Bolton MA, Pruzinsky T, Cash TF, Persing JA. Measuring outcomes in plastic surgery: Body image and quality of life in abdominoplasty patients. *Plast Reconstr Surg*. 2003;112(2):619–625. doi:10.1097/01.PRS.0000070972.57000.08
19. Slade GD, Spencer AJ. Development and evaluation of the Oral Health Impact Profile. *Community Dent Health*. 1994;11(1):3–11. PMID:8193981.
20. Eslamipour F, Najimi A, Tadayonfard A, Azamian Z. Impact of orthognathic surgery on quality of life in patients with dentofacial deformities. *Int J Dent*. 2017;2017:4103905. doi:10.1155/2017/4103905
21. Love J, Dropmann D, Selker R. Jamovi v. 2.3.21. The Jamovi Project; 2022. https://www.jamovi.org/download.html. Accessed January 8, 2022.
22. Alanko O, Tuomisto MT, Peltomäki T, Tolvanen M, Soukka T, Svedström-Oristo AL. A longitudinal study of changes in psychosocial well-being during orthognathic treatment. *Int J Oral Maxillofac Surg*. 2017;46(11):1380–1386. doi:10.1016/j.ijom.2017.05.004
23. Antoun JS, Fowler PV, Jack HC, Farella M. Oral health-related quality of life changes in standard, cleft, and surgery patients after orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 2015;148(4):568–575. doi:10.1016/j.ajodo.2015.03.028
24. Baherimoghaddam T, Tabrizi R, Naseri N, Pouzesh A, Oshagh M, Torkan S. Assessment of the changes in quality of life of patients with class II and III deformities during and after orthodontic-surgical treatment. *Int J Oral Maxillofac Surg*. 2016;45(4):476–485. doi:10.1016/j.ijom.2015.10.019
25. Chaurasia N, Upadhyaya C, Srivastava S, Dulal S. Assessment of changes in quality of life in patients with dentofacial deformities after orthognathic surgery: A study in Nepalese population. *J Oral Maxillofac Surg Med Pathol*. 2018;30(2):111–114. doi:10.1016/j.ajoms.2017.10.005
26. Choi WS, Lee S, McGrath C, Samman N. Change in quality of life after combined orthodontic-surgical treatment of dentofacial deformities. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol*. 2010;109(1):46–51. doi:10.1016/j.tripleo.2009.08.019
27. Tabaie SRS, Tabaie ES, Sadrabadi FS, Rahmatinejad P, Valizadeh S, Pouyanfar H. The role of orthodontic treatment on patient's mental health, body image, and oral health-related quality of life orthodontic treatment and its effects on psychological factors. *Iran J Health Psychol*. 2022;5(2):37–46. doi:10.30473/IJOHP.2022.58607.1176

28. Geramy A, Mazaheri Nazarifar A, Saffar Shahroudi A, Sheikhzadeh S. Oral health-related quality of life following orthognathic surgery for class III correction its relationship with cephalometric changes. *Int J Oral Maxillofac Surg*. 2019;48(11):1434–1439. doi:10.1016/j.ijom.2019.03.899
29. Ilic J, Cutovic T, Kozomara R, Radojicic J, Adamovic T, Lovric J. Assessment of the impact of orthodontic-surgical treatment on the quality of life of patients with mandibular prognathism. *Vojnosanit Pregl*. [in press]
30. Kavin T, Jagadesan AG, Venkataraman S. Changes in quality of life and impact on patients' perception of esthetics after orthognathic surgery. *J Pharm Bioall Sci*. 2012;4(6):290. doi:10.4103/0975-7406.100276
31. Kiyak HA. Does orthodontic treatment affect patients' quality of life? *J Dent Educ*. 2008;72(8):886–894. doi:10.1002/j.0022-0337.2008.72.8.tb04564.x
32. Lancaster L, Salaita RD, Swamy C, et al. Effects of orthognathic surgery on quality of life compared with nonsurgical controls in an American population. *Am J Orthod Dentofacial Orthop*. 2020;158(4):555–563. doi:10.1016/j.ajodo.2019.09.020
33. Lee S, McGrath C, Samman N. Impact of orthognathic surgery on quality of life. *J Oral Maxillofac Surg*. 2008;66(6):1194–1199. doi:10.1016/j.joms.2008.01.006
34. Murphy C, Kearns G, Sleeman D, Cronin M, Allen PF. The clinical relevance of orthognathic surgery on quality of life. *Int J Oral Maxillofac Surg*. 2011;40(9):926–930. doi:10.1016/j.ijom.2011.04.001
35. Rezaei F, Masalehi H, Golshah A, Imani MM. Oral health related quality of life of patients with class III skeletal malocclusion before and after orthognathic surgery. *BMC Oral Health*. 2019;19(1):289. doi:10.1186/s12903-019-0989-9
36. Silva I, Cardemil C, Kashani H, et al. Quality of life in patients undergoing orthognathic surgery: A two-centered Swedish study. *J Craniomaxillofac Surg*. 2016;44(8):973–978. doi:10.1016/j.jcms.2016.04.005
37. Soh CL, Narayanan V. The impact of surgical correction on the quality of life in patients with dentofacial deformity: A prospective study. *J Oral Maxillofac Surg Med Pathol*. 2015;27(3):323–327. doi:10.1016/j.ajoms.2014.03.003
38. Sun H, Shang HT, He LS, Ding MC, Su ZP, Shi YL. Assessing the quality of life in patients with dentofacial deformities before and after orthognathic surgery. *J Oral Maxillofac Surg*. 2018;76(10):2192–2201. doi:10.1016/j.joms.2018.03.026
39. Zhang M. *Changes in Children's Oral Health Related Quality of Life Following Orthodontic Treatment* [postgraduate thesis]. Hong Kong: University of Hong Kong; 2007: doi:10.5353/th_b3893854
40. Jacobson A. Psychologic implications of orthognathic surgery in patients with skeletal class II or class III malocclusions. *Am J Orthod Dentofacial Orthop*. 2002;122(6):679. doi:10.1067/mod.2002.128732
41. Jung MH. Quality of life and self-esteem of female orthognathic surgery patients. *J Oral Maxillofac Surg*. 2016;74(6):1240.e1–1240.e7. doi:10.1016/j.joms.2016.01.046
42. Takatsuji H, Kobayashi T, Kojima T, et al. Effects of orthognathic surgery on psychological status of patients with jaw deformities. *Int J Oral Maxillofac Surg*. 2015;44(9):1125–1130. doi:10.1016/j.ijom.2015.02.003
43. Brunault P, Battini J, Potard C, et al. Orthognathic surgery improves quality of life and depression, but not anxiety, and patients with higher preoperative depression scores improve less. *Int J Oral Maxillofac Surg*. 2016;45(1):26–34. doi:10.1016/j.ijom.2015.07.020
44. Motegi E, Hatch JP, Rugh JD, Yamaguchi H. Health-related quality of life and psychosocial function 5 years after orthognathic surgery. *Am J Orthod Dentofacial Orthop*. 2003;124(2):138–143. doi:10.1016/S0889-5406(03)00391-3
45. Bortoluzzi MC, de Camargo Smolarek P, Claudino M, Campagnoli EB, Manfro R. Impact of dentofacial deformity on quality of life: Age and gender differences evaluated through OQLQ, OHIP and SF36. *J Oral Maxillofac Res*. 2015;6(3):e3. doi:10.5037/jomr.2015.6303
46. Tachiki C, Nishii Y, Takaki T, Sueishi K. Condition-specific quality of life assessment at each stage of class III surgical orthodontic treatment: A prospective study. *Bull Tokyo Dent Coll*. 2018;59(1):1–14. doi:10.2209/tdcpublication.2016-0042

# Decreased miR-127 promotes the occurrence of breast cancer via increasing the expression of SPP1

*Guijiang Wei[1–3,B,D], *Meiying Tan[1–3,A,B], *Chunfang Wang[1–3,C,E], Lina Liang[1–3,A,F]

[1] Center for Clinical Laboratory Diagnosis and Research, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China
[2] Baise Key Laboratory of Clinical Molecular Diagnosis, Research and Development for High Incidence Diseases, China
[3] Key Laboratory of Clinical Molecular Diagnosis and Research for High Incidence Diseases in Western Guangxi Universities, Baise, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1113–1123

**Address for correspondence**
Lina Liang
E-mail: 247949108@qq.com

**Funding sources**
The study was funded by the Natural Science Foundation of Guangxi (grant No. 2019JJB140033) and the Baise Scientific Research and Technology Development Project (grant No. 20192101).

**Conflict of interest**
None declared

*Guijiang Wei, Meiying Tan and Chunfang Wang contributed equally to this work.

Received on April 24, 2022
Reviewed on July 11, 2022
Accepted on February 12, 2023

Published online on March 15, 2023

## Abstract

**Background.** The expression of miR-127 has been reported to be decreased in the breast tissue of patients with breast cancer (BRC). However, the mechanism of miR-127 involvement in the pathogenesis of BRC is still unclear and requires urgent clarification.

**Objectives.** To explore the role of miR-127 in the pathogenesis of BRC.

**Materials and methods.** In this study, we measured the expression of miR-127 in blood samples of 60 BRC patients and 60 controls, investigated the influence of miR-127 on the viability and apoptosis of MCF-7 and MDA-231 cells, identified a miR-127 target gene, and determined the expression level of the target gene in the blood samples of BRC patients and controls.

**Results.** We found that miR-127 expression was significantly decreased in the plasma of BRC patients compared to controls. Additionally, the upregulation of miR-127 in MCF-7 and MDA-231 cells inhibited their proliferation and promoted their apoptosis. Conversely, the downregulation of miR-127 promoted cell proliferation and inhibited their apoptosis. The *SPP1* was successively predicted and validated as a target gene of miR-127. Finally, the expression level of *SPP1* was significantly increased in the plasma of BRC patients compared to controls.

**Conclusions.** Our study demonstrated that decreased miR-127 may promote BRC cell proliferation, inhibit apoptosis and promote the occurrence of BRC through increasing the SPP1 expression level.

**Key words:** pathogenesis, breast cancer, SPP1, miR-127

**Cite as**
Wei G, Tan M, Wang C, Liang L. Decreased miR-127 promotes the occurrence of breast cancer via increasing the expression of SPP1. *Adv Clin Exp Med*. 2023;32(10):1113–1123. doi:10.17219/acem/161161

**DOI**
10.17219/acem/161161

# Background

Cancer is a cause of a great economic burden and a severe public health crisis.[1] Among various kinds and types of cancers, breast cancer (BRC) is the most prevalent among females worldwide.[2] Although the mortality rate of BRC dropped by 40% from 1989 to 2017 in the USA, the incidence rate has still been rising by 0.3% per year.[3] In China, BRC accounted for approx. 16.5% of new female cancer incidence and 7.82% of female cancer mortality in 2014.[4] Traditionally, high body mass index (BMI), advanced age, as well as high plasma glucose and alcohol consumption were considered to be related to the etiology of BRC.[5,6] In addition, similar to other cancers, the etiology of BRC was closely related to the aberrant expression of multiple genes, both coding[7,8] and non-coding.[9,10] Recently, the role of non-coding gene expression, in particular microRNAs (miRs), in the occurrence of BRC has gradually become a focus of research into the etiology of BRC.[11,12] The miRs often consist of 18–25 nucleotides. They mainly regulate the translation of coding RNAs via biding to their 3' untranslated regions, and affect the functions of coding RNAs in various pathophysiological processes.[13–15]

The miR-127 is a miR located at the q32 region of chromosome 1. The *miR-127* gene encodes 2 kinds of mature miRs, namely miR-127-3p[16,17] and miR-127-5p. In this study, miR-127-5p was the object of our research. According to previous studies, miR-127 was involved in the pathogenesis of several diseases such as osteoarthritis,[18,19] severe pneumonia[20] and premature ovarian insufficiency,[21] among others. Regarding cancers, the expression of miR-127 was found to be decreased in blood samples of patients suffering from endometrial adenocarcinoma[22] and colon cancer,[23] according to the data from next-generation sequencing. Moreover, miR-127 was reported to participate in the occurrence and progression of squamous cell carcinoma,[24,25] gastric cancer,[26] cervical cancer,[27] and hepatocellular carcinoma,[28–30] through the regulation of different target genes such as *ADCY7*, *CDC6*, *SORT1*, and *FOXD1*. Recently, a decrease in miR-127 expression was also reported in the BRC tissues when compared to paracarcinoma tissues, which may be caused by the hypermethylation of the *miR-127* gene.[31] Additionally, an RNA-protein nanoplex including miR-127 was reported to be effective in the inhibition of tumor growth in a mouse model of BRC.[32] Self-assembling nanoplexes were formed by mixing the CXCR4-scFv-protamine fusion protein with miR-127. Although the results of these studies appear promising for the development of diagnostic and therapeutic applications in BRC, the expression of miR-127 in the breast tissues or blood samples of BRC patients has yet to be verified, and the mechanism of miR-127 in the pathogenesis of BRC requires urgent clarification.

Herein, we measured the expression of miR-127 in blood samples of BRC patients from Guangxi region in China, investigated the influence of miR-127 on the viability and apoptosis of MCF-7 and MDA-231 cells, and identified the target gene of miR-127. Finally, we verified the expression level of the target gene in the blood samples of BRC patients and revealed a preliminary mechanism of miR-127 involvement in the pathogenesis of BRC.

# Objectives

This study aimed to explore the role of miR-127 in the pathogenesis of BRC and to identify a potential diagnostic or therapeutic biomarker for BRC.

# Materials and methods

## Study population

We performed a case-control study, with the approval from the Ethics Committee of Youjiang Medical University for Nationalities (approval No. 2020036688), and received written informed consent from all BRC patients and controls examined. The BRC patients (n = 60) were recruited from the Department of Breast and Thyroid Surgery (Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China) from January 2020 to June 2020. In the same period, data of age-matched controls (n = 60) were collected from the Department of Physical Examination of the same hospital. Clinical parameters, including age, progesterone receptor status, estrogen receptor status, and clinical stage were collected. Cases from families with a history of BRC, recurrent BRC or other types of cancers were removed from the study population. Controls whose family members suffered from cancer, breast lesions or mastitis, or other major human diseases such as stroke, were also removed from the study population.

## qRT-PCR

A total RNA extraction kit (Qiagen, Hilden, Germany) was employed for extracting total RNA from the plasma of clinical samples or BRC cell lines. The extraction product was immediately stored at –80°C. Following complementary DNA strand synthesis from total RNA, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed on a PCR machine (Bio-Rad, Hercules, USA) in triplicate, according to the manufacturer's instructions (Qiagen). The primers for qRT-PCR were supplied by RiboBio (Guangzhou, China). The cel-miR-39 was used as the internal control, and the relative miR-127 level was calculated using the $2^{-\Delta\Delta CT}$ method.

## Cell transfection

Two typical BRC cell lines MCF-7 and MDA-231, were purchased from Guangdao Corporation (Shanghai, China).

The MCF-7 BRC cell line was isolated from the pleural effusion of a 69-year-old Caucasian female patient with BRC and retained multiple characteristics of differentiated mammary epithelium. The MDA-231 BRC cell line was isolated from the pleural effusion of a 51 year-old Caucasian female patient with BRC and barely expressed epidermal growth factor receptor and transforming growth factor-α receptor. It could form poorly differentiated adenocarcinoma in nude mice and BALB/c mice treated with amyotrophic lateral sclerosis (ALS). Cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Carlsbad, USA), which was supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, USA), 80 U/mL penicillin (Gibco) and 100 μg/mL streptomycin (Gibco) under sterile conditions at 37°C and 5% $CO_2$. The miR-127 mimics (5'-UAGUCUCGGGAGACUCGAAGUC-3'), inhibitors (5'-GACUUCGAGUCUCCCGAGACUA-3') and negative control (5'-UAGUCUCGGGAGACUCACUACC-3') were supplied by RiboBio. Before the transfection, MCF-7 and MDA-231 cells were transferred to a 6-well plate with $3\times10^5$ cells per well and cultured until 30% confluence. Subsequently, miR-127 mimics, inhibitors and negative control were transfected into MCF-7 and MDA-231 cells with Lipofectamine™ 3000, according to the manufacturer's instructions. Finally, qRT-PCR was used to examine the effect of cell transfection.

## Cell viability measurement

The viability of MCF-7 and MDA-231 cells was measured using Cell Counting Kit-8 (CCK-8) assay (TransGen Biotech, Beijing, China). Cells transfected with miR-127 mimics, inhibitors and negative control were seeded into a 96-well plate with $4\times10^3$ cells per well, and were cultured under sterile conditions at 37°C and 5% $CO_2$. At 0, 24, 48, and 72 h, 100 μL of CCK-8 solution was added into partial wells of the plate and then incubated at 37°C for 2 h. Finally, the absorbance was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad). Each experiment was performed in triplicate.

## Cell apoptosis measurement

The apoptosis rates of MCF-7 and MDA-231 cells were measured with flow cytometry using Annexin-V-FITC/PI kit provided by Solarbio (Beijing, China). Cells transfected with miR-127 mimics, inhibitors and negative control were inoculated into a 96-well plate as above for 48 h, then collected and washed twice with cold 1× phosphate-buffered saline (PBS) and adjusted to $1\times10^6$ cells/mL using 1× binding buffer. Subsequently, 5 μL of Annexin-V-FITC and 5 μL of propidium iodide (PI) were successively added into 100 μL of cell suspension. Then, the mixture was incubated at 25°C for 15 min in the dark. Finally, flow cytometry was used to analyze the apoptosis rate of cells. Each experiment was performed in triplicate.

## Western blotting

A western blotting kit (Solarbio) was used to examine the expression of protein. The harvested cells were washed twice with cold 1× PBS. Then, cold 1× lysate solution containing phenylmethylsulfonyl fluoride was added, and samples were kept on ice for 30 min. The samples were centrifuged (10,000 × g for 10 min at 4°C) and the concentration of extracted proteins was determined using a bicinchoninic acid assay (BCA). Then, proteins were separated with a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) assay and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were completely soaked in 1× PBS with 5% bovine serum albumin (BSA) and 0.1% Tween, and shaken gently at 4°C overnight. Afterward, the membranes were incubated in the primary antibodies, namely anti-Bcl-2, anti-BAX, anti-Cytochrome c (anti-Cyt-c), anti-cleaved caspase-3, anti-β-actin, and anti-SPP1 antibodies, which were diluted in 1× PBS with 5% BSA and 0.1% Tween at 4°C overnight. After washing with 1× PBS and 0.1% Tween 5 times, the membranes were incubated in the secondary horseradish peroxidase (HRP)-linked antibodies (1:5000 diluted) at 25°C for 60 min. After washing 5 times with 1× PBS and 0.1% Tween, the bands on the membranes were detected with enhanced chemiluminescence and analyzed using ImageJ software (National Institutes of Health, Bethesda, USA). The β-actin was used as the internal control, and each experiment was performed in triplicate.

## Dual-luciferase reporter assay

The *SPP1* was predicted as the target gene of miR-127 according to online bioinformatics software (TargetScan; https://www.targetscan.org) analysis and a search of previous literature.[33] The DNA fragments including wild-type and mutant binding sites of the 3'UTR of SPP1 were synthesized by Sangon Biotech (Shanghai, China) and then inserted into the psiCHECK-2 vector. Human embryonic kidney 293 cells were inoculated in a 24-well plate and cultured under sterile conditions at 37°C and 5% $CO_2$. After the cells reached 70% confluence, 500 ng of recombinant psiCHECK-2 vector and 5 pmol miR-127 mimics or negative control were transfected into human embryonic kidney 293 cells using Lipofectamine™ 3000 transfection system (Invitrogen). After 48 h, firefly luciferase activity and Renilla luciferase activity were measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, USA). Each experiment was performed in triplicate.

## Enzyme-linked immunosorbent assay

An ELISA kit (Shunran Biotechnology, Xinzheng, China) was utilized to measure SPP1 levels in the plasma of BRC patients and controls. Plasma was diluted 1:3

Table 1. Clinical parameters of BRC patients and controls

| Variable | | Controls (n = 60) | BRC (n = 60) | p-value |
|---|---|---|---|---|
| Age [years] (M ±SD) | | 50.40 ±11.70 | 49.98 ±8.26 | 0.824# |
| Estrogen receptor (%) | positive | – | 28 (46.67) | – |
| | negative | – | 32 (53.33) | – |
| Progesterone receptor (%) | positive | – | 24 (40.00) | – |
| | negative | – | 36 (60.00) | – |
| Clinical stages (%) | I–III | – | 47 (78.34) | – |
| | IV | – | 13 (21.66) | – |

# t-test; M ±SD – mean ± standard deviation; BRC – breast cancer.

in a dilution buffer and successively added into a 96-well plate by a micropipette. The mixture was incubated at 37°C for 30 min and then discarded. The wells were washed 5 times with 1× washing buffer, then the HRP-linked secondary antibody was added and incubated at 37°C for 30 min. After washing 5 times with 1× washing buffer, 50 µL of tetramethylbenzidine solution was added to the wells and incubated at 37°C for 15 min in the dark. Finally, 50 µL of terminating solution was added to the wells, and the absorbance was read at 450 nm with an ELISA reader (Bio-Rad).

## Statistical analyses

The IBM SPSS v. 21 software (IBM Corp., Armonk, USA) was used for statistical analysis. Data were presented as the mean ± standard deviation (M ±SD). The normality distribution and the homogeneity of variance were evaluated using the Kolmogorov–Smirnov test and Levene's test, respectively. Discrete variables including age and relative miR-127 level as well as SPP1 level of cases and controls were compared using the Student's t-test, whereas continuous variables including the optical density (OD) 450 nm values, the apoptosis rates, the relative luciferase activity, and the expression levels of Bcl-2, BAX, Cyt-c, cleaved caspase-3, and SPP1 were compared using one-way analysis of variance (ANOVA) followed by the Tukey's post hoc analysis. A value of $p < 0.05$ was considered statistically significant. Clinical parameters of BRC patients and controls are presented in Table 1. The results of the normality distribution and the homogeneity of variance analyses are shown in Supplementary Table 1. The results of the ANOVA followed by the post hoc analysis are shown in Supplementary Table 2.

# Results

## Clinical parameters and relative miR-127 level of BRC patients and controls

The age of BRC patients was not significantly different from that of controls (Student's t-test, $t = 0.252$, degrees of freedom (df) = 118, $p = 0.824$) (Table 1). The percentages of BRC patients with positive estrogen receptor and progesterone receptor were 46.67% and 40.00%, respectively. The BRC patients at clinical stage IV accounted for 21.66% of cases. The relative miR-127 level was significantly decreased in the plasma of BRC patients compared to controls (Student's t-test, $t = 3.061$, df = 118, $p = 0.006$) (Fig. 1).

## miR-127 inhibited viability and promoted apoptosis in MCF-7 and MDA-231 cells

As demonstrated using the ANOVA test, there was a statistically significant effect of the treatments on the relative miR-127 level in both MCF-7 ($F(3,8) = 55.436$, $p < 0.001$) and MDA-231 cells ($F(3,8) = 75.642$, $p < 0.001$) (Fig. 2A,B). When compared with controls, the relative miR-127 level in MCF-7 and MDA-231 cells was significantly upregulated after the cells were transfected with miR-127 mimics. However, the relative miR-127 level in MCF-7 and MDA-231 cells was downregulated after the cells were transfected with miR-127 inhibitors.

The treatment had a statistically significant effect on both MCF-7 viability (24h-F (3,8) = 102.191, $p < 0.001$; 48h-F (3,8) = 307.466, $p < 0.001$; 72h-F (3,8) = 256.069, $p < 0.001$) and MDA-231 (24h-F (3,8) = 208.510, $p < 0.001$; 48h-F (3,8) = 222.030, $p < 0.001$; 72h-F (3,8) = 96.235,

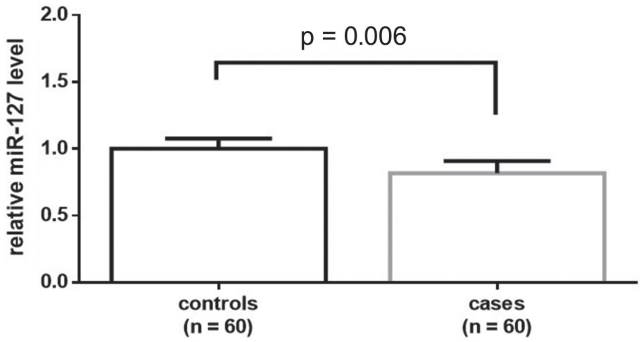



Fig. 1. Relative miR-127 level in the plasma of controls and breast cancer (BRC) patients. The relative miR-127 level was significantly decreased in the plasma of BRC patients compared to controls. The upper whisker represents the 95% confidence interval (95% CI) value

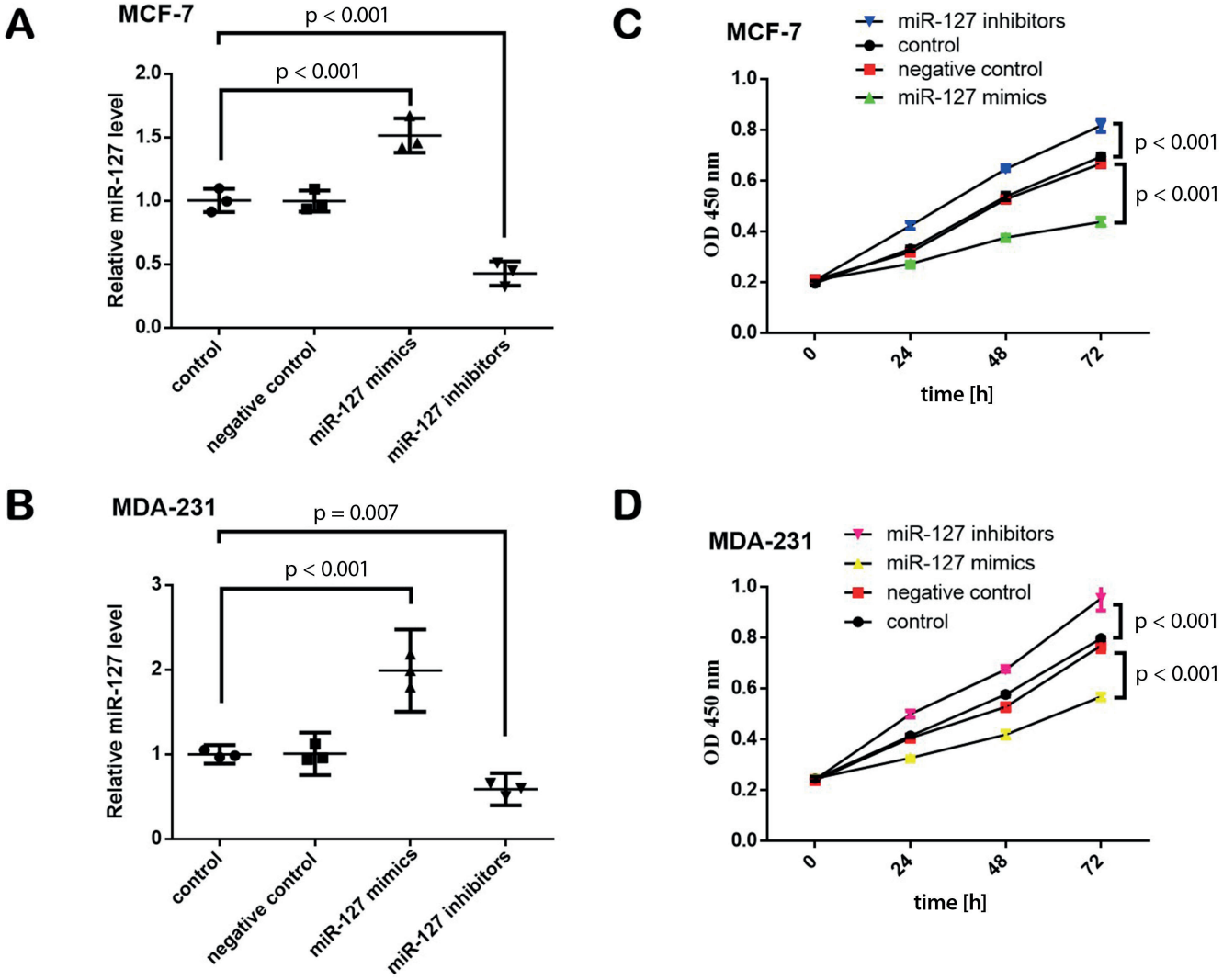



**Fig. 2.** miR-127 inhibited viability in MCF-7 and MDA-231 cells. A. The relative miR-127 level in MCF-7 cells after transfection with miR-127 mimics or inhibitors; B. Relative miR-127 level in MDA-231 cells after transfection with miR-127 mimics or inhibitors; C. Viability of MCF-7 cells after transfection with miR-127 mimics or inhibitors; D. Viability of MDA-231 cells after transfection with miR-127 mimics or inhibitors. Whiskers represents the 95% confidence interval (95% CI) values

OD – optical density.

$p < 0.001$) viability (Fig. 2C,D). When compared with controls, the viability of MCF-7 and MDA-231 cells was inhibited after the cells were transfected with miR-127 mimics. Conversely, the viability of MCF-7 and MDA-231 cells was promoted after the cells were transfected with miR-127 inhibitors.

Moreover, the treatments had a statistically significant effect on both the MCF-7 apoptosis rate ($F(3,8) = 169.603$, $p < 0.001$) and the MDA-231 apoptosis rate ($F(3,8) = 279.029$, $p < 0.001$) (Fig. 3,4). When compared with controls, the apoptosis rate of MCF-7 and MDA-231 cells was significantly increased after the cells were transfected with miR-127 mimics. In contrast, the apoptosis rate of MCF-7 and MDA-231 cells was inhibited after the cells were transfected with inhibitors.

## MiR-127 decreased the expression level of Bcl-2 and increased the expression levels of BAX, Cyt-c and cleaved caspase-3 in MCF-7 and MDA-231 cells

The effect of the treatments on the expression levels of Bcl-2 ($F(3,8) = 46.271$, $p < 0.001$), BAX ($F(3,8) = 255.628$, $p < 0.001$), Cyt-c ($F(3,8) = 137.014$, $p = 0.001$), and cleaved caspase-3 ($F(3,8) = 255.751$, $p < 0.001$) in MCF-7 cells was statistically significant (Fig. 5). A similar effect was found in the expression levels of Bcl-2 ($F(3,8) = 118.263$, $p < 0.001$), BAX ($F(3,8) = 650.679$, $p < 0.001$), Cyt-c ($F(3,8) = 209.512$, $p < 0.001$), and cleaved caspase-3 ($F(3,8) = 128.638$, $p < 0.001$) in MDA-231 cells (Fig. 6).

When compared with controls, the expression of Bcl-2 was significantly decreased, but the expression levels of BAX, Cyt-c and cleaved caspase-3 were significantly

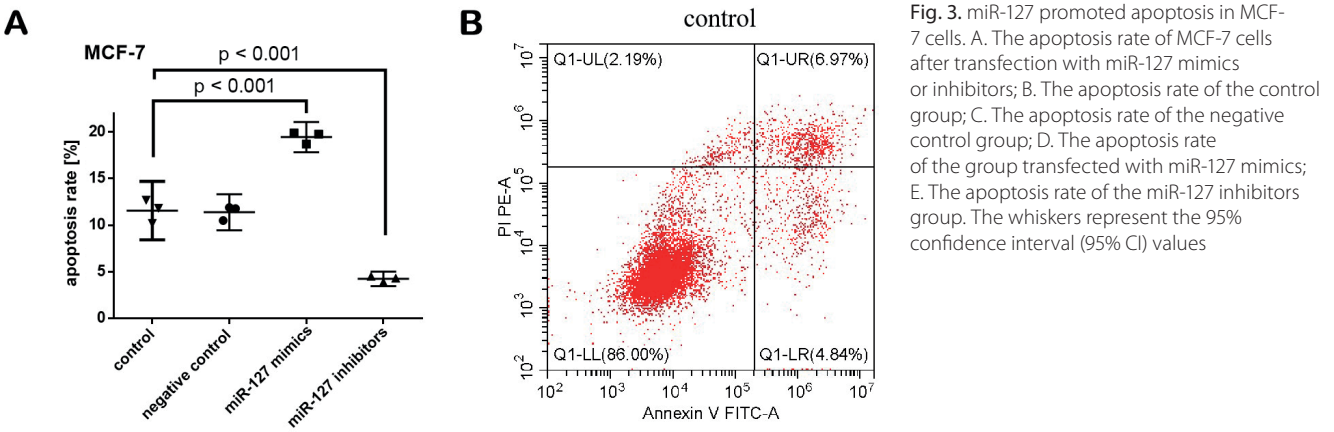



Fig. 3. miR-127 promoted apoptosis in MCF-7 cells. A. The apoptosis rate of MCF-7 cells after transfection with miR-127 mimics or inhibitors; B. The apoptosis rate of the control group; C. The apoptosis rate of the negative control group; D. The apoptosis rate of the group transfected with miR-127 mimics; E. The apoptosis rate of the miR-127 inhibitors group. The whiskers represent the 95% confidence interval (95% CI) values

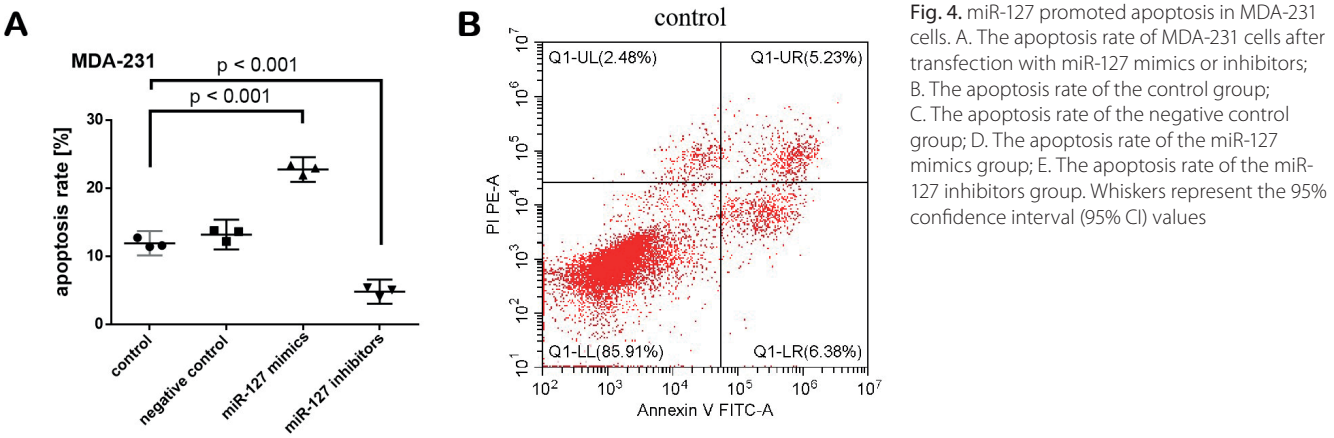



Fig. 4. miR-127 promoted apoptosis in MDA-231 cells. A. The apoptosis rate of MDA-231 cells after transfection with miR-127 mimics or inhibitors; B. The apoptosis rate of the control group; C. The apoptosis rate of the negative control group; D. The apoptosis rate of the miR-127 mimics group; E. The apoptosis rate of the miR-127 inhibitors group. Whiskers represent the 95% confidence interval (95% CI) values

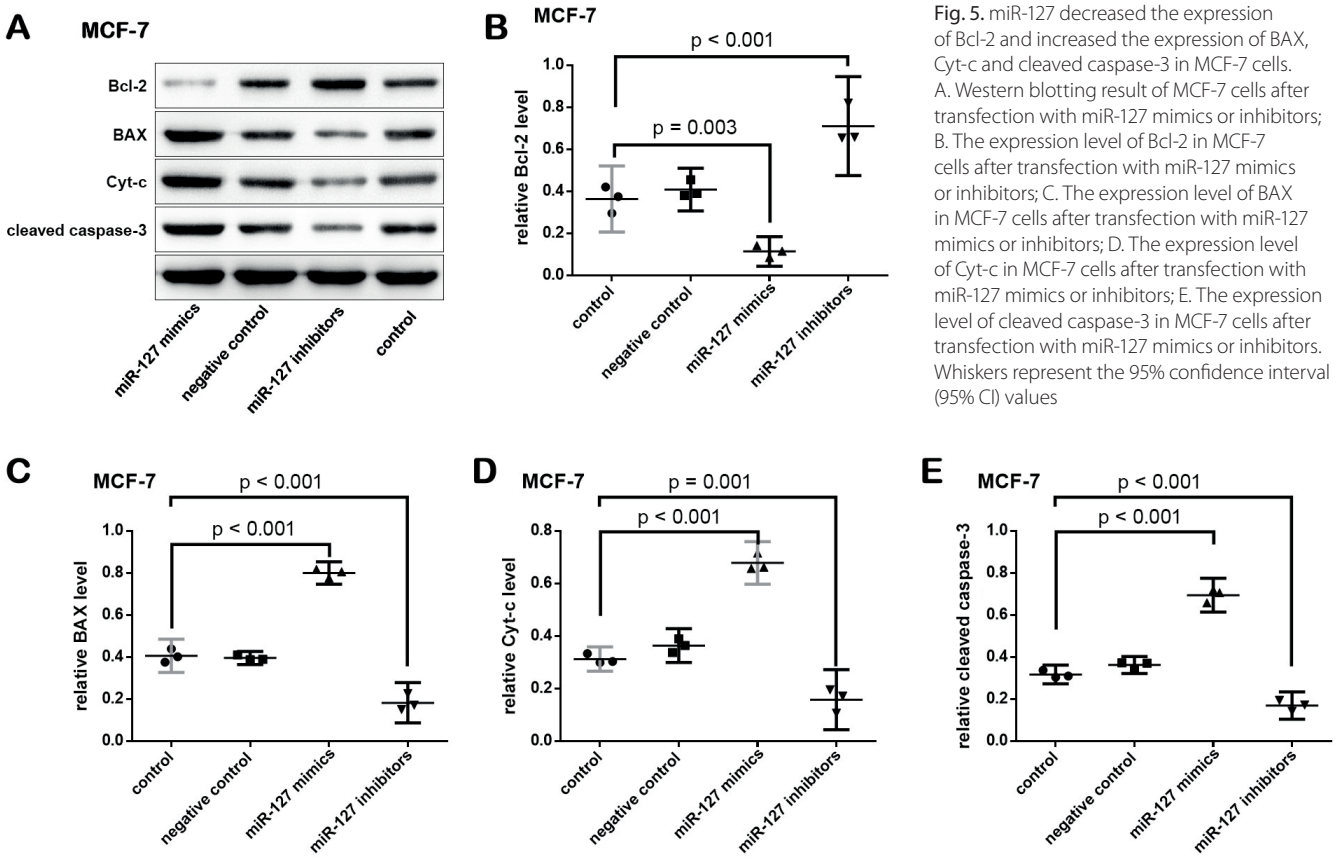



Fig. 5. miR-127 decreased the expression of Bcl-2 and increased the expression of BAX, Cyt-c and cleaved caspase-3 in MCF-7 cells. A. Western blotting result of MCF-7 cells after transfection with miR-127 mimics or inhibitors; B. The expression level of Bcl-2 in MCF-7 cells after transfection with miR-127 mimics or inhibitors; C. The expression level of BAX in MCF-7 cells after transfection with miR-127 mimics or inhibitors; D. The expression level of Cyt-c in MCF-7 cells after transfection with miR-127 mimics or inhibitors; E. The expression level of cleaved caspase-3 in MCF-7 cells after transfection with miR-127 mimics or inhibitors. Whiskers represent the 95% confidence interval (95% CI) values

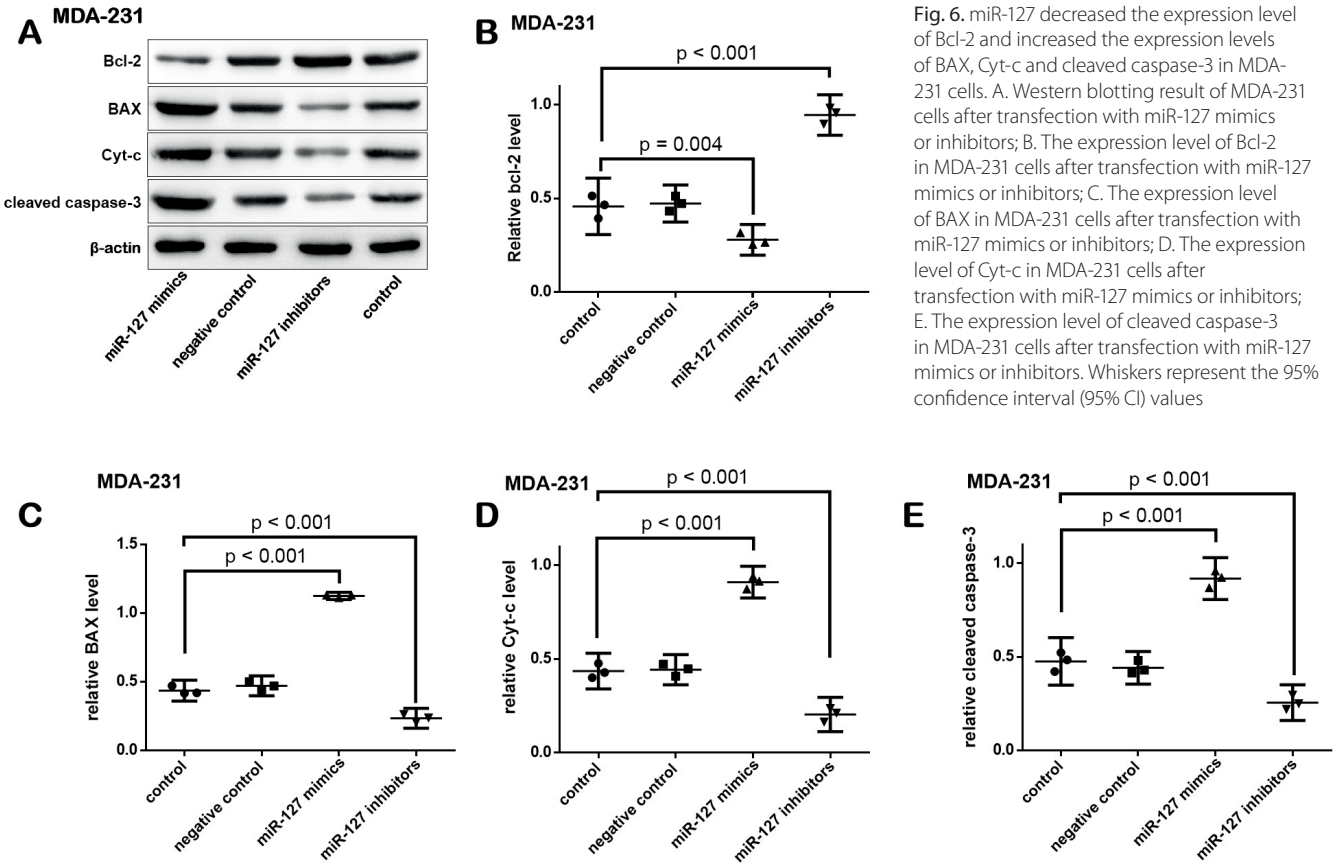



Fig. 6. miR-127 decreased the expression level of Bcl-2 and increased the expression levels of BAX, Cyt-c and cleaved caspase-3 in MDA-231 cells. A. Western blotting result of MDA-231 cells after transfection with miR-127 mimics or inhibitors; B. The expression level of Bcl-2 in MDA-231 cells after transfection with miR-127 mimics or inhibitors; C. The expression level of BAX in MDA-231 cells after transfection with miR-127 mimics or inhibitors; D. The expression level of Cyt-c in MDA-231 cells after transfection with miR-127 mimics or inhibitors; E. The expression level of cleaved caspase-3 in MDA-231 cells after transfection with miR-127 mimics or inhibitors. Whiskers represent the 95% confidence interval (95% CI) values

increased after MCF-7 and MDA-231 cells were transfected with miR-127 mimics. Conversely, the expression level of Bcl-2 was increased, but the expression levels of BAX, Cyt-c and cleaved caspase-3 were decreased after the cells were transfected with miR-127 inhibitors.

## miR-127 inhibited SPP1 expression level through targeting 3'UTR of SPP1

With the help of online bioinformatic software (TargetScan) analysis and previous literature,[31] *SPP1* was predicted as the target gene of miR-127. The predicted binding site between miR-127 and the 3'UTR of MTHFR is shown in Fig. 7. Subsequently, the relationship between miR-127 and SPP1 was validated using dual-luciferase reporter assay. The results showed that miR-127 mimics significantly decreased the relative luciferase activity of SPP1-wild type instead of SPP1-mutant type compared to miR-127 inhibitors (ANOVA, F (3,8) = 30.884, p = 0.001). Furthermore, miR-127 could inhibit SPP1 expression level by targeting the 3' untranslated region of SPP1, making *SPP1* a target gene of miR-127.

## miR-127 decreased the relative SPP1 level in MCF-7 and MDA-231 cells and SPP1 was increased in the plasma of BRC patients and controls

A statistically significant effect of the treatments on both the relative SPP1 level in MCF-7 cells (F (3,8) = 165.636, p < 0.001) and MDA-231 cells (F (3,8) = 73.849, p < 0.001) was found (Fig. 8). When compared with controls,

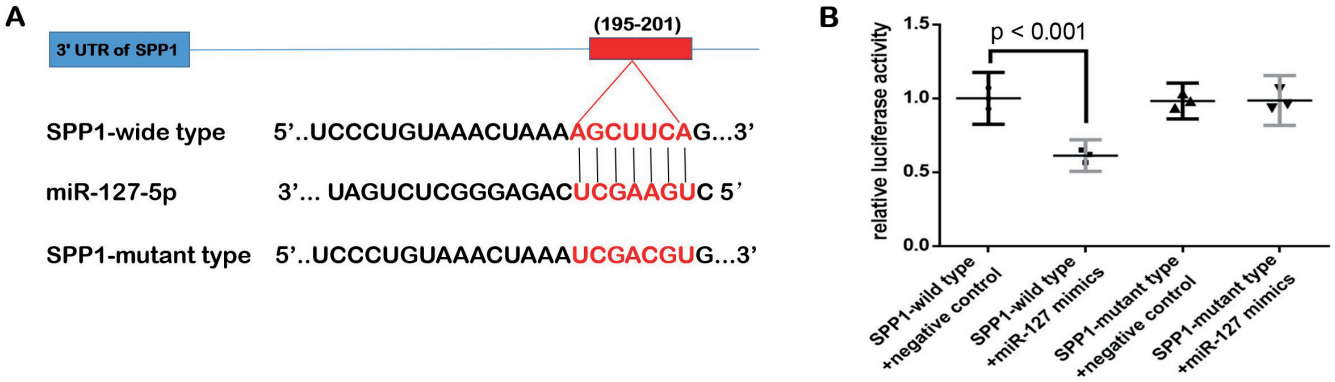



Fig. 7. miR-127 inhibited SPP1 expression level through targeting the 3' untranslated region of SPP1. A. The predicted binding site between miR-127 and the 3' untranslated region of MTHFR; B. Relative luciferase activity of the SSP1-wild type and miR-127 mimics group was significantly lower than in other groups. Whiskers represent the 95% confidence interval (95% CI) values

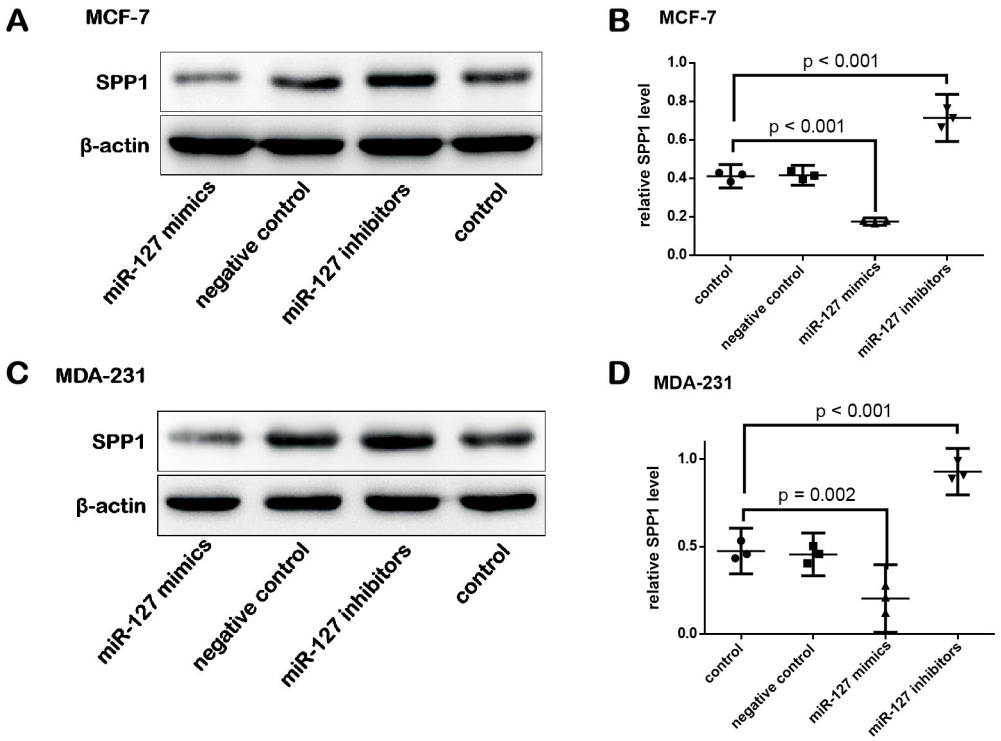



Fig. 8. miR-127 decreased the expression level of SPP1 in MCF-7 and MDA-231 cells. A. Western blotting result of MCF-7 cells after transfection with miR-127 mimics or inhibitors; B. The expression level of SPP1 in MCF-7 cells after transfection with miR-127 mimics or inhibitors; C. Western blotting result of MDA-231 cells after transfection with miR-127 mimics or inhibitors; D. The expression level of SPP1 in MDA-231 cells after transfection with miR-127 mimics or inhibitors. Whiskers represent the 95% confidence interval (95% CI) values

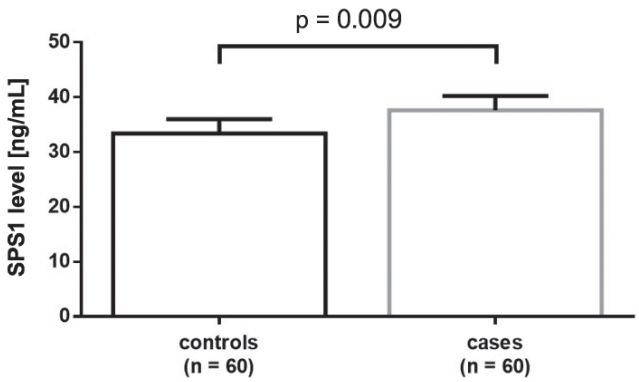



Fig. 9. SPP1 expression in the plasma of controls and breast cancer (BRC) patients. The SPP1 level was significantly increased in the plasma of BRC patients compared to controls. Upper whisker represents a 95% confidence interval (95% CI) value

the relative SPP1 level in MCF-7 and MDA-231 cells was significantly decreased after transfection with miR-127 mimics. Conversely, the relative SPP1 level in MCF-7 and MDA-231 cells was increased after cells were transfected with miR-127 inhibitors. The SPP1 level was significantly increased in the plasma of BRC patients compared to controls (Student's t-test, t = 2.292, df = 118, p = 0.009) (Fig. 9).

## Discussion

Breast cancer is the most prevalent cancer type among female adults in the whole world. According to previous studies, the aberrant expression of non-coding genes like microRNAs was closely related to the etiology of BRC. The miR-127 was a kind of microRNA firstly reported to arise from the region q32 of chromosome 1 and its distinctive expression patterns might be associated with the karyotype of acute myeloid leukemia.[34,35] Based on the evidence from previous studies, the aberrant expression of miR-127 was correlated with various diseases, such as osteoarthritis. Recently, the expression level of miR-127 was also reported to be decreased in the BRC tissues compared to para-carcinoma tissues. In our study, the expression level of miR-127 in the blood samples of BRC patients from Guangxi, China was firstly measured. Similar to the study above, the relative miR-127 level was found to be significantly decreased in the plasma of BRC patients compared to controls, and the dysregulation of miR-127 in the pathogenesis of BRC was further verified. However, the effect of the dysregulation of miR-127 in the pathogenesis of BRC was not clear.

According to research on esophageal squamous cell carcinoma and hepatocellular carcinoma, the upregulation of miR-127 could inhibit the proliferation of cancer cells. As for BRC, a designed RNA-protein nanoplex including miR-127 could effectively inhibit tumor growth in a BRC mouse model. In the current study, 2 typical BRC cell lines, MCF-7 and MDA-231, were chosen to investigate the influence of miR-127 on the viability and apoptosis of BRC cells. The miR-127 successfully inhibited cell viability and promoted apoptosis in MCF-7 and MDA-231 cells. We further explored the influence of miR-127 on the expression levels of pro-proliferation factors such as Bcl-2, and pro-apoptosis factors such as BAX, Cyt-c and cleaved caspase-3 in MCF-7 and MDA-231 cells. We found that miR-127 decreased the expression of Bcl-2 and increased the levels of BAX, Cyt-c and cleaved caspase-3 in MCF-7 and MDA-231 cells. These data highlight that the upregulation of miR-127 in MCF-7 and MDA-231 cells inhibited their proliferation and promoted their apoptosis, but the downregulation of miR-127 promoted their proliferation and inhibited their apoptosis. However, the mechanism of miR-127 in the regulation of viability and apoptosis needs further clarification.

Previously, some studies reported that miR-127 might participate in the occurrence and progression of several kinds of cancer like squamous cell carcinoma via regulating some target genes like ADCY7. However, the target gene of miR-127 and its role in the pathogenesis of BRC have not been reported. With the help of online bioinformatic software analysis, previous literature[33] and dual-luciferase reporter assay, *SPP1* was successfully predicted and validated as a target gene of miR-127. To further explore the influence of miR-127 on SPP1, we measured the expression of SPP1 in MCF-7 and MDA-231 cells after miR-127 was up- or downregulated. The miR-127 decreased the expression level of SPP1 in MCF-7 and MDA-231 cells. The *SPP1* is a gene located in the q22 region of chromosome 4 and has been investigated by numerous groups regarding the pathogenesis of various cancers, including BRC. Qin et al. reported that SPP1 was upregulated in the blood samples of patients with nasopharyngeal carcinoma, and when upregulated in CEN-2Z cancer cells, it promoted their proliferation and suppressed their apoptosis.[36] Similarly, Liu et al. mentioned that SPP1 downregulation in human renal cancer cells inhibited their proliferation and increased their apoptosis.[37] In addition, miR-181a was found to promote the apoptosis of cervical cancer cells by inhibiting the expression level of SPP1, and SPP1 could facilitate chemotherapy resistance of BRC cells by preventing the activation of caspase-3 into cleaved caspase-3.[38] In our study, the expression level of SPP1 was significantly increased in the plasma of BRC patients compared to controls. Based on the evidence above, we could make preliminary conclusions that the downregulation of miR-127 increased the expression level of SPP1, promoted BRC cell proliferation, inhibited their apoptosis, and, finally, promoted the occurrence of BRC.

### Limitations

All the patients and controls in our study were enrolled in the Affiliated Hospital of Youjiang Medical University for Nationalities. However, the data collected from other research centers will be examined in the future by our team in cooperation with researchers in other countries.

Second, due to limited funding and research resources, the role of miR-127 in the pathogenesis of BRC was explored in clinical samples and cellular models, but not in animal models, and the sample size was limited in our in vitro experiments. The data from animal models will be collected, and a larger sample size in the cellular experiments will be further explored in future studies.

# Conclusions

In summary, our study measured the expression of miR-127 in blood samples of BRC patients from Guangxi, China, investigated the influence of miR-127 on the viability and apoptosis of BRC cell lines, and revealed a target gene of miR-127, potentially highlighting the mechanism involving miR-127 in the pathogenesis of BRC. These outcomes may provide critical information for a new explanation of BRC pathogenesis and a potential diagnostic or therapeutic biomarker.

## Supplementary data

The Supplementary Tables are available at https://doi.org/10.5281/zenodo.7624083. The package contains the following files:

Supplementary Table 1. Normality distribution and the homogeneity of variance.

Supplementary Table 2. Results of ANOVA followed by the post hoc analysis.


### ORCID iDs

Guijiang Wei https://orcid.org/0000-0001-8856-2101
Meiying Tan https://orcid.org/0009-0006-9476-1450
Chunfang Wang https://orcid.org/0000-0001-6055-4564
Lina Liang https://orcid.org/0000-0001-7573-8147


### References


1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
2. Azamjah N, Soltan-Zadeh Y, Zayeri F. Global trend of breast cancer mortality rate: A 25-year study. *Asian Pac J Cancer Prev*. 2019;20(7):2015–2020. doi:10.31557/APJCP.2019.20.7.2015
3. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(6):438–451. doi:10.3322/caac.21583
4. Li H, Zheng RS, Zhang SW, et al. Incidence and mortality of female breast cancer in China, 2014 [in Chinese]. *Zhonghua Zhong Liu Za Zhi*. 2018;40(3):166–171. doi:10.3760/cma.j.issn.0253-3766.2018.03.002
5. Escala-Garcia M, Morra A, Canisius S, et al. Breast cancer risk factors and their effects on survival: A Mendelian randomisation study. *BMC Med*. 2020;18(1):327. doi:10.1186/s12916-020-01797-2
6. Li N, Deng Y, Zhou L, et al. Global burden of breast cancer and attributable risk factors in 195 countries and territories, from 1990 to 2017: Results from the Global Burden of Disease Study 2017. *J Hematol Oncol*. 2019;12(1):140. doi:10.1186/s13045-019-0828-0
7. Ugalde-Morales E, Grassmann F, Humphreys K, et al. Association between breast cancer risk and disease aggressiveness: Characterizing underlying gene expression patterns. *Int J Cancer*. 2021;148(4):884–894. doi:10.1002/ijc.33270
8. Barros-Oliveira M da C, Costa-Silva DR, dos Santos AR, Pereira RO, Soares-Júnior JM, da Silva BB. Influence of *CYP19A1* gene expression levels in women with breast cancer: A systematic review of the literature. *Clinics (Sao Paulo)*. 2021;76:e2846. doi:10.6061/clinics/2021/e2846
9. Crudele F, Bianchi N, Reali E, Galasso M, Agnoletto C, Volinia S. The network of non-coding RNAs and their molecular targets in breast cancer. *Mol Cancer*. 2020;19(1):61. doi:10.1186/s12943-020-01181-x
10. Liu J, Zhao G, Liu XL, et al. Progress of non-coding RNAs in triple-negative breast cancer. *Life Sci*. 2021;272:119238. doi:10.1016/j.lfs.2021.119238
11. Bahreini F, Rayzan E, Rezaei N. MicroRNA-related single-nucleotide polymorphisms and breast cancer. *J Cell Physiol*. 2021;236(3):1593–1605. doi:10.1002/jcp.29966
12. Sabit H, Cevik E, Tombuloglu H, Abdel-Ghany S, Tombuloglu G, Esteller M. Triple negative breast cancer in the era of miRNA. *Crit Rev Oncol Hematol*. 2021;157:103196. doi:10.1016/j.critrevonc.2020.103196
13. Chen PS, Lin SC, Tsai SJ. Complexity in regulating microRNA biogenesis in cancer. *Exp Biol Med (Maywood)*. 2020;245(5):395–401. doi:10.1177/1535370220907314
14. Dexheimer PJ, Cochella L. MicroRNAs: From mechanism to organism. *Front Cell Dev Biol*. 2020;8:409. doi:10.3389/fcell.2020.00409
15. Annese T, Tamma R, De Giorgis M, Ribatti D. MicroRNAs biogenesis, functions and role in tumor angiogenesis. *Front Oncol*. 2020;10:581007. doi:10.3389/fonc.2020.581007
16. Chen J, Wang M, Guo M, Xie Y, Cong YS. MiR-127 regulates cell proliferation and senescence by targeting BCL6. *PLoS One*. 2013;8(11):e80266. doi:10.1371/journal.pone.0080266
17. Wang S, Li H, Wang J, Wang D, Yao A, Li Q. Prognostic and biological significance of microRNA-127 expression in human breast cancer. *Dis Markers*. 2014;2014:401986. doi:10.1155/2014/401986
18. Zhou ZB, Huang GX, Fu Q, et al. CircRNA33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. *Mol Ther*. 2019;27(3):531–541. doi:10.1016/j.ymthe.2019.01.006
19. Li Z, Yuan B, Pei Z, et al. Circ_0136474 and MMP-13 suppressed cell proliferation by competitive binding to miR-127-5p in osteoarthritis. *J Cell Mol Med*. 2019;23(10):6554–6564. doi:10.1111/jcmm.14400
20. Chen C, Lin S, Zhou L, et al. MicroRNA-127-5p attenuates severe pneumonia via tumor necrosis factor receptor-associated factor 1. *Exp Ther Med*. 2020;20(3):2856–2862. doi:10.3892/etm.2020.8997
21. Zhang X, Dang Y, Liu R, Zhao S, Ma J, Qin Y. MicroRNA-127-5p impairs function of granulosa cells via *HMGB2* gene in premature ovarian insufficiency. *J Cell Physiol*. 2020;235(11):8826–8838. doi:10.1002/jcp.29725
22. Tang FH, Chang WA, Tsai EM, Tsai MJ, Kuo PL. Investigating novel genes potentially involved in endometrial adenocarcinoma using next-generation sequencing and bioinformatic approaches. *Int J Med Sci*. 2019;16(10):1338–1348. doi:10.7150/ijms.38219
23. Pathak S, Meng WJ, Nandy SK, et al. Radiation and SN38 treatments modulate the expression of microRNAs, cytokines and chemokines in colon cancer cells in a p53-directed manner. *Oncotarget*. 2015;6(42):44758–44780. doi:10.18632/oncotarget.5815
24. Cai L, Wang Y, Wu J, Wu G. Hsa_circ_0008234 facilitates proliferation of cutaneous squamous cell carcinoma through targeting miR-127-5p to regulate ADCY7. *Arch Dermatol Res*. 2022;314(6):541–551. doi:10.1007/s00403-021-02261-8
25. Xiong G, Diao D, Lu D, et al. Circular RNA circNELL2 acts as the sponge of miR-127-5p to promote esophageal squamous cell carcinoma progression. *Onco Targets Ther*. 2020;13:9245–9255. doi:10.2147/OTT.S247847
26. Liang M, Yao W, Shi B, et al. Circular RNA hsa_circ_0110389 promotes gastric cancer progression through upregulating SORT1 via sponging miR-127-5p and miR-136-5p. *Cell Death Dis*. 2021;12(7):639. doi:10.1038/s41419-021-03903-5
27. Chang S, Sun L, Feng G. SP1-mediated long noncoding RNA POU3F3 accelerates the cervical cancer through miR-127-5p/FOXD1. *Biomed Pharmacother*. 2019;117:109133. doi:10.1016/j.biopha.2019.109133
28. Su Y, Xu C, Liu Y, Hu Y, Wu H. Circular RNA hsa_circ_0001649 inhibits hepatocellular carcinoma progression via multiple miRNAs sponge. *Aging (Albany NY)*. 2019;11(10):3362–3375. doi:10.18632/aging.101988
29. Zhang W, Zhu L, Yang G, et al. Hsa_circ_0026134 expression promoted TRIM25- and IGF2BP3-mediated hepatocellular carcinoma cell proliferation and invasion via sponging miR-127-5p. *Biosci Rep*. 2020;40(7):BSR20191418. doi:10.1042/BSR20191418
30. Huan L, Bao C, Chen D, et al. MicroRNA-127-5p targets the biliverdin reductase B/nuclear factor-κB pathway to suppress cell growth in hepatocellular carcinoma cells. *Cancer Sci*. 2016;107(3):258–266. doi:10.1111/cas.12869

31. Pronina IV, Loginov VI, Burdennyy AM, et al. DNA methylation contributes to deregulation of 12 cancer-associated microRNAs and breast cancer progression. *Gene*. 2017;604:1–8. doi:10.1016/j.gene.2016.12.018
32. Deci MB, Liu M, Gonya J, et al. Carrier-free CXCR4-targeted nanoplexes designed for polarizing macrophages to suppress tumor growth. *Cel Mol Bioeng*. 2019;12(5):375–388. doi:10.1007/s12195-019-00589-w
33. Tu M, Li Y, Zeng C, et al. MicroRNA-127-5p regulates osteopontin expression and osteopontin-mediated proliferation of human chondrocytes. *Sci Rep*. 2016;6(1):25032. doi:10.1038/srep25032
34. Song G, Wang L. MiR-433 and miR-127 arise from independent overlapping primary transcripts encoded by the miR-433-127 locus. *PLoS One*. 2008;3(10):e3574. doi:10.1371/journal.pone.0003574
35. Dixon-McIver A, East P, Mein CA, et al. Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia. *PLoS One*. 2008;3(5):e2141. doi:10.1371/journal.pone.0002141
36. Qin H, Wang R, Wei G, et al. Overexpression of osteopontin promotes cell proliferation and migration in human nasopharyngeal carcinoma and is associated with poor prognosis. *Eur Arch Otorhinolaryngol*. 2018;275(2):525–534. doi:10.1007/s00405-017-4827-x
37. Liu H, Chen A, Guo F, Yuan L. Influence of osteopontin short hairpin RNA on the proliferation and invasion of human renal cancer cells. *J Huazhong Univ Sci Technolog Med Sci*. 2010;30(1):61–68. doi:10.1007/s11596-011-0111-7
38. Graessmann M, Berg B, Fuchs B, Klein A, Graessmann A. Chemotherapy resistance of mouse WAP-SVT/t breast cancer cells is mediated by osteopontin, inhibiting apoptosis downstream of caspase-3. *Oncogene*. 2007;26(20):2840–2850. doi:10.1038/sj.onc.1210096

# Initial clinical, laboratory and radiological features of SARS-CoV-2-infected patients and their impact on the course of the disease

Mateusz Maligłówka[1,A–D], Łukasz Bułdak[1,A,C–F], Marcin Cyrnek[1,B,D], Marcin Hachuła[1,B,D], Michał Kosowski[1,B,D], Marcin Basiak[1,C,E], Witold Szkróbka[1,C,E], Joanna Bosowska[2,B,C], Maciej Cebula[2,B,C], Michał Holecki[3,E,F], Bogusław Okopień[1,E,F]

[1] Department of Internal Medicine and Clinical Pharmacology, School of Medicine, Medical University of Silesia, Katowice, Poland

[2] Department of Radiodiagnostics, Invasive Radiology and Nuclear Medicine, Department of Radiology and Nuclear Medicine, School of Medicine, Medical University of Silesia, Katowice, Poland

[3] Department of Internal Medicine, Autoimmune and Metabolic Diseases, School of Medicine, Medical University of Silesia, Katowice, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1125–1132

**Address for correspondence**
Mateusz Maligłówka
E-mail: mmaliglowka@sum.edu.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on February 17, 2022
Reviewed on October 26, 2022
Accepted on February 12, 2023

Published online on March 30, 2023

## Abstract

**Background.** On March 11, 2020, coronavirus disease (COVID-19) was declared a global threat by the World Health Organization (WHO). It quickly became apparent that reducing inpatient mortality rates and early phase prediction of possible deterioration or severe disease course relied on finding more specific biomarkers.

**Objectives.** This retrospective study assessed initial clinical, laboratory and radiological features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients and explored their impact on mortality and the course of the disease. Such efforts aimed to facilitate the identification of high-risk patients and to improve the formulation of treatment plans for these individuals.

**Materials and methods.** The cohort comprised 111 consecutive adult inpatients diagnosed with COVID-19 and hospitalized in the Internal Medicine Ward of the University Clinical Center of prof. K. Gibiński of the Medical University of Silesia in Katowice, Poland, a COVID-19 Treatment Unit, between November 16, 2020 and February 15, 2021. All available clinical, laboratory and radiological findings were extracted from electronic records and assessed as possible risk factors for poor prognosis.

**Results.** Clinicasl and radiological features with higher frequency in COVID-19 non-survivors included older age, history of smoking, concomitant cardiovascular diseases, low oxygen saturation ($SpO_2$), and high infection risk assessed on admission as well as high opacity score, percentage of opacity and percentage of high opacity in computed tomography. Non-survivors had decreased serum lymphocytes, monocytes, calcium, magnesium, and hemoglobin oxygen saturation. They also had increased red cell distribution width (RDW), C-reactive protein (CRP), procalcitonin, alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), D-dimer, troponin, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels, as well as a base deficit.

**Conclusions.** This retrospective study identified several markers associated with a fatal course of COVID-19. The early assessment of SARS-CoV-2-infected inpatients should consider these markers.

**Key words:** COVID-19, SARS-CoV-2, markers of poor prognosis

**Cite as**
Maligłówka M, Bułdak Ł, Cyrnek M, et al. Initial clinical, laboratory and radiological features of SARS-CoV-2-infected patients and their impact on the course of the disease. *Adv Clin Exp Med*. 2023;32(10):1125–1132. doi:10.17219/acem/161158

**DOI**
10.17219/acem/161158

# Background

A novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China and caused coronavirus disease (COVID-19), which became a health concern at the end of 2019. On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic.[1] Epidemiological reports from the WHO, as of July 6, 2021, confirmed over 180 million COVID-19 diagnoses worldwide, and more than 3.9 million infected patients died.[2] The constant spread of novel viral mutations, inadequate prophylactic methods and the lack of highly effective targeted treatment caused a growing number of hospitalizations and the breakdown of the healthcare systems in many countries.

The most common symptoms of COVID-19 are fever, cough, fatigue, anorexia, shortness of breath, and muscle pain.[3] According to the available data, typical changes in laboratory tests include leukopenia with decreased lymphocyte count and elevated serum levels of C-reactive protein (CRP), lactate dehydrogenase (LDH) and D-dimers.[4,5] Typical radiological manifestations presented in high-resolution computed tomography (HRCT) include ground-glass opacities (GGO), consolidations, crazy paving, and reticular patterns.[6,7]

Approximately 15% of patients suffer from severe COVID-19 symptoms that require oxygen support, and 5% develop critical complications such as acute respiratory distress syndrome (ARDS), sepsis, multiple organ failure, and thromboembolism.[8] There is a link between older age (over 60 years), smoking, obesity, pregnancy, and concomitant diseases such as diabetes, hypertension, chronic diseases of the heart, lungs, and kidneys, and severe disease and death.[8–10] Furthermore, high sequential organ failure assessment (SOFA) scores and D-dimer levels above 1 μg/mL are associated with poor prognosis.[11]

The treatment of COVID-19 patients depends on the phase of the disease. According to the recommendations of the Polish Association of Epidemiologists and Infectiologists, there are 4 clinical stages of COVID-19:

Stage 1. Asymptomatic or mildly symptomatic, oxygen saturation ($SpO_2$) ≥ 95%, and no hospitalization is necessary;

Stage 2. Fully symptomatic (viral multiplication), $SpO_2$ drops below 95%, usually during the 1st week after disease onset, and hospitalization is required;

Stage 3. Respiratory failure (cytokine storm), $SpO_2$ < 90%, usually occurs in the 2nd week after disease onset, and hospitalization is required;

Stage 4. ARDS, mechanical ventilation and intensive care unit treatment are required.[12]

According to the international guidelines of contagious disease associations, there is some evidence for the efficacy of anti-inflammatory drugs (glucocorticoids (GKs) and tocilizumab), antiviral drugs (remdesivir), anticoagulants (low-molecular-weight heparin), and oxygen ($O_2$) supplementation, especially in patients with a severe condition.[13]

The reduction of COVID-19 mortality was one of the main objectives during the pandemic and relied on finding specific markers capable of predicting possible worsening or severe disease course (including death) at an early stage. Therefore, this retrospective study compared the clinical, laboratory and imaging results of survivors and non-survivors hospitalized for COVID-19 in order to identify clinical features and early markers of poor prognosis. Clustering of the biomarkers should constitute a warning sign and may lead to the earlier introduction of a more aggressive therapy, which may improve patient outcomes.

# Objectives

To identify potential biomarkers of severe SARS-CoV-2 infection among clinical, laboratory and radiological features at the beginning of the treatment process.

# Materials and methods

## Study design

This retrospective study included 111 adult inpatients diagnosed as SARS-CoV-2 nucleic acid-positive using real-time polymerase chain reaction (PCR; 78 patients) or tested SARS-CoV-2 antigen-positive using antigen test (33 patients) between November 16, 2020 and February 15, 2021. Patients were admitted to the Internal Medicine Ward of the University Clinical Center of prof. K. Gibiński of the Medical University of Silesia in Katowice, Poland, which was converted into a COVID-19 Treatment Unit.

A screening tool devised by Grzesiowski (Table 1) and modified for the initial assessment of all patients admitted to our hospital was used to estimate the risk of infection.[14] The screening tool estimated the probability of infectious complications in the hospitalized subjects, though it was not specifically devised or validated for use in research concerning SARS-CoV-2-like infections. A score of at least 1 point designated a patient to the high-risk infection group. Analyses at the central laboratory utilized commercially available equipment: Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan), ACL 500 (Werfen, Barcelona, Spain), Rapid Point 500 (Siemens Healthcare, Erlangen, Germany), and Cobas PRO (Roche Diagnostics GmbH, Mannheim, Germany).

A validated image processing plug-in package, Siemens Syngo.via® (Siemens Healthcare, Erlangen, Germany), was used for assessing HRCT scans with a VB50. Parenchymal lung changes caused by SARS-CoV-2 and observed as an abnormal increase in lung density were measured in Hounsfield units (HU) and defined as opacities.[15]

Table 1. Risk factors for infection on admission to hospital

| Risk factors for infection on admission to hospital | Points |
|---|---|
| Age >75 years | 0/1 |
| Transfer from other ward or hospitalization in the last 6 months | 0/1 |
| Surgery/invasive test in the last 6 months | 0/1 |
| Alert pathogen colonization | 0/1 |
| Presence of catheters | 0/1 |
| Skin injury | 0/1 |
| Unconsciousness/aspiration/sudden cardiac arrest/ immobilization | 0/1 |
| Antimicrobial therapy in the last 3 months | 0/1 |
| Current immunosuppressive therapy/radiotherapy/ chemotherapy | 0/1 |
| Metabolic disease (i.e., diabetes, obesity, uremia) | 0/1 |
| Malnutrition | 0/1 |
| Current neoplastic disease | 0/1 |
| COPD/asthma/respiratory failure | 0/1 |
| Total | 0–13 |

Low infection risk = 0; high infection risk ≥1; COPD – chronic obstructive pulmonary disease.

A semi-quantitative scoring system devised by Pan et al.[6] classified the opacity score by assessing the involvement of each lobe as 0 (no involvement), 1 (<5% involvement), 2 (6–25% involvement), 3 (26–49% involvement), 4 (50–75% involvement), or 5 (>75% involvement). The total opacity score was the sum of the score of each lobe, and ranged from 0 (no involvement) to 25 (maximum involvement). A HU threshold of –200 was applied by default for the high-density opacities. The approximate density determined for healthy lung parenchyma was between –700 and –600 HU.[16]

The 2 study groups included survivors (n = 76) and non-survivors (n = 35). The definition of survivors was patients discharged without symptoms of COVID-19 after isolation. Non-survivors were patients who died during isolation. According to the Chief Sanitary Inspectorate Guidelines, the minimum isolation for symptomatic patients was 13 days from symptom occurrence, with at least 3 symptom-free days. In cases of persistent clinical features of COVID-19, the isolation was prolonged.[17]

According to the Ethics Committee of the Medical University of Silesia (Katowice, Poland), this retrospective study did not require the approval of the Ethics Committee (statement No. PCN/0022/KB/62121).

## Statistical analyses

All available data were collated in a Microsoft Excel 365 spreadsheet (Microsoft Corp., Redmond, USA) and transferred to the Statistica software package v. 13.0 (StatSoft Inc., Tulsa, USA) and Plus Set v. 5.0 (TIBCO Software Inc., Palo Alto, USA). Data are presented in tables as mean with 95% confidence interval (95% CI) for normal distribution or median with interquartile range (IQR) for non-normal distribution. The Shapiro–Wilk test and visual inspection of histograms assessed the distribution of continuous variables. Normally distributed data had the Shapiro–Wilk test $p \geq 0.05$ and a dome-shaped histogram distribution, while data with a non-normal distribution had a value of $p < 0.05$. The Student's t-test was used to compare means of continuous data between survivors and non-survivors, and the Mann–Whitney U test was used to evaluate medians of non-normal distributions. At the same time, the Fisher's exact test was employed to compare categorical variables. A value of $p < 0.05$ indicated statistical significance.

# Results

## Clinical features

This retrospective study included a total of 111 inpatients with a mean age of 68.3 (65.9–71.3) years, with 51 females and 60 males classified as survivors (n = 76) or non-survivors (n = 35). The mean age of non-survivors (73.2 (69.1–77.4) years) was significantly higher (p = 0.019) than that of survivors (66.1 (65.5–69.7) years). The medical history of non-survivors indicated a significantly greater incidence of cardiovascular and metabolic diseases such as hypertension, ischemic heart disease, heart failure, and type 2 diabetes (88.6% compared to 68.4%; p = 0.033). Meanwhile, non-survivors used nicotine more often (60% compared to 35.6%; p = 0.023) and had an increased risk of infection (62.9% compared to 38.2%; p = 0.033) on admission to the hospital (Table 2). However, treatment before the infection with angiotensin-converting-enzyme inhibitors (ACEIs), angiotensin-II-receptor antagonists (sartans), β-blockers, direct oral anticoagulants (DOACs), vitamin K antagonists (VKAs), mineralocorticoid receptor antagonists (MRAs), or antiplatelet drugs, did not significantly differ between survivors and non-survivors.

One of the most vital elements for selecting high-risk patients was infection risk assessment on admission, based on data gathered during anamnesis (Table 1). It revealed a higher probability of COVID-19-related death in patients at an increased risk of infection due to clinical predispositions. Moreover, physical examination revealed significantly lower peripheral capillary hemoglobin $SpO_2$ values in survivors (94% (87%–97%) compared to 96% (93%–97%); p = 0.046).

## Laboratory findings

Initial laboratory findings in non-survivors revealed significantly lower lymphocyte ($0.76\times10^9$/L (0.48–1.33) compared to $1.15\times10^9$/L (0.69–1.57); p = 0.031) and monocyte ($0.39\times10^9$/L (0.28–0.75) compared to $0.6\times10^9$/L (0.45–0.835); p = 0.021) counts, significantly higher red cell distribution width (RDW) (47.05 (43–50) fL compared to 43.7

Table 2. Clinical features of coronavirus disease (COVID-19) patients

| Clinical feature | Survivors (n = 76) | Non-survivors (n = 35) | test value | df | p-value | Statistical test |
|---|---|---|---|---|---|---|
| Age [years] (mean and 95% CI) | 66.1 (62.5–69.7) | 73.2 (69.1–77.4) | −2.382 (t value) | 109 | 0.019 | Student's t-test |
| Cardiovascular and metabolic diseases (hypertension or coronary artery disease or heart failure or type 2 diabetes) | 52/76 (68.4%) | 31/35 (88.6%) | – | 1 | 0.033 | Fisher's test |
| Nicotine use | 27/76 (35.6%) | 21/35 (60.0%) | – | 1 | 0.023 | |
| High risk for infection | 29/76 (38.2%) | 22/35 (62.9%) | – | 1 | 0.033 | |
| $SpO_2$ [%] (median and 25%–75% Q) | 96 (93–97) | 94 (87–97) | 1.999 (U value) | N/A | 0.046 | Mann–Whitney U test |

$SpO_2$ – oxygen saturation; 95% CI – 95% confidence interval; df – degrees of freedom; N/A – not applicable.

(39.8–50.4) fL; p = 0.021), and significantly higher serum levels of CRP (80.3 (40.5–128) ng/mL compared to 45.7 (9.6–88.8) mg/L; p = 0.003), procalcitonin (0.39 (0.199–1.04) ng/mL compared to 0.17 (0.068–0.347) ng/mL; p = 0.001) and D-dimers (2409 (1170–11,412) ng/mL compared to 1410.5 (867–2849) ng/mL; p = 0.026) than those in survivors.

Disturbances in plasma electrolytes manifested in non-survivors as lower levels of total serum calcium (8.32 (8.02–8.62) mmol/L compared to 8.66 (8.47–8.83) mmol/L; p = 0.043) and magnesium (1.69 (1.61–1.92) mmol/L compared to 1.94 (1.73–2.09) mmol/L; p = 0.037). Other laboratory findings reflecting internal organ function in non-survivors included significant elevations in serum alkaline phosphatase (ALP) activity (97 (78–145) U/L compared to 68 (55–102) U/L; p = 0.027), creatinine concentration (1.19 (0.85–1.83) mg/dL compared to 0.99 (0.75–1.21) mg/dL; p = 0.012), blood urea nitrogen (BUN) (28.4 (17.4–59.4) mg/dL compared to 15.84 (12.2–24.7) mg/dL; p = 0.012), troponin (43.25 (27.6–78.6) ng/L compared to 22.7 (10.2–36.3) ng/L; p = 0.001), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (3734.5 (740–12,080) pg/mL compared to 792 (154–1952) pg/mL; p = 0.003). Furthermore, acid-base balance assessment of arterial blood samples revealed significantly lower hemoglobin $SpO_2$ (88.85% (77.8–93.5%) compared to 94.2% (89.4–95.7%); p = 0.039) and higher base excess in non-survivors (5 (2.45–9.25) mmol/L compared to 2.6 (0.8–3.1) mmol/L; p = 0.022), as compared with survivors (Table 3).

## Computed tomography findings

Initial inpatient computer tomography (CT) records (n = 83) (61 from survivors and 22 from non-survivors) provided opacity scores and their derivatives. The group of non-survivors had a significantly higher opacity score (7 (4–11) compared to 4 (2–7); p = 0.004), percentage of opacity (16.0% (6.4–41.4%) compared to 7.9% (1.3–19.1%); p = 0.001) and volume of opacity (609.9 (259.1–1209.2) mL compared to 279.6 (41.3–685.9) mL; p = 0.011), as well as a higher percentage of high opacity (5.3% (1.5–17.2%) compared to 1.6% (0.1–5.2%); p = 0.003) and volume of high opacity (200.8 (61.1–548.8) mL compared to 43.3 (3.2–163.5) mL; p = 0.002) than survivors (Table 4).

## Therapeutic interventions

Intravenous drug use (GKs, including 4 mg of dexamethasone daily and 200 mg remdesivir on day 1, followed by a maintenance dose of 100 mg for 4 days) and $O_2$ therapy in the study group followed the recommendations of the Polish Association of Epidemiologists and Infectiologists. In detail, GKs are indicated for SARS-CoV-2-infected patients at stages 2, 3 and 4, whereas remdesivir is indicated for stage 2.[12] Remdesivir was used to a similar extent in both groups (28.8% compared to 34.4%; p = 0.647), while dexamethasone was used much more often in non-survivors (90.6% compared to 49.3%; p < 0.001). The passive $O_2$ supply used a facial mask with $O_2$ flow up to 15 L/min and was more frequent in non-survivors (88.6% compared to 53.3%; p < 0.001), as was mechanical ventilation (31.4% compared to 2.7%; p < 0.001). Breathing support methods were not evaluated due to the limited access to high-flow $O_2$ therapy and noninvasive ventilation devices during the first stages of the pandemic.

# Discussion

This retrospective analysis described the clinical, laboratory and radiological parameters of COVID-19 patients and aimed to identify differences that may affect the course of the disease in survivors and non-survivors. Our observations appear to confirm prior reports on the COVID-19 risk factors for severe disease or death, including older age, history of smoking and concomitant cardiovascular diseases.[8,9,18] On the other hand, obesity was not a risk factor for a poor prognosis. However, this may be due to insufficient number of participants with a body mass index (BMI) >30 (15%).

Taking into consideration the ability of SARS-CoV-2 to induce silent hypoxemia, measuring $SpO_2$ using a pulse oximeter is indispensable to physical examination.[19] In the current study, pulse oximetry demonstrated

**Table 3.** Laboratory findings of coronavirus disease (COVID-19) patients

| Laboratory markers | Reference range | Survivors (n = 76) | Non-survivors (n = 35) | test value | df | p-value | Statistical test |
|---|---|---|---|---|---|---|---|
| Lymphocyte count [×10$^9$/L] | 1.5–3.5 | 1.15 (0.69–1.57) | 0.76 (0.48–1.33) | 786.0 | N/A | 0.031 | Mann–Whitney U test |
| Monocyte count [×10$^9$/L] | 0.2–0.8 | 0.6 (0.45–0.835) | 0.39 (0.28–0.75) | 765.0 | N/A | 0.021 | |
| RDW-SD [fL] | 36.3–47.3 | 43.7 (39.8–50.4) | 47.05 (43–50) | 921.5 | N/A | 0.021 | |
| C-reactive protein [mg/L] | <5 | 45.7 (9.6–88.8) | 80.3 (40.5–128) | 856.0 | N/A | 0.003 | |
| Procalcitonin [ng/mL] | <0.5 | 0.17 (0.068–0.347) | 0.39 (0.199–1.04) | 468.0 | N/A | 0.001 | |
| ALP [U/L] | 40–129 | 68 (55–102) | 97 (78–145) | 488.0 | N/A | 0.027 | |
| Creatinine [mg/dL] | 0.67–1.17 | 0.99 (0.75–1.21) | 1.19 (0.85–1.83) | 854.0 | N/A | 0.012 | |
| BUN [mg/dL] | 44081.00 | 15.84 (12.2–24.67) | 28.365 (17.41–59.35) | 238.5 | N/A | 0.012 | |
| D-dimer [ng/mL] | <500 | 1410.5 (867–2849) | 2409 (1170–11,412) | 735.0 | N/A | 0.026 | |
| Magnesium [mg/dL] | 1.6–2.4 | 1.94 (1.73–2.09) | 1.69 (1.61–1.92) | 292.0 | N/A | 0.037 | |
| Calcium [mg/dL] | 8.8–10.2 | 8.66 (8.47–8.83) | 8.32 (8.02–8.62) | 2.064 | 109 | 0.043 | t-test |
| Troponin [ng/L] | <14.5 | 22.7 (10.2–36.3) | 43.25 (27.6–78.6) | 429.5 | N/A | 0.001 | Mann–Whitney U test |
| NT-proBNP [pg/mL] | <125 | 792 (154–1952) | 3734.5 (740–12,080) | 282.0 | N/A | 0.003 | |
| Hemoglobin oxygen saturation, % | >96 | 94.2 (89.4–95.7) | 88.85 (77.8–93.5) | 87.5 | N/A | 0.039 | |
| Base excess [mmol/L] | (–2.0)–3.0 | 2.6 (0.8–3.1) | 5 (2.45–9.25) | 81.0 | N/A | 0.022 | |

ALP – alkaline phosphatase; BUN – blood urea nitrogen; NT-proBNP – N-terminal pro B-type natriuretic peptide; RDW-SD – red blood cell distribution width standard deviation; df – degrees of freedom; N/A – not applicable.

**Table 4.** Computed tomography findings of coronavirus disease (COVID-19) patients

| CT findings | Survivors (n = 61) | Non-survivors (n = 22) | U value | p-value | Statistical test |
|---|---|---|---|---|---|
| Opacity score | 4 (2–7) | 7 (4–11) | –2.88 | 0.004 | Mann–Whitney U test |
| Volume of opacity [mL] | 279.6 (41.3–685.9) | 609.9 (259.1–1209.2) | –2.53 | 0.011 | |
| Opacity [%] | 7.9 (1.3–19.1) | 16.0 (6.4–41.4) | –2.62 | 0.001 | |
| Volume of high opacity [mL] | 43.3 (3.2–163.5) | 200.8 (61.1–548.8) | –3.14 | 0.002 | |
| High opacity [%] | 1.6 (0.1–5.2) | 5.3 (1.5–17.2) | –2.97 | 0.003 | |
| Mean HU total | –742.8 (–803.2––683.9) | –675.1 (–753.1––556.7) | –2.52 | 0.012 | |
| Mean HU of opacity | –486.6 (–551.4––375.1) | –396.4 (–493.4––310.8) | –2.15 | 0.031 | |

CT – computed tomography; HU – Hounsfield units.

significantly lower hypoxemia in non-survivors, and arterial blood gas analysis confirmed this finding. These observations highlight the necessity of early evaluation of arterial blood gases in severely ill patients and support using pulse oximeters as a readily available patient screening tool. Despite the limitations of this method among patients with peripheral circulation disturbances or varnished nails, it appears to be the simplest and fastest way to predict a severe course of COVID-19.[20–22] However, identifying the exact $SpO_2$ cutoff point for determining the severity of the prognosis requires further investigations.

In earlier studies, typical changes in peripheral blood cell counts associated with the severity of COVID-19 included increased leukocyte and neutrophil counts with concomitant lymphopenia.[23,24] Leukocytosis and neutrophilia indicate an increased inflammatory status in patients with severe disease. Both groups had similar white blood count (WBC), though non-survivors had significantly lower lymphocyte levels.[18] Lymphopenia is the most characteristic blood cell change associated with COVID-19 severity. It is caused by multiple factors, including direct attachment of the virus to lymphocytes with subsequent impairment of their immune functioning or inflammatory mediator-induced injury.[24]

Non-survivors had significantly lower peripheral blood monocyte counts than survivors. Monocytes and macrophages are attracted to the alveolar spaces of the lungs, are the first cells to respond to viruses, and are responsible for the local antiviral response. The SARS-CoV-2 infects monocytes and macrophages in an angiotensin-converting enzyme 2 (ACE2)-dependent or -independent manner and inactivates them, allowing viruses to spread through different tissues.[25] A detailed explanation of antiviral immune responses could simplify the development of an effective COVID-19 treatment method.

The widespread distribution of red blood cells in non-survivors was the only significant disturbance in erythroid lineage, which is consistent with previous observations

associated with impaired synthesis of mature red blood cells due to hypoxia.[26]

The COVID-19-infected inpatients, both survivors and non-survivors, had elevated serum levels of CRP and procalcitonin, which is consistent with previous studies.[23] These simple parameters are examples of cytokine storm indicators that are useful for monitoring the course of the disease.[27]

The SARS-CoV-2-induced coagulopathy, revealed in laboratory tests as increased serum levels of D-dimers, results in an increased thromboembolic risk and complications, such as pulmonary embolism.[18] The significantly higher serum D-dimer levels in non-survivors on admission, their potential use as markers of poor prognosis, and the correlation between their level and disease severity are consistent with previous studies.[11,28]

Hyponatremia, hypokalemia and hypocalcemia are the most common electrolyte disturbances in COVID-19 patients.[29] The current study revealed lower serum levels of calcium and magnesium in non-survivors. Possible explanations for these electrolyte imbalances include gastrointestinal and renal loss due to SARS-CoV-2 invasion, hypoxic damage to the central nervous system, and the actions of pro-inflammatory cytokines.[29]

Liver injury in the course of SARS-CoV-2 infection, manifested by elevated serum levels of alanine transaminase (ALT), aspartate aminotransferase (AST), ALP, gamma-glutamyl transpeptidase (GGTP), LDH, and total bilirubin, is frequently observed and its risk and severity are related to the condition of infected patients.[30–32] Moreover, serum levels of LDH are one of the most valuable factors for predicting mortality.[33] In the current study, non-survivors had higher serum levels of LDH, though the difference was non-significant ($p < 0.08$), and significantly higher ALP ($p < 0.03$). The causes of liver damage are still unclear but are mainly related to general hypoxia, direct viral cytotoxicity through ACE2 receptors on the surface of hepatic cells, and a hepatocyte cell membrane dysfunction-induced cytokine storm.[34,35]

Concomitant kidney disease is another risk factor for death during SARS-CoV-2 infection,[36] with acute kidney injury (AKI) observed in up to 46% of patients hospitalized for COVID-19.[37] This study demonstrated significantly higher serum creatinine levels and BUN in non-survivors. Other studies observed pathological changes in tubular kidney regions and urine samples.[38,39]

Despite the fact that the heart contains ACE2 receptors, direct myocardial SARS-CoV-2 infection data are limited.[40] Elevated levels of heart damage and overload enzymes, including troponin and NT-proBNP, may be secondary to the general condition, circulating cytokines and endotoxin levels of patients with severe disease.[41] Moreover, significantly higher levels of troponin and NT-proBNP in non-survivors suggest their association with COVID-19 severity and mortality.[42]

In our study, the results of CT examinations of hospitalized patients are consistent with previous reports. A higher opacity score and percentage of opacity correlate with disease severity and poor prognosis.[43] Therefore, we recommend HRCT as a simple imaging test for infected patients.

Data from the current study suggest that no single abnormality demonstrates a strong relationship with the course of the disease and that numerous factors are responsible for multiple organ damage. Future studies should seek to develop a prospective prediction model for fatal COVID-19.

The delay between symptom onset and hospital admission in the group of non-survivors might explain no difference in remdesivir use between survivors and non-survivors. The early stage of the disease was consistent with the signs and symptoms of pneumonia, which justified the use of remdesivir. Most patients received GK treatment, which may reflect the more severe clinical condition and the development of a systemic inflammatory response (tocilizumab was unavailable). The increased frequency of $O_2$ support and mechanical ventilation use in non-survivors confirmed the severity of the disease.

## Limitations

Limitations to consider when interpreting the results include the retrospective design of the study, which meant that the groups were not homogenous in terms of clinical features. Furthermore, not all laboratory and imaging tests were available for every patient. There were also slight differences between the onset of symptoms and the day of admission to the hospital.

# Conclusions

This retrospective study aimed to find the most useful biomarkers for predicting the severity and poor prognosis of COVID-19 inpatients using medical parameters assessed on admission to the hospital. In this regard, the most valuable parameters appear to be advanced age, a history of cardiovascular disease, nicotine addiction, low peripheral $SpO_2$, low lymphocyte count, low monocyte count, and high CRP, PCT, D-dimer, ALP, creatinine, magnesium, calcium, troponin, NT-proBNP, and oxyhemoglobin levels, as well as a high opacity score in CT. The deterioration of organ functions observed during clinical examination, laboratory tests and radiological scans at the beginning of infection do not predict a severe course of the disease with certainty. Nevertheless, combining biomarkers and symptoms increases the risk of a fatal COVID-19 outcome. However, a full explanation of COVID-19 pathogenesis should facilitate the search for novel early biomarkers of severe disease. Further prospective studies are required to develop a predictive outcome model based on data collected on admission. Such an attempt would require cumulative analysis of numerous reports, similar to this study.

## Supplementary material

The Supplementary files are available at https://doi.org/10.5281/zenodo.7693350. The package contains the following files:

Supplementary Table 1. Data concerning laboratory findings in COVID-19 patients with and without statistical significance.

Supplementary Table 2. Data concerning statistical assesment of the equality of variances in clinical and laboratory parameters of COVID-19 patients.

### ORCID iDs

Mateusz Maligłówka https://orcid.org/0000-0002-3898-8785
Łukasz Bułdak https://orcid.org/0000-0002-2017-5516
Marcin Cyrnek https://orcid.org/0009-0002-4658-231X
Marcin Hachuła https://orcid.org/0000-0001-9023-676X
Michał Kosowski https://orcid.org/0000-0003-2597-463X
Marcin Basiak https://orcid.org/0000-0003-0674-5612
Witold Szkróbka https://orcid.org/0000-0001-9256-568X
Joanna Bosowska https://orcid.org/0000-0001-9583-1832
Maciej Cebula https://orcid.org/0000-0002-4917-8526
Michał Holecki https://orcid.org/0000-0002-3289-1050
Bogusław Okopień https://orcid.org/0000-0001-7228-2906

### References

1. Jin Y, Yang H, Ji W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. *Viruses*. 2020;12(4):372. doi:10.3390/v12040372
2. World Health Organization. Weekly epidemiological update on COVID-19: July 6, 2021. Geneva, Switzerland: World Health Organization; 2021. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---6-july-2021. Accessed July 10, 2021.
3. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506. doi:10.1016/S0140-6736(20)30183-5
4. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet*. 2020;395(10223):507–513. doi:10.1016/S0140-6736(20)30211-7
5. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061–1069. doi:10.1001/jama.2020.1585
6. Pan F, Zheng C, Ye T, et al. Different computed tomography patterns of coronavirus disease 2019 (COVID-19) between survivors and non-survivors. *Sci Rep*. 2020;10(1):11336. doi:10.1038/s41598-020-68057-4
7. Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): A pictorial review. *Eur Radiol*. 2020;30(8):4381–4389. doi:10.1007/s00330-020-06801-0
8. World Health Organization. COVID-19 clinical management living guidance. Geneva, Switzerland: World Health Organization; 2021. https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1. Accessed May 10, 2021.
9. World Health Organization. WHO statement: Tobacco use and COVID-19. Geneva, Switzerland: World Health Organization; 2021. https://www.who.int/news/item/11-05-2020-who-statement-tobacco-use-and-covid-19. Accessed May 12, 2021.
10. Allotey J, Fernandez S, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: Living systematic review and meta-analysis. *BMJ*. 2020;370:m3320. doi:10.1136/bmj.m3320
11. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet*. 2020;395(10229):1054–1062. doi:10.1016/S0140-6736(20)30566-3
12. Flisiak R, Parczewski M, Horban A, et al. Management of SARS-CoV-2 infection: Recommendations of the Polish Association of Epidemiologists and Infectiologists. Annex no. 2 as of October 13, 2020. *Pol Arch Intern Med*. 2020;130(10):915–918. doi:10.20452/pamw.15658
13. Flisiak R, Horban A, Jaroszewicz J, et al. Management of SARS-CoV-2 infection: Recommendations of the Polish Association of Epidemiologists and Infectiologists as of April 26, 2021. *Pol Arch Intern Med*. 2021;131(5):487–496. doi:10.20452/pamw.15979
14. Grzesiowski P. Ocena ryzyka zakażenia przy przyjęciu do szpitala – propozycja formularza. *Biuletyn Stowarzyszenia Higieny Lecznictwa Kwartalnik*. 2009;4:27–28.
15. Klein JS, Brant WE, Helms CA, Vinson EN, eds. *Brant and Helms Fundamentals of Diagnostic Radiology*. 5th ed. Philadelphia, USA: Wolters Kluwer; 2019. ISBN:978-1-4963-6739-6.
16. Kazerooni EA, Gross BH. *Cardiopulmonary Imaging*. Philadelphia, USA: Lippincott Williams & Wilkins; 2004. ISBN:978-0-7817-3655-8.
17. Chief Sanitary Inspectorate. Polish guidelines for quarantine and isolation of COVID-19 as of September 2, 2020 [in Polish]. Warszawa, Poland: Chief Sanitary Inspectorate; 2020. https://www.gov.pl/web/gis/zasady-odbywania-kwarantanny-i-izolacji-obowiazujace-od-2-wrzesnia-2020-r. Accessed January 21, 2021.
18. Gao Y, Ding M, Dong X, et al. Risk factors for severe and critically ill COVID-19 patients: A review. *Allergy*. 2021;76(2):428–455. doi:10.1111/all.14657
19. Dhont S, Derom E, Van Braeckel E, Depuydt P, Lambrecht BN. The pathophysiology of 'happy' hypoxemia in COVID-19. *Respir Res*. 2020;21(1):198. doi:10.1186/s12931-020-01462-5
20. Liu S, Luo H, Wang Y, et al. Clinical characteristics and risk factors of patients with severe COVID-19 in Jiangsu province, China: A retrospective multicentre cohort study. *BMC Infect Dis*. 2020;20(1):584. doi:10.1186/s12879-020-05314-x
21. Duan J, Wang X, Chi J, et al. Correlation between the variables collected at admission and progression to severe cases during hospitalization among patients with COVID-19 in Chongqing. *J Med Virol*. 2020;92(11):2616–2622. doi:10.1002/jmv.26082
22. Xie J, Covassin N, Fan Z, et al. Association between hypoxemia and mortality in patients with COVID-19. *Mayo Clin Proc*. 2020;95(6):1138–1147. doi:10.1016/j.mayocp.2020.04.006
23. Zhang J, Cao Y, Tan G, et al. Clinical, radiological, and laboratory characteristics and risk factors for severity and mortality of 289 hospitalized COVID-19 patients. *Allergy*. 2021;76(2):533–550. doi:10.1111/all.14496
24. Ou M, Zhu J, Ji P, et al. Risk factors of severe cases with COVID-19: A meta-analysis. *Epidemiol Infect*. 2020;148:e175. doi:10.1017/S095026882000179X
25. Jafarzadeh A, Chauhan P, Saha B, Jafarzadeh S, Nemati M. Contribution of monocytes and macrophages to the local tissue inflammation and cytokine storm in COVID-19: Lessons from SARS and MERS, and potential therapeutic interventions. *Life Sci*. 2020;257:118102. doi:10.1016/j.lfs.2020.118102
26. Wang C, Zhang H, Cao X, et al. Red cell distribution width (RDW): A prognostic indicator of severe COVID-19. *Ann Transl Med*. 2020;8(19):1230–1230. doi:10.21037/atm-20-6090
27. Azkur AK, Akdis M, Azkur D, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy*. 2020;75(7):1564–1581. doi:10.1111/all.14364
28. Yao Y, Cao J, Wang Q, et al. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: A case control study. *J Intensive Care*. 2020;8:49. doi:10.1186/s40560-020-00466-z
29. Lim JH, Jung HY, Choi JY, et al. Hypertension and electrolyte disorders in patients with COVID-19. *Electrolyte Blood Press*. 2020;18(2):23–30. doi:10.5049/EBP.2020.18.2.23
30. Zhao X, Lei Z, Gao F, Xie Q, Jang K, Gong J. The impact of coronavirus disease 2019 (COVID-19) on liver injury in China: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2021;100(4):e24369. doi:10.1097/MD.0000000000024369
31. An YW, Song S, Li WX, et al. Liver function recovery of COVID-19 patients after discharge, a follow-up study. *Int J Med Sci*. 2021;18(1):176–186. doi:10.7150/ijms.50691
32. Benedé-Ubieto R, Estévez-Vázquez O, Flores-Perojo V, et al. Abnormal liver function test in patients infected with coronavirus (SARS-CoV-2): A retrospective single-center study from Spain. *J Clin Med*. 2021;10(5):1039. doi:10.3390/jcm10051039

33. Yan L, Zhang HT, Goncalves J, et al. An interpretable mortality prediction model for COVID-19 patients. *Nat Mach Intell*. 2020;2(5):283–288. doi:10.1038/s42256-020-0180-7
34. Jothimani D, Venugopal R, Abedin MF, Kaliamoorthy I, Rela M. COVID-19 and the liver. *J Hepatol*. 2020;73(5):1231–1240. doi:10.1016/j.jhep.2020.06.006
35. Bangash MN, Patel J, Parekh D. COVID-19 and the liver: Little cause for concern. *Lancet Gastroenterol Hepatol*. 2020;5(6):529–530. doi:10.1016/S2468-1253(20)30084-4
36. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int*. 2020;97(5):829–838. doi:10.1016/j.kint.2020.03.005
37. Chan L, Chaudhary K, Saha A, et al. AKI in hospitalized patients with COVID-19. *J Am Soc Nephrol*. 2021;32(1):151–160. doi:10.1681/ASN.2020050615
38. Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int*. 2020;98(1):219–227. doi:10.1016/j.kint.2020.04.003
39. Nogueira SÁR, de Oliveira SCS, de Carvalho AFM, et al. Renal changes and acute kidney injury in COVID-19: A systematic review. *Rev Assoc Med Bras*. 2020;66(Suppl 2):112–117. doi:10.1590/1806-9282.66.s2.112
40. Imazio M, Klingel K, Kindermann I, et al. COVID-19 pandemic and troponin: Indirect myocardial injury, myocardial inflammation or myocarditis? *Heart*. 2020;106(15):1127–1131. doi:10.1136/heartjnl-2020-317186
41. Shi S, Qin M, Cai Y, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *Eur Heart J*. 2020;41(22):2070–2079. doi:10.1093/eurheartj/ehaa408
42. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. 2020;5(7):811–818. doi:10.1001/jamacardio.2020.1017
43. Francone M, Iafrate F, Masci GM, et al. Chest CT score in COVID-19 patients: Correlation with disease severity and short-term prognosis. *Eur Radiol*. 2020;30(12):6808–6817. doi:10.1007/s00330-020-07033-y

# Application of low-dose hydro-CT using SAFIRE in the evaluation of esophageal cancer

Milena Celmer[A–D], Krzysztof Międzybrodzki[B,D,E], Mateusz Patyk[D,E], Patryk Pozowski[B,C], Urszula Zaleska-Dorobisz[E,F]

Department of General and Paediatric Radiology, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1133–1138

**Address for correspondence**
Milena Celmer
E-mail: milena.celmer1@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on October 12, 2022
Reviewed on December 19, 2022
Accepted on February 12, 2023

Published online on April 7, 2023

## Abstract

**Background.** The esophageal cancer treatment strategy depends on the tumor stage according to the tumor, node and metastasis (TNM) classification. One of the methods recommended for esophageal cancer assessment is computed tomography (CT). The CT imaging is especially important for patients with contraindications for gastroscopy, which is the primary method used for assessing esophageal diseases.

**Objectives.** The aim of this retrospective study was to evaluate the inter-rater reliability of low-dose hydro-CT with a sinogram-affirmed iterative reconstruction algorithm (SAFIRE) used for the staging of esophageal cancer by 2 independent radiologists. We also evaluated the application of this method for the diagnosis of esophageal cancer.

**Materials and methods.** Low-dose hydro-CT was performed in 65 patients, and the raw data were reconstructed with SAFIRE. Obtained images were retrospectively interpreted by 2 independent and experienced radiologists. Histopathological results were used as the reference standard. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in the diagnosis of esophageal cancer were calculated for hydro-CT. The examination of the inter-rater reliability level in the assessment of the esophageal cancer stage in the TNM classification was performed by calculating Cohen's kappa coefficient (κ) with square weights and standard errors (SEs) for kappa. Independence tests were also performed (Fisher's exact test – two-tailed, and Pearson's $\chi^2$ test).

**Results.** For the diagnosis of esophageal cancer with hydro-CT, a sensitivity of 93%, a specificity of 100%, a PPV of 100%, and a NPV of 88% were observed. In the statistical analyses for the T, N and M stages, κ values greater than 0.90 and significance levels of $p < 0.001$ were obtained.

**Conclusions.** Hydro-CT using low-dose techniques may be a valuable diagnostic method for staging and diagnosis of esophageal cancer, especially in patients with contraindications for invasive procedures.

**Key words:** multi-detector computed tomography, esophageal diseases, esophageal neoplasms, TNM staging

**Cite as**
Celmer M, Międzybrodzki K, Patyk M, Pozowski P, Zaleska-Dorobisz U. Application of low-dose hydro-CT using SAFIRE in the evaluation of esophageal cancer. *Adv Clin Exp Med*. 2023;32(10):1133–1138. doi:10.17219/acem/161160

**DOI**
10.17219/acem/161160

# Background

The development of multi-detector computed tomography (MDCT) imaging in recent years has enabled high-resolution images to be obtained with a very short scan time. As a result, we have been given new, wide-ranging imaging capabilities for the gastrointestinal tract, including the esophagus.[1–3]

According to the GLOBOCAN 2018 statistics, esophageal cancer ranks 7$^{th}$ on the list of the most common cancers in the world, and the MDCT study is one of the most important tools indicated for its evaluation.[4] The use of water in MDCT hydrography (hydro-CT) as an oral neutral contrast agent allows for optimal distension of the esophageal walls and facilitates the diagnosis of small tumor lesions. It also improves the visual differentiation of pathological tumor tissues from the normal walls of the esophagus and surrounding structures in CT examinations with intravascular iodine contrast agents.[1,2]

Due to the increasing use of MDCT in clinical practice, it was necessary to reduce radiation doses in accordance with the ALARA (as low as reasonably achievable) principle.[5,6] Low-dose CT protocols are also used in hydro-CT studies. Obtaining high-quality images despite reduced radiation doses is possible thanks in part to improved raw data processing techniques (e.g., iterative reconstruction algorithms).[7] Reducing exposure to ionizing radiation is particularly important for patients undergoing multiple CT scans and for patients undergoing radiotherapy.[4] In patients with esophageal cancer, radiotherapy may be an important element of therapy, and CT examinations may be useful in the initial staging assessment and then during the follow-up.[8,9]

Although endoscopic biopsy is the gold standard in esophageal cancer diagnosis,[10] there is a group of patients with contraindications for endoscopy, including endoscopic ultrasound (EUS; uncooperative patients), or for biopsy (in the case of active bleeding, coagulopathy or patient instability). In these patients, low-dose hydro-CT with the use of iterative image reconstruction may be an alternative method for esophageal cancer diagnostics.

# Objectives

The aim of this retrospective study was to evaluate the inter-rater reliability for the staging of esophageal cancer according to the tumor, node and metastasis (TNM) classification performed by 2 independent radiologists based on low-dose hydro-CT. We also evaluated the application of this method in the diagnosis of esophageal cancer.

# Materials and methods

## Study design

Low-dose hydro-CT was performed in 65 patients (44 men and 21 women, mean age: 64.3 years). The main indications for hydro-CT were the evaluation of benign lesions found in endoscopy or the staging of esophageal cancer in patients before treatment or after neoadjuvant radiotherapy. In our study, all endoscopies were performed by 3 different surgeons experienced in esophagoscopy (>8 years of experience) at our institution. In all cases, during the endoscopy, multiple biopsies (5–10 per procedure) were performed. Written informed consent was obtained from all patients, and this study was approved by the Bioethical Committee of Wroclaw Medical University (Wrocław, Poland).

## CT protocol and image analysis

All images were obtained with patients in the supine position using a 128-slice CT scanner (SOMATOM Definition AS+; Siemens Healthcare, Erlangen, Germany). The CT parameters were as follows: tube potential of 120 kV, tube current modulation (Care Dose 4D), collimation of 128 × 0.6 mm, a pitch of 0.8, and a gantry rotation time of 0.5 s. Raw data were reconstructed with a sinogram-affirmed iterative reconstruction algorithm (SAFIRE; Siemens Healthcare) and a strength setting of 3 using a high spatial resolution kernel (I30). The scan range covered the area of the thorax and the upper part of the abdominal cavity.

Before CT scanning, all patients ingested water orally in a volume of 0.75–1.0 L (within 10 min before starting the scanning), followed by 0.25 L (immediately before scanning) to distend the esophagus and gastroesophageal junction walls. They were also administered 20 mg of butylscopolamine intravenously (10 min before scanning) to reduce gastrointestinal tract motility. The ionic contrast medium (Ultravist 370; Bayer Healthcare, Leverkusen, Germany) was administered intravenously at a dose of 1.5 mL/kg of body weight, at a rate of 3.0–3.5 mL/s. Dynamic enhanced phases were then performed with scan delay times of 25 s and 50 s.

The obtained images were retrospectively interpreted by 2 independent and experienced (5 and 10 years of experience) radiologists who were blinded to the endoscopy results. They used a diagnostic workstation (Syngo.via; Siemens Healthcare) and maximum intensity projection reconstructions or multiplanar reconstructions for coronal, sagittal and axial images.

## Statistical analyses

The results of the esophageal biopsy were treated as a standard of reference. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in the diagnosis of esophageal cancer were calculated for hydro-CT

Table 1. Computed tomography (CT) criteria for tumor, node and metastasis (TNM) staging of esophageal cancer used in the study

| Stage | | Characteristics |
|---|---|---|
| T stage | T0 | no visible changes in the wall of the esophagus; surrounding adipose tissue unchanged |
| | T1/2 | esophageal wall thickening and transmural contrast enhancement with smooth external outlines; surrounding adipose tissue unchanged or visible desmoplastic reaction; no or slight stenosis of the esophagus |
| | T3 | usually large tumor with contrast enhancement and significant thickening of the esophageal wall with irregular outer border; infiltration of the surrounding adipose tissue; moderate or severe stenosis |
| | T4 | infiltration of surrounding organs |
| N stage | N0 | no enlarged lymph nodes have been found (short-axis diameter of imaged lymph nodes less than 1 cm) |
| | N1 | enlarged lymph nodes present (cervical, mediastinal, epigastric; short-axis diameter of lymph nodes at least 1 cm) |
| M stage | M0 | no evidence of metastases |
| | M1 | distant metastases present |

The criteria are based on the studies by Ba-Ssalamah et al.[3] and Prokop and Galanski.[11]

(the calculations take into account the results obtained by the more experienced of the 2 radiologists). Additionally, we evaluated the compatibility level of the 2 independent radiologists in assessing the stage of esophageal cancers according to the TNM classification (presented in Table 1) using Cohen's kappa coefficient (κ) and independence tests (two-tailed Fisher's exact test and Pearson's $\chi^2$ test). A value of $p < 0.05$ was considered statistically significant. The radiation dose was estimated using the volume CT dose index ($CTDI_{vol}$). CTDI is a standardized measure of radiation dose output of a CT scanner which allows the user to compare radiation output of different CT scanners. Calculations and statistical analysis of the results were performed using Microsoft Excel 2019 (Microsoft, Redmond, USA) and Statistica v. 13 (StatSoft Inc., Tulsa, USA).

## Results

The diagnosis of esophageal cancer was confirmed in a histopathological examination in 43 (66.2%) patients. In the remaining 22 cases (33.8%), benign lesions were diagnosed with endoscopy (7 cases of hernia hiatus esophagi, 2 cases of achalasia, 1 benign polyp, and 12 cases of esophagitis). In 40 cases (93%), a correct diagnosis of esophageal cancer was made using hydro-CT. For the diagnosis of esophageal cancer with hydro-CT, a sensitivity of 93%, a specificity of 100%, a PPV of 100%, and a NPV of 88% were observed.

In the statistical analyses for the T stage, $\kappa = 0.966$ and a significance level of $p < 0.0001$ (in Pearson's $\chi^2$ independence test) were obtained. These results indicate the compatibility of the opinion of the 2 radiologists in the assessment of the T stage of esophageal cancer (Table 2). For the evaluation of enlarged lymph nodes (N stage), a statistically significant, very strong relationship was also observed between readers 1 and 2. In this case, $\kappa = 0.952$ and a significance level of $p < 0.001$ (two-tailed Fisher's exact test) were obtained (Table 3). In 100% of cases, the readers agreed in their assessment of distant metastases (M stage).

The $CTDI_{vol}$ was in the range of 3.32–7.08 mGy per scan.

Table 2. Number classifications of tumor size (T stage) observed by the 2 radiologists and the estimated value of Cohen's kappa coefficient (κ) with square weights and standard errors (SEs)

| Reader 1 | Reader 2 | | | |
|---|---|---|---|---|
| | T0 | T1/2 | T3 | T4 |
| T0 | 3 | 0 | 0 | 0 |
| T1/2 | 2 | 17 | 0 | 0 |
| T3 | 0 | 0 | 13 | 0 |
| T4 | 0 | 0 | 0 | 8 |

n = 43; Cohen's kappa coefficient $\kappa = 0.966$; SE = 0.022; $p < 0.0001$.

Table 3. Number of classifications of the enlarged lymph nodes (N stage) observed by the 2 radiologists and the estimated value of Cohen's kappa coefficient (κ) with square weights and standard errors (SEs)

| Reader 2 | Reader 1 | |
|---|---|---|
| | N0 | N1 |
| N0 | 25 | 0 |
| N1 | 1 | 17 |

n = 43; Cohen's kappa coefficient $\kappa = 0.952$; SE = 0.048; $p < 0.001$.

## Discussion

The main objective of the present study was to estimate the level of compatibility between 2 radiologists (inter-rater reliability) in the classification of the stage of esophageal cancer according to the TNM system. For this purpose, κ value was calculated. In the test materials for categories T, N and M, κ values of 0.90 were obtained, indicating very good compatibility between the 2 readers (Fig. 1–3). This result indicates the possibility of unambiguous assessments of the local stage of esophageal cancer in a hydro-CT study. Importantly, these results suggest that it is possible to accurately assess the stage of esophageal cancer, which is crucial in deciding on the proper therapeutic procedure.

Confirmation of these conclusions can be found in other studies. Ba-Ssalamah et al. evaluated the usefulness of MDCT hydrography in the assessment of category T in esophageal cancer.[1] In this study, images obtained

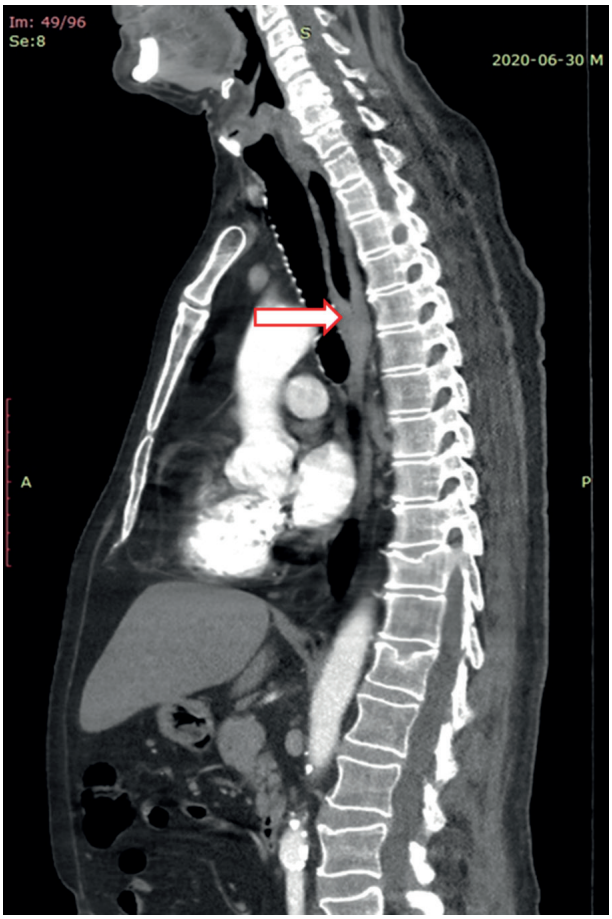



Fig. 1. Hydro-computed tomography (CT) image in sagittal reconstructions in a patient with T1/2 esophageal cancer shows the esophageal wall thickening 10 mm in depth with a smooth outer border in the thoracic part of the esophagus

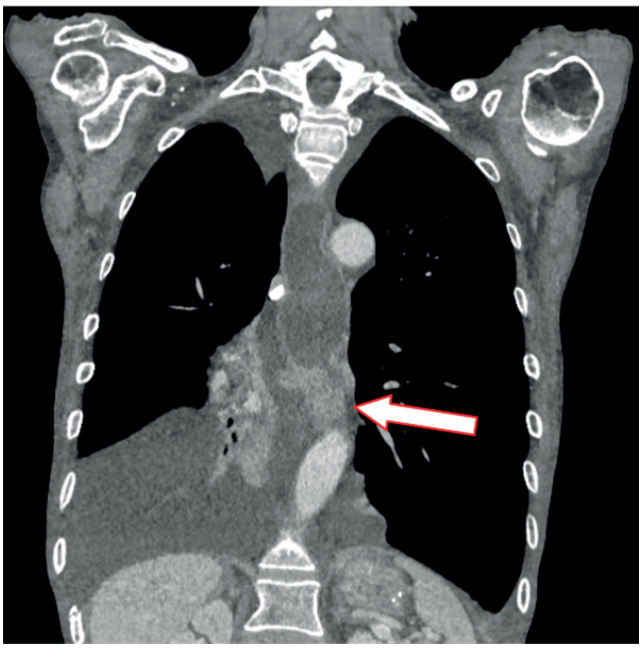


Fig. 2. Hydro-computed tomography (CT) image of the esophagus in coronal reconstruction shows an irregular, circumferential wall thickening up to 11 mm in depth in the proximal and middle third of the esophagus, with inhomogeneous enhancement and blurred outer borders in terms of the T3 tumor

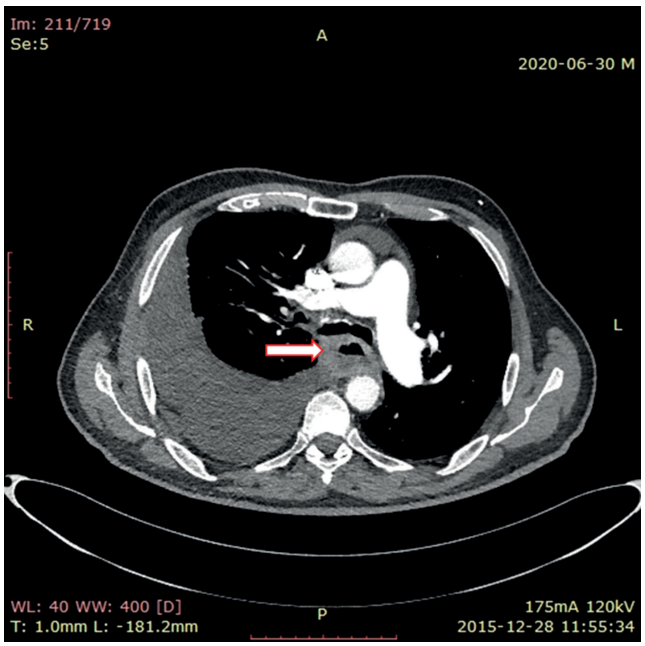



Fig. 3. Hydro-computed tomography (CT) image of the esophagus in axial reconstruction in a patient with a T4 stage of esophageal cancer and a large esophageal diverticulum filled with content located supravascularly on the right side. The tumor causes a concentric thickening of the esophageal wall at the level of the tracheal bifurcation with proximal dilatation of the esophagus, with a broad contact with the thoracic aorta and the Th8 vertebra, and with infiltration of the right intermediate bronchus (causing atelectasis in the right lower lobe), the pericardium and the outflow of the pulmonary veins

using hydro-CT performed before and after surgery in 131 patients were evaluated by 2 independent radiologists and compared with the results of a histopathological examination of the material removed during the operation. Very good compatibility was achieved between the readers, with a weighted κ ratio of 0.93 and κ unbalanced at 0.89. The results obtained by both readers showed a high sensitivity for hydro-CT (95%) and a high PPV (96%) in the assessment of the T stage. Staging was properly assessed in 76.3% and 68.7% of patients by investigators 1 and 2, respectively.

In this study, we also evaluated the application of low-dose hydro-CT using SAFIRE in the diagnosis of esophageal cancer. Our results suggest a high utility for this imaging modality. However, it should be noted that the assessment of the T stage and diagnosis of esophageal cancer using hydro-CT may have some limitations.[3,12] One of them is the poor contrast of the different layers of the esophageal wall, which makes it difficult to identify early forms of esophageal tumors and differentiate them from benign esophagitis. Due to this limitation, endoscopy with biopsy should be performed in each patient, and hydro-CT may be considered a useful method in the diagnosis of esophageal cancer only for patients with a contraindication to endoscopy or biopsy.

The latest, 8$^{th}$ edition of the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) guidelines recommends EUS as a method

of choice for the clinical evaluation of the T stage.[13] Shim et al. presented a comparison of the accuracy of EUS and CT examinations in the assessment of esophageal cancer advancement.[14] In the conclusions of their report, they suggest that EUS examination is not superior to chest CT for diagnosing the T stage in esophageal cancers. Other authors report that EUS is less accurate in staging cancer after radiotherapy and chemotherapy. It is also limited by the inability to assess lumen-occluding tumors in the esophagus.[15–17] At the same time, the advantage of hydro-CT in detecting distant metastases, such as lung or liver metastases (stage M), should be emphasized. Thus, it allows for a complete assessment of esophageal cancer advancement according to the TNM system.

According to the AJCC/UICC guidelines, EUS, MDCT and fluorodeoxyglucose positron emission tomography are basic imaging techniques that do not differ significantly in accuracy, sensitivity or specificity in the assessment of regional lymph nodes in patients with esophageal cancer.[12]

It is commonly accepted that hydro-CT scans are performed in the supine or prone position, depending on the location of the lesion, on the dependent side.[1,18] Our experience shows that the supine position for scanning is better tolerated and more comfortable for patients whose stomach is bloated with a large amount of water. Some authors claim that there is no need to use the prone position for scanning in clinical practice.[11,19–21] Therefore, in our study, the scanning was performed only in the supine position.

## Limitations

Our study has some limitations. These include the inability to compare the results obtained from hydro-CT with the intraoperative and postoperative histopathological evaluation of tumor tissues. The patient group was heterogeneous and some patients were treated only with radiotherapy.

The study group was relatively small and consisted mainly of patients with advanced cancer lesions, as esophageal cancer screening is not performed in our population. Therefore, it should be noted that our results confirm the usefulness of hydro-CT in the diagnosis and evaluation of esophageal cancer, usually only in advanced cases.

In this study, we used the low-dose scanning technique, which in some cases may reduce the quality of the obtained images and affect the evaluation of the primary tumor and metastatic lesions, especially in the liver. Some authors, based on objective and subjective analyses of image quality, suggest that a combination of Care Dose 4D and SAFIRE reduces the radiation dose by an average of 74.85% while maintaining image quality.[7] However, they noted the unclear impact of different imaging conditions and the use of post-processing techniques on the accuracy of the diagnosis, which led them to believe that research into the use of this method should be continued.

# Conclusions

In conclusion, hydro-CT using low-dose techniques may be a valuable diagnostic method for staging and diagnosis of esophageal cancer, especially for patients with contraindications to invasive procedures (endoscopy, biopsy or EUS). Conducting studies on a larger group of patients using the latest low-dose techniques and iterative algorithms (third-generation CT scanners) may contribute to the wider use of MDCT for the evaluation of esophageal cancer in the future.


## ORCID iDs

Milena Celmer https://orcid.org/0000-0001-8575-1388
Krzysztof Międzybrodzki https://orcid.org/0000-0001-9837-2758
Mateusz Patyk https://orcid.org/0000-0001-7316-1890
Patryk Pozowski https://orcid.org/0000-0002-4230-2168
Urszula Zaleska-Dorobisz https://orcid.org/0000-0001-5035-485X


## References


1. Ba-Ssalamah A, Matzek W, Baroud S, et al. Accuracy of hydro-multidetector row CT in the local T staging of oesophageal cancer compared to postoperative histopathological results. *Eur Radiol*. 2011;21(11):2326–2335. doi:10.1007/s00330-011-2187-2
2. Kim SH, Lee JM, Han JK, et al. Three-dimensional MDCT imaging and CT esophagography for evaluation of esophageal tumors: Preliminary study. *Eur Radiol*. 2006;16(11):2418–2426. doi:10.1007/s00330-006-0337-8
3. Ba-Ssalamah A, Zacherl J, Noebauer-Huhmann IM, et al. Dedicated multidetector CT of the esophagus: Spectrum of diseases. *Abdom Imaging*. 2009;34(1):3–18. doi:10.1007/s00261-007-9290-5
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
5. Luo LN, He LJ, Gao XY, et al. Evaluation of preoperative staging for esophageal squamous cell carcinoma. *World J Gastroenterol*. 2016;22(29):6683–6689. doi:10.3748/wjg.v22.i29.6683
6. Hendee WR, Marc Edwards F. ALARA and an integrated approach to radiation protection. *Semin Nucl Med*. 1986;16(2):142–150. doi:10.1016/S0001-2998(86)80027-7
7. Wang L, Gong S, Yang J, et al. CARE Dose 4D combined with sinogram-affirmed iterative reconstruction improved the image quality and reduced the radiation dose in low dose CT of the small intestine. *J Appl Clin Med Phys*. 2019;20(1):293–307. doi:10.1002/acm2.12502
8. Zhou Y, Liu D, Hou P, et al. Low-dose spectral insufflation computed tomography protocol preoperatively optimized for T stage esophageal cancer: Preliminary research experience. *World J Gastroenterol*. 2018;24(36):4197–4207. doi:10.3748/wjg.v24.i36.4197
9. Hong SJ, Kim TJ, Nam KB, et al. New TNM staging system for esophageal cancer: What chest radiologists need to know. *Radiographics*. 2014;34(6):1722–1740. doi:10.1148/rg.346130079
10. Graham DY, Schwartz JT, Cain GD, Gyorkey F. Prospective evaluation of biopsy number in the diagnosis of esophageal and gastric carcinoma. *Gastroenterology*. 1982;82(2):228–231. doi:10.1016/0016-5085(82)90009-9
11. Prokop M, Galanski M. Przewód pokarmowy. In: Gołębiowski M, Bekiesińska-Figatowska M. eds. *Spiralna i wielorzędowa tomografia komputerowa człowieka*. 2nd ed. Warszawa, Poland: MediPage; 2007:537–585. ISBN:978-83-89769-23-7.
12. Weaver SR, Blackshaw GRJC, Lewis WG, et al. Comparison of special interest computed tomography, endosonography and histopathological stage of oesophageal cancer. *Clin Radiol*. 2004;59(6):499–504. doi:10.1016/j.crad.2003.11.016
13. Rice TW, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: Application to clinical practice. *Ann Cardiothorac Surg*. 2017;6(2):119–130. doi:10.21037/acs.2017.03.14

14. Shim HI, Lee DH, Cho JH, et al. Comparing the accuracy of EUS and CT in staging of esophageal cancer. *J Clin Oncol*. 2020;38(4 Suppl):286. doi:10.1200/JCO.2020.38.4_suppl.286
15. Yang GY, Wagner TD, Jobe BA, Thomas CR. The role of positron emission tomography in esophageal cancer. *Gastrointest Cancer Res*. 2008; 2(1):3–9. PMID:19259317.
16. Lightdale CJ, Kulkarni KG. Role of endoscopic ultrasonography in the staging and follow-up of esophageal cancer. *J Clin Oncol*. 2005; 23(20):4483–4489. doi:10.1200/JCO.2005.20.644
17. Lee G, I H, Kim SJ, et al. Clinical implication of PET/MR imaging in preoperative esophageal cancer staging: Comparison with PET/CT, endoscopic ultrasonography, and CT. *J Nucl Med*. 2014;55(8):1242–1247. doi:10.2967/jnumed.114.138974
18. Lee JE, Kim YH, Shin HH, Jeong WG, Na KJ. The diagnostic utility of prone position chest CT for the evaluation of esophageal cancer. *Chonnam Med J*. 2020;56(1):68–74. doi:10.4068/cmj.2020.56.1.68
19. Li R, Chen T, Wang L, et al. Quantitative measurement of contrast enhancement of esophageal squamous cell carcinoma on clinical MDCT. *World J Radiol*. 2012;4(4):179–185. doi:10.4329/wjr.v4.i4.179
20. Karmazanovsky GG, Buryakina S, Kondratiev E, Yang Q, Ruchkin D, Kalinin D. Value of two-phase dynamic multidetector computed tomography in differential diagnosis of post-inflammatory strictures from esophageal cancer. *World J Gastroenterol*. 2015;21(29): 8878–8887. doi:10.3748/wjg.v21.i29.8878
21. Kumano S, Okada M, Shimono T, et al. T-staging of gastric cancer of air-filling multidetector-row CT: Comparison with hydro-multidetector-row CT. *Eur J Radiol*. 2012;81(11):2953–2960. doi:10.1016/j.ejrad.2011.12.039

# Pan-cancer analysis of the oncogenic effects of G-protein-coupled receptor kinase-interacting protein-1 and validation on liver hepatocellular carcinoma

*Tao Wang[1,A], *Kun Su[1,C,D], Lianming Wang[1,C], Yanmei Shi[2,D], Yichun Niu[2,B], Yahao Zhou[3,B], Ayong Wang[3,C], Tao Wu[1,E,F]

[1] Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Kunming Medical University, China
[2] Department of Gastroenterology, The Second Affiliated Hospital of Kunming Medical University, China
[3] Department of Hepatobiliary Surgery, Puer People's Hospital, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1139–1147

**Address for correspondence**
Tao Wu
E-mail: taowubio@outlook.com

**Funding sources**
The study was supported by the major provincial science and technology projects of Yunnan (grant No. 202002AA100007).

**Conflict of interest**
None declared

*Tao Wang and Kun Su contributed equally to this work.

Received on May 18, 2022
Reviewed on June 13, 2022
Accepted on February 12, 2023

Published online on March 30, 2023

**Cite as**
Wang T, Su K, Wang L, et al. Pan-cancer analysis of the oncogenic effects of G-protein-coupled receptor kinase-interacting protein-1 and validation on liver hepatocellular carcinoma. *Adv Clin Exp Med*. 2023;32(10):1139–1147. doi:10.17219/acem/161157

**DOI**
10.17219/acem/161157



## Abstract

**Background.** Despite G-protein-coupled receptor kinase-interacting protein-1 (GIT1) being recognized as a new promoter gene in some types of cancer, its effect on human pan-cancers and liver hepatocellular carcinoma (LIHC) remains unclear.

**Objectives.** To elucidate the molecular mechanisms of GIT1 in pan-cancer and LIHC.

**Materials and methods.** Various bioinformatics approaches were utilized to elucidate the oncogenic effects of GIT1 on human pan-cancers.

**Results.** The GIT1 was aberrantly expressed in pan-cancers and associated with the clinical stage. Moreover, the upregulation of GIT1 expression was indicative of poor overall survival (OS) in patients with LIHC, skin cutaneous melanoma (SKCM) and uterine corpus endometrial carcinoma (UCEC), as well as of poor disease-free survival (DFS) in patients with LIHC and UCEC. Furthermore, GIT1 levels were correlated with cancer-associated fibroblasts (CAFs) in adrenocortical carcinoma (ACC), cervical squamous cell carcinoma (CESC) and LIHC. The analysis of single-cell sequencing data revealed an association of GIT1 levels with apoptosis, cell cycle and DNA damage. In addition, multivariate Cox analysis indicated that high GIT1 levels were an independent risk factor for shorter OS in patients with LIHC. Finally, the gene set enrichment analysis revealed INFLAMMATORY_RESPONSE pathway and IL2_STAT5_SIGNALING to be the most enriched in LIHC.

**Conclusions.** Our data demonstrate the oncogenic effects of GIT1 on various cancers. We believe that GIT1 can serve as a biomarker for LIHC.

**Key words:** pan-cancer analysis, GIT1, oncogene, liver hepatocellular carcinoma

# Background

Liver cancers are associated with elevated mortality rates across the world,[1–3] and while significant advancements have been made in surgical techniques, chemotherapy and other treatment approaches, the 5-year survival rate remains far from satisfactory.[4–6] Moreover, liver cancer is the most common type of cancer in China. Specifically, cancer recurrence at the intermediate or advanced stage occurs in approx. half of the patients. Considering the increase in the incidence and mortality rates of liver cancer, it is crucial to identify new prognostic biomarkers.

G-protein-coupled receptor kinase-interacting protein-1 (GIT1) has been shown to repress the β2-adrenergic receptor pathway and stimulate receptor phosphorylation. Many proteins interact with GIT1 via its various domains. Notably, GIT1 is essential for focal cell migration, adhesion and the development of lamellipodia. The principal roles of GIT1 include focal adhesion remodeling,[7] receptor internalization and transmission of cellular signals.[8] The GIT1 is widely expressed in the brain, liver, lungs, nerves, and blood vessels.[9,10] The expression of GIT1 is upregulated in breast cancer, while its downregulation has been found to regulate the cell progression of breast cancer.[11] The GIT1 can stimulate tumor development by activating extracellular signal-regulated kinase signaling in hepatocellular carcinoma.[12,13] Moreover, GIT1 participates in epithelial–mesenchymal transition and promotes the invasion of oral squamous cell carcinoma.[14] Interestingly, this protein is involved in a number of varied cellular processes, including enhancing neurite and spine maturation,[15] mediating vascular intima and pulmonary vasculature development,[16] as well as cell migration and adhesion.[17] While the overexpression of GIT1 has been shown to regulate chondrocyte proliferation and apoptosis via integrin-β1, it also increases autophagy via disruption of the Beclin-1 and Bcl-2 interaction in osteoclast. Mechanistically, GIT1 achieves these outcomes by altering ERK1/2, AKT, NF-κB, and Notch expression, and accelerating lung cancer cell migration and metastasis via Rac1/Cdc42 signal, which further validates its participation in cancer occurrence and development.[18–21] A previous study found that the suppression of GIT1 inhibits breast cancer cell invasion and metastasis via the upregulation of miR-149.[22] Recently, a report demonstrated that GIT1 is reduced in ER(–) breast cancer when compared to ER(+) cancer, and that higher GIT1 expression implied a better prognosis in ER(–) breast cancer patients.[11] Thus, GIT1 appears to have distinct functions in the growth and migration of breast cancer cells. However, its roles and mechanisms in pan-cancer demand further investigations.

Herein, we investigated various cancers for GIT1 expression and patient survival data. To elucidate the mechanisms of GIT1 and the associated proteins, we performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA). Furthermore, we evaluated the association between GIT1 levels and immune infiltration. Finally, single-cell sequencing results were assessed to examine the GIT1 expression in cells in related tumors.

# Objectives

This study aimed to measure the expression of GIT1 in various cancers and the association between GIT1 levels and immune infiltration.

# Materials and methods

## Pattern of GIT1 expression based on the pan-cancer study

The GIT1 level patterns in cancer and corresponding samples were obtained using ONCOMINE (http://www.oncomine.org/resource/login.html) and TIMER2.0 (http://timer.comp-genomics.org/). For ONCOMINE, the parameters were set as p = 0.001, fold change: 2.0 and gene ranking: top 10%. The GIT1 level patterns in different cancer stages were acquired using the "Stage plots" module of GEPIA2 (http://gepia2.cancer-pku.cn/#index).

## Survival and prognosis

Both overall survival (OS) and disease-free survival (DFS) results were obtained through the GEPIA website.[22] High and low GIT1 expression groups were established based on the median level of GIT1. The association between GIT1 levels and pan-cancer survival outcome was detected using the log-rank test. Furthermore, Cox regression examining GIT1 levels and the clinical variables was used to detect the effects of GIT1 on the prognostic value of liver hepatocellular carcinoma (LIHC) patients. Calibration curves and the concordance index (C-index) were evaluated by comparing predicted probabilities with the observed events.

## GIT1-associated functional enrichment

Proteins interacting with GIT1 were analyzed using the STRING tool (http://string.embl.de/)[23] under the setting of no more than 100 interactors and low confidence (0.150) to obtain the potent GIT1-binding proteins. Furthermore, the top 100 genes demonstrating an expression profile similar to that of GIT1 in various cancers were analyzed with the GEPIA2 tool. Then, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed using proteins interacting with GIT1, together with the top 100 genes, using the DAVID software. A p-value of <0.01 was considered statistically significant.

## Immune infiltration

The relationship between GIT1 expression, immune infiltration and cancer-associated fibroblasts (CAFs) was analyzed with TIMER[24] using Spearman's correlation based on the ranked values. The p-values and partial correlation values were measured employing the purity-adjusted Spearman's rank correlation test, and data were visualized with heat maps and scatter plots. Furthermore, the relationship between GIT1 levels and various tumor immune subtypes was investigated through the TISDB tool (http://cis.hku.hk/TISIDB/index), and the distribution of the 6 immune subtypes was determined. The TISDB is an online tool for cross-linking studies of tumors and immunity, which contains data from PubMed, The Cancer Genome Atlas (TCGA) and other public databases.[25,26]

## Single-cell sequencing results

The distinct functional states of various cancer cells at single-cell level,[27] and the association of GIT1 levels and pan-cancer functional status were obtained through the "correlation plot" module of CancerSEA (http://biocc.hrbmu.edu.cn/CancerSEA).[28] The threshold for the association between GIT1 and cancer functional states was set as a correlation strength >0.3 and a p-value <0.05.

## GSEA

The GSEA is a method to demonstrate that the expression of a given gene set is overrepresented. The GSEA was employed to evaluate distinct functions among the high- and low-risk score subgroups, using the hallmark gene set h.all.v7.0.symbols.gmt. Gene sets with |normalized enrichment score (NES)| > 1, nominal (NOM) $p < 0.01$ and false discovery rate (FDR) $q < 0.25$ were considered significant.

## Statistical analyses

To assess the different levels of GIT1 in normal and pan-cancer samples, we used Wilcoxon rank-sum test. Cancer patient survival was detected with the Kaplan–Meier curve, and Spearman's rank correlation coefficient was used to measure the correlation between the 2 groups. The statistical analysis was performed using R software v. 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) and the 'edgeR' package. A value of $p < 0.05$ was considered statistically significant.

# Results

## Abnormal expression of GIT1 in different cancers

The GIT1 expression patterns were evaluated in pan-cancer through TIMER2.0, which includes data about gene expression patterns in normal and pan-cancer samples. We found that GIT1 expression levels were significantly upregulated in various cancers, including LIHC and lung adenocarcinoma (LUAD), among others (Fig. 1A).

Next, we used GEPIA2 to investigate the correlation between GIT1 levels and clinical stage. An association between GIT1 levels and clinical stage for glioblastoma multiforme, head and neck squamous cell carcinoma, kidney chromophobe (KICH), LIHC, lung squamous cell carcinoma, and others was found (Fig. 1B). Collectively, these data indicate that GIT1 expression is upregulated in pan-cancer and that GIT1 can be a promotor of pan-cancers.

Based on the above data, it became evident that GIT1 is involved in both pan-cancer and LIHC development and can thus serve as a potential biomarker.

## Relationship between GIT1 levels and patient prognosis

To study the correlation between GIT1 levels and patient prognosis, we used GEPIA2 to conduct a survival investigation. The obtained data showed that the overexpression of GIT1 was indicative of poor OS in patients with LIHC ($p = 0.002$), skin cutaneous melanoma (SKCM) ($p = 0.026$) and uterine corpus endometrial carcinoma (UCEC) ($p = 0.006$). Conversely, better OS was found in patients with kidney renal clear cell carcinoma (KIRC) ($p = 0.011$) and glioma ($p < 0.001$) (Fig. 2A). Furthermore, the overexpression of GIT1 was associated with poor DFS in patients with LIHC and UCEC, and improved DFS in those with KIRC, SKCM and glioma (Fig. 2B). These data indicate that there is a close association of GIT1 overexpression with poor survival outcomes in some types of cancers, including LIHC.

## Protein–protein interaction and enrichment pathway analyses

Unfortunately, the mechanism underlying GIT1-mediated oncogenesis remains unknown. To examine the protein–protein interaction (PPI) network and enrichment signal of GIT1, proteins that bind GIT1 were obtained from the STRING database, and the database was verified using the experimental setup. Eleven proteins were found to interact with GIT1, namely ADRBK1, ARHGEF6, ARHGEF7, CAMK4, ERC2, LPXN, PAK1, PAK2, PPFIA1, PTK2, and PXN (Fig. 3A).

Then, the top 100 proteins that closely interacted with GIT1 were found using GEPIA2, with PXN found to be common to both methods. Furthermore, GO and KEGG pathway enrichment analyses indicated that the above genes were involved in several cellular processes, including regulation of GTPase activity, microtubule polymerization/depolymerization, protein kinase activator activity, and Ras GTPase binding, among others (Fig. 3B,C). In addition, KEGG data revealed that GIT1 participated

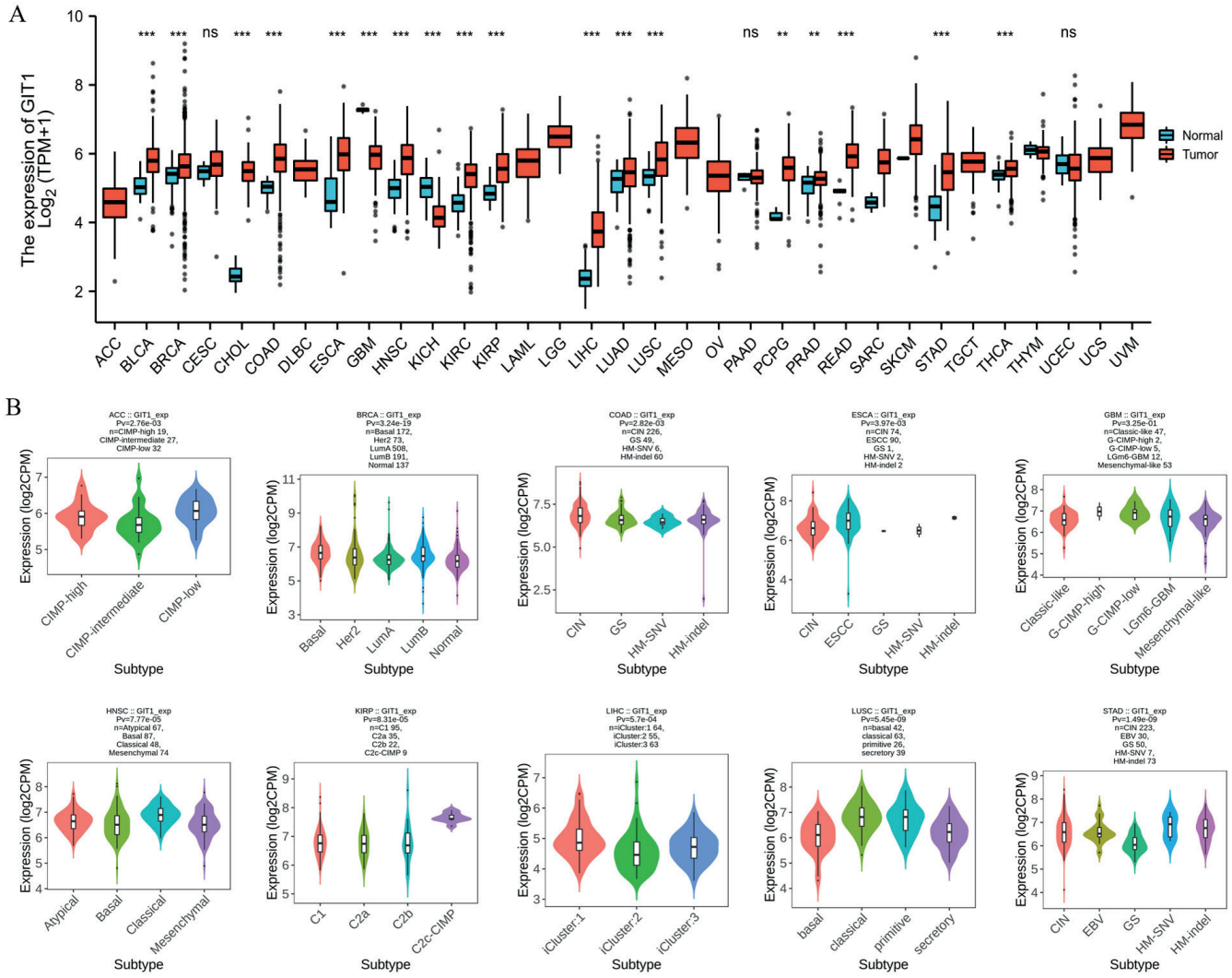



Fig. 1. Abnormal G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression patterns in various cancers. A. GIT1 levels in various cancers were presented as box plots using The Cancer Genome Atlas (TCGA) database via TIMER using R v. 3.6.3 software (Wilcoxon rank-sum test). Data in the box plot are shown as the median. The box and whisker plots were used to gain an in-depth understanding of the GIT1 level patterns in pan-cancer; B. Analysis of GIT1 levels in different clinical stages of various cancers using GEPIA2, according to TCGA data

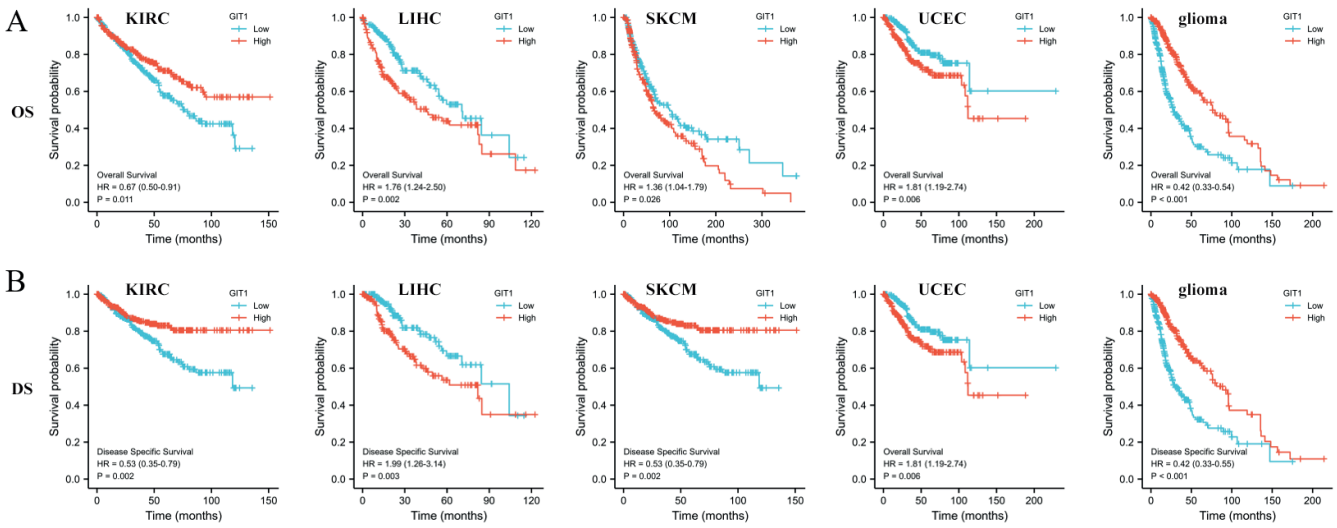



Fig. 2. Prognostic value of G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression in different tumors based on The Cancer Genome Atlas (TCGA) database. Correlations of GIT1 expression and overall survival (OS) (A) and disease-free survival (DFS) (B) were analyzed using the Kaplan–Meier plotter via GEPIA. The median expression of GIT1 was used to separate the high and low GIT1 expression groups

high – high-GIT1 expression group; low – low-GIT1 expression group; KIRC – kidney renal clear cell carcinoma; LIHC – liver hepatocellular carcinoma; SKCM – skin cutaneous melanoma; UCEC – uterine corpus endometrial carcinoma.

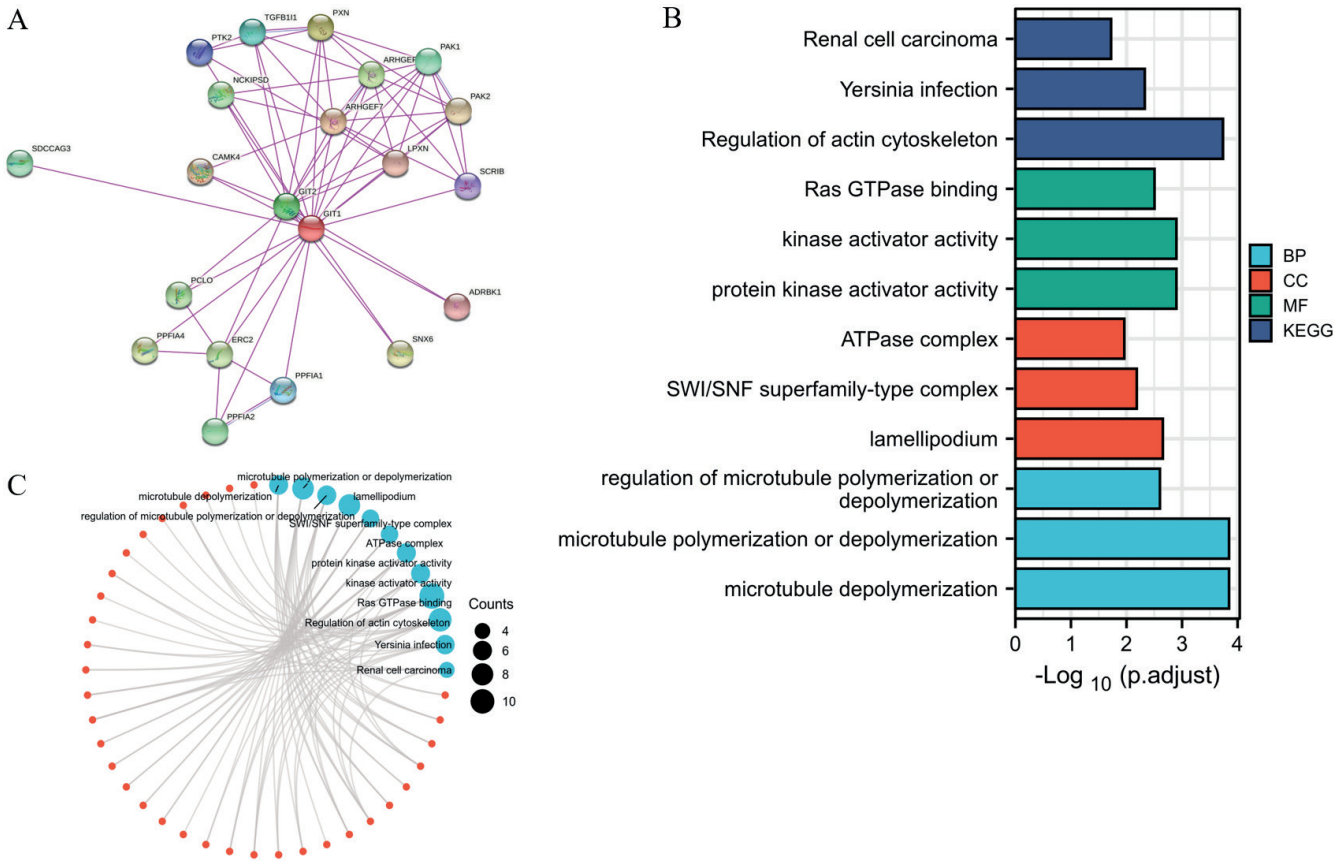



Fig. 3. Protein–protein interaction (PPI) and enrichment signal pathway study of G-protein-coupled receptor kinase-interacting protein-1 (GIT1). A. The combining genes of GIT1 were measured using the STRING website by setting the parameter of "no more than 100 interactors" via the STRING tool; B,C. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis based on GIT1-combining proteins and cooperating genes

MF – molecular function; bp – biological process; CC – cell component; SWI/SNF – yeast mating-type switching/sucrose non-fermenting.

in tumorigenesis of renal cell carcinoma, regulation of the actin cytoskeleton, focal adhesion, and other signaling pathways (Fig. 3B,C). Thus, these data found that GIT1 together with its closely interacting partner proteins correlated with focal adhesion and regulation of the actin cytoskeleton, which implied an increased complexity of the GIT1-mediated signal network.

## Relationship between GIT1 levels and tumor microenvironment

To explain the effect of GIT1 expression on the immune microenvironment, TIMER was applied to study the association between GIT1 levels and tumor microenvironment (TME) characteristics in various cancers. We found that GIT1 levels were correlated with CAFs in adrenocortical carcinoma (ACC), cervical squamous cell carcinoma (CESC) and LIHC (Fig. 4A).

To further explore the relationship between GIT1 levels and CAFs, we examined biomarkers of CAF levels in different cancers and found that GIT1 expression was associated with the C1–C6 immune subtypes (Fig. 4B). Interestingly, the GIT1 levels were also connected with those immune subtypes in LIHC.

## GIT1 expression pattern at the single-cell level and its relationship with biological functions

We validated GIT1 expression at the single-cell level across pan-cancers and determined its association with biological functions. The GIT1 levels were found to be positively correlated with acute lymphocytic leukemia (ALL), LUAD and ovarian serous cystadenocarcinoma (OV) apoptosis. Specifically, GIT1 expression was correlated with the LUAD cell cycle and retinoblastoma DNA damage (Fig. 5A).

There was an association between GIT1 levels and proliferation, epithelial–mesenchymal transition and metastasis in ALL (Fig. 5B). Moreover, t-distributed stochastic neighbor embedding (t-SNE) diagrams revealed GIT1 expression patterns in single cells in ALL, colorectal cancer (CRC), LUAD, and glioma (Fig. 5C). Collectively, these data suggest that GIT1 participates in mediating cancer development.

## Cox regression study

A nomogram was developed for internal validation, and a predictive model was prepared (Fig. 6A). We found

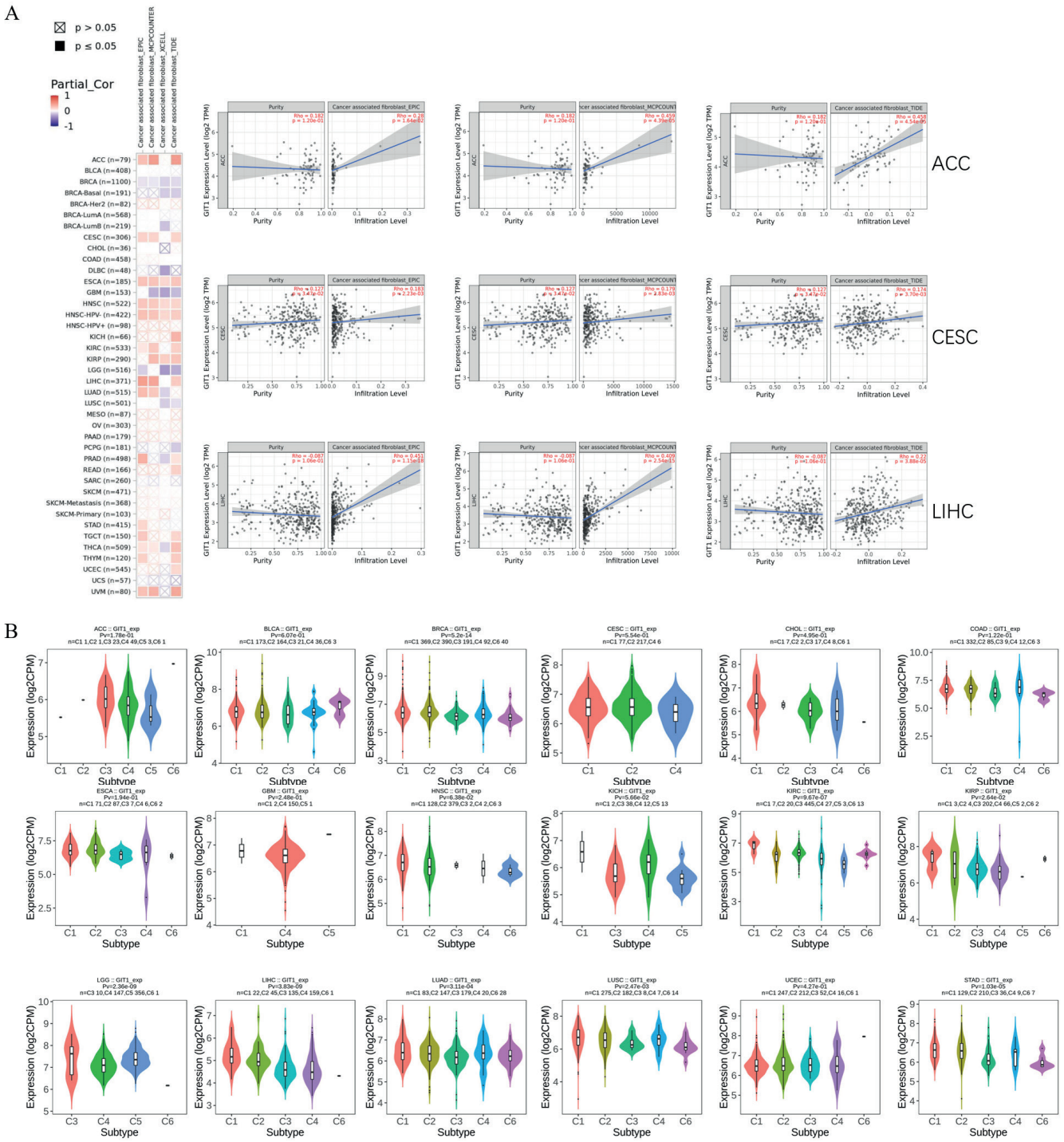



Fig. 4. The association between G-protein-coupled receptor kinase-interacting protein-1 (GIT1) expression and cancer-associated fibroblasts (CAFs). A. Different algorithms (EPIC, MCPCOUNTER, XCELL, and TIDE) were applied to confirm any potential correlation. The association between GIT1 levels and CAFs was obtained from TIMER. The p-values and the correlation values were acquired using the partial Spearman's correlation test with the "purity adjustment" option; B. GIT1 expression in various cancer immune subtypes was obtained from TISDB

ACC – adrenocortical carcinoma; LIHC – liver hepatocellular carcinoma; CESC – cervical squamous cell carcinoma.

that the C-index of the nomogram was 0.669 (95% confidence interval (95% CI): 0.637–0.701), and the calibration curve displayed the nomogram's desirable prediction for 1–5-year clinical consequences (Fig. 6B). Altogether, these data indicate that GIT1 may be a potent biomarker for LIHC.

## GSEA

The GSEA revealed that the INFLAMMATORY_RESPONSE pathway and IL2_STAT5_SIGNALING were the most enriched in LIHC (Fig. 6C,D). Taken together, obtained data revealed that GIT1 expression was associated with specific gene signatures of these key pathways in LIHC.

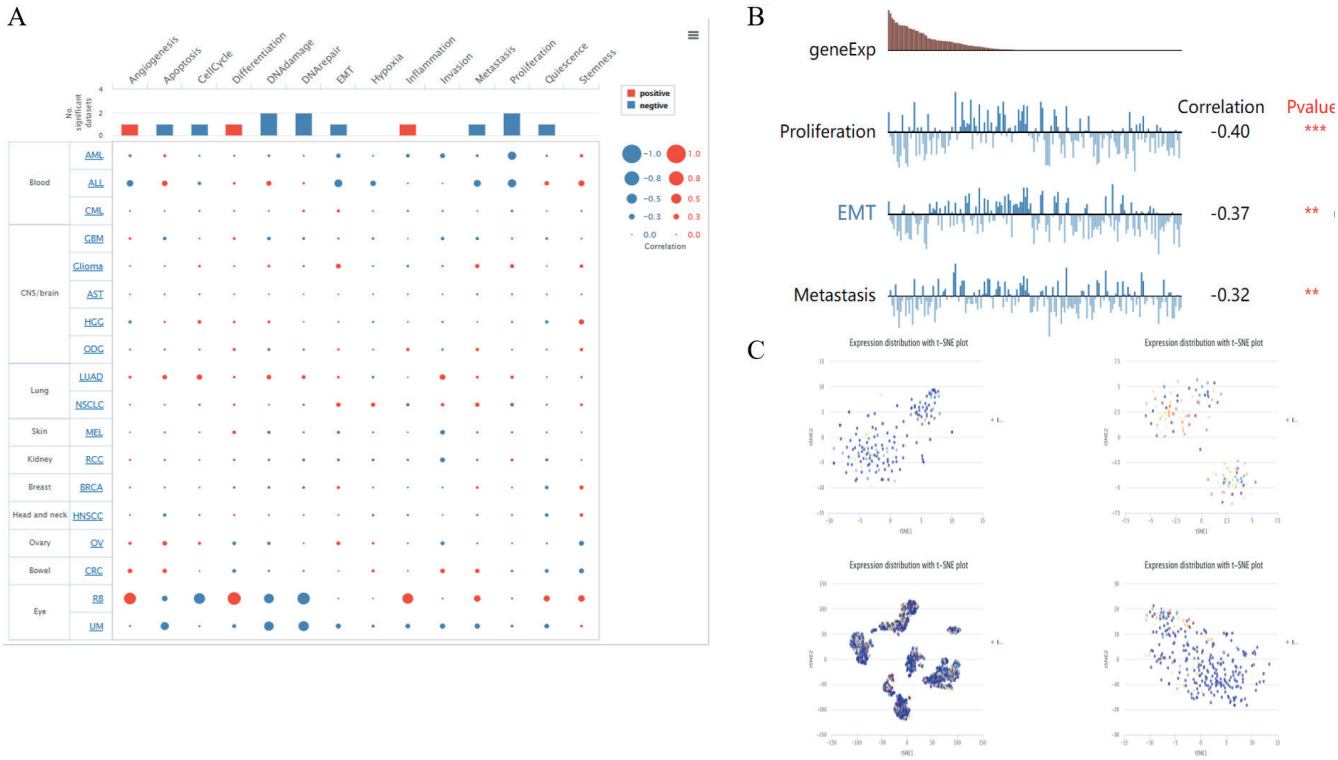



**Fig. 5.** Level of G-protein-coupled receptor kinase-interacting protein-1 (GIT1) in single-cell data and the association of GIT1 with cancer function. A. The association of GIT1 levels and pan-cancer function was shown using the CancerSEA database. Red plots indicated a positive association, whereas blue plots indicated a negative correlation; B. The association between GIT1 level and the different functions obtained from CancerSEA; C. T-distributed stochastic neighbor embedding (t-SNE) diagrams highlighted GIT1 levels in single cancer cells

EMT – epithelial–mesenchymal transition.

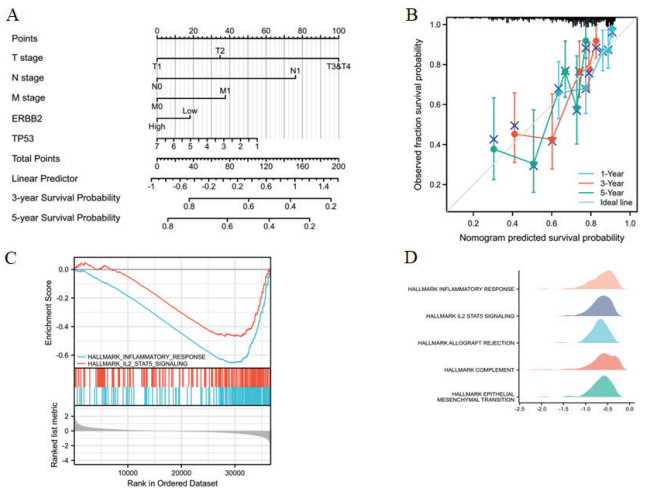



**Fig. 6.** Formation and verification of a nomogram for liver hepatocellular carcinoma (LIHC) based on G-protein-coupled receptor kinase-interacting protein-1 (GIT1) levels and the Gene Set Enrichment Analysis (GSEA) data. A. Nomogram for calculating the possibility of 1–5-year overall survival (OS) in LIHC; B. Calibration plots confirming the effectiveness of nomograms for OS in LIHC patients. Calibration curve for the OS nomogram model; C,D. The GSEA results presented the relevant enrichment signal. Gene sets with |NES| > 1, NOM p < 0.01 and FDR q < 0.25 were regarded as significant

NES – normalized enrichment score; NOM – nominal; FDR – false discovery rate.

## Discussion

Previous studies have reported a close relationship between aberrant gene expression and the development of pan-cancers. Investigations into pan-cancer provide deep insights into the molecular mechanism underlying different malignancies and are useful to identify new therapeutic markers for cancer treatment.[29] Therefore, we investigated the expression and predictive significance of GIT1 in different tumors.

Firstly, we found that GIT1 is aberrantly expressed in different cancers, including LIHC. To further examine the prognostic value of GIT1, a Kaplan–Meier survival study was performed, which revealed an association between high GIT1 levels and the poor outcomes associated with pan-cancers, including LIHC. Thus, we found that the overexpression of GIT1 could be an independent indicator of poor prognosis in patients with LIHC and other cancers. Moreover, Cox regression analysis verified that GIT1 overexpression may be a risk factor for LIHC. Thus, our data suggested that GIT1 is a pro-oncogene in pan-cancers.

Recently, Chen et al. described a prognostic model for OS which included age and other factors for pan-cancer based on GIT1 expression.[30] In accordance with that model, we developed a prognostic nomogram model including clinical stage and GIT1 levels, which may increase

the accuracy of classifying high-risk cases. This model further assessed the relationship between clinical features and GIT1 levels in cases of LIHC, and demonstrated that increased GIT1 levels were associated with the clinical stage. The results revealed that GIT1 could act as a potent biomarker for different cancers, especially LIHC.

Additionally, the enrichment analyses revealed that GIT1 may impact cancer development through the regulation of focal adhesions and the actin cytoskeleton, together with their associated pathways. Chen et al. have shown that these signals have a key role in the development of pan-cancers.[31]

The TME has been shown to promote crosstalk between cancer cells and other cell types. In fact, CAFs have been reported to have a functional role in stimulating tumorigenesis. Thus, the signature of pan-CAF is associated with poor survival in cancer. Interestingly, other studies have suggested that CAFs inhibit cancer development, which implies that they have an antitumor effect.[32–34] Our results indicated an association between GIT1 levels and CAFs in different cancers, and therefore we believe that GIT1 mediates the development of pan-cancers. However, the molecular mechanism regarding how GIT1 modulates CAFs warrants further investigation. The well-defined immune subtype in various cancers could improve the effectiveness of targeted immune treatment. We found that GIT1 is aberrantly expressed in various immune subtypes of pan-cancer, which potentially makes it an important target in immune therapies aimed at various cancers.

Considering the complex nature of cancer cells, the utilization of single-cell transcriptomic data is a valuable method of examining various types of cancers. To elucidate the effect of GIT1 on pan-cancer progression, the CancerSEA website was used. The GIT1 expression was found to be positively associated with ALL, LUAD and OV apoptosis, and specifically positively associated with the LUAD cell cycle. Furthermore, an association was found between GIT1 levels and cell proliferation, epithelial–mesenchymal transition, and metastasis in ALL. However, the mechanism underlying GIT1 in pan-cancer warrants further investigation.

Finally, GSEA results indicated that GIT1 was associated with the inflammatory response pathway and IL2/STAT5 signaling in LIHC. These signals have been shown to be actively involved in the development of pan-cancers, including LIHC.

Recently, advances in the prediction abilities of computational biology began to offer new understanding of biomarkers and non-coding RNAs connected to pan-cancers, including ceRNA network prediction. A previous report presented data highlighting that GIT1 was involved in the ceRNA network, and, consequently, more research is necessary to investigate the role of GIT1 in ceRNA interaction.

## Limitations

Our findings were mostly obtained from online tools, and more results based on clinical cases are needed to further authenticate our findings. Furthermore, in vitro and in vivo analyses should be performed to confirm the role of GIT in LIHC progression.

# Conclusions

We comprehensively investigated the effects of GIT1 on various cancers. Our findings revealed that GIT1 was overexpressed in different cancers, including LIHC, which was in turn associated with a poor prognosis. Furthermore, GIT1 was shown to mediate pan-cancer development, namely LIHC progression, through the regulation of focal adhesion and the actin cytoskeleton, inflammatory response pathways, and IL2/STAT5 signaling. Further studies are needed to elucidate the molecular mechanisms underlying GIT1, which appears valuable for cancer-targeted therapy.

## Availability of data and materials

The datasets generated and/or analyzed in this study are available in the TCGA database (https://portal.gdc.cancer.gov/).


### ORCID iDs

Tao Wang https://orcid.org/0009-0005-3799-6782
Kun Su https://orcid.org/0009-0006-2387-1441
Lianming Wang https://orcid.org/0009-0009-3706-0510
Yanmei Shi https://orcid.org/0009-0005-3990-6998
Yichun Niu https://orcid.org/0009-0008-0242-8496
Yahao Zhou https://orcid.org/0009-0005-2388-9994
Ayong Wang https://orcid.org/0009-0006-8482-1691
Tao Wu https://orcid.org/0009-0002-1260-3645


### References


1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer*. 2021;149(4):778–789. doi:10.1002/ijc.33588
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
4. Chen J, Liang H, Lu J, He Y, Lai R. Probiotics improve postoperative adaptive immunity in colorectal cancer patients: A systematic review and meta-analysis. *Nutr Cancer*. 2022;74(8):2975–2982. doi:10.1080/01635581.2022.2056619
5. Hong R, Sun H, Li D, et al. A review of biosensors for detecting tumor markers in breast cancer. *Life (Basel)*. 2022;12(3):342. doi:10.3390/life12030342
6. Zhang X, Yang Z, An Y, et al. Clinical benefits of PD-1/PD-L1 inhibitors in patients with metastatic colorectal cancer: A systematic review and meta-analysis. *World J Surg Onc*. 2022;20(1):93. doi:10.1186/s12957-022-02549-7
7. Zhang N, Cai W, Ma Y, Yin G, Nagel D, Berk B. GIT1 is a novel MEK1-ERK1/2 scaffold that localizes to focal adhesions. *Cell Biol Int*. 2009;34(1):41–47. doi:10.1042/CBI20090016

8. Segura I, Essmann CL, Weinges S, Acker-Palmer A. Grb4 and GIT1 transduce ephrinB reverse signals modulating spine morphogenesis and synapse formation. *Nat Neurosci*. 2007;10(3):301–310. doi:10.1038/nn1858
9. Li L, Tang P, Zhou Z, et al. GIT1 regulates angiogenic factor secretion in bone marrow mesenchymal stem cells via NF-κB/Notch signalling to promote angiogenesis. *Cell Prolif*. 2019;52(6):e12689. doi:10.1111/cpr.12689
10. Kim H, Kim JI, Kim H, Kim JW, Kim BN. Interaction effects of *GIT1* and *DRD4* gene variants on continuous performance test variables in patients with ADHD. *Brain Behav*. 2017;7(9):e00785. doi:10.1002/brb3.785
11. Zhang S, Miyakawa A, Wickström M, et al. GIT1 protects against breast cancer growth through negative regulation of Notch. *Nat Commun*. 2022;13(1):1537. doi:10.1038/s41467-022-28631-y
12. Wang G, Bai X, Jiang G, et al. GIT1 overexpression promotes epithelial-mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. *Bioengineered*. 2021;12(1):30–43. doi:10.1080/21655979.2020.1855914
13. Chen J, Yang P, Yang J, Wen Z, Zhang B, Zheng X. GIT1 is a novel prognostic biomarker and facilitates tumor progression via activating ERK/MMP9 signaling in hepatocellular carcinoma. *Onco Targets Ther*. 2015;8:3731–3742. doi:10.2147/OTT.S96715
14. Huang WC, Chan SH, Jang TH, et al. miRNA-491-5p and GIT1 serve as modulators and biomarkers for oral squamous cell carcinoma invasion and metastasis. *Cancer Res*. 2014;74(3):751–764. doi:10.1158/0008-5472.CAN-13-1297
15. Hong ST, Mah W. A critical role of GIT1 in vertebrate and invertebrate brain development. *Exp Neurobiol*. 2015;24(1):8–16. doi:10.5607/en.2015.24.1.8
16. Pang J, Hoefen R, Pryhuber GS, et al. G-protein-coupled receptor kinase interacting protein-1 is required for pulmonary vascular development. *Circulation*. 2009;119(11):1524–1532. doi:10.1161/CIRCULATIONAHA.108.823997
17. Zhu J, Zhou Q, Xia Y, et al. GIT/PIX condensates are modular and ideal for distinct compartmentalized cell signaling. *Mol Cell*. 2020;79(5):782.e6–796.e6. doi:10.1016/j.molcel.2020.07.004
18. Xu R, Xu R, Wang Y, Wang W, Jiang L, Gong S. G-protein-coupled receptor kinase-interacting protein 1 (GIT1) promotes head and neck squamous cell carcinoma metastases via activating the PI3K/AKT/mTOR signal pathway. *Comput Math Methods Med*. 2022;2022:6881932. doi:10.1155/2022/6881932
19. Ye X, Chen X. miR-149-5p inhibits cell proliferation and invasion through targeting GIT1 in medullary thyroid carcinoma. *Oncol Lett*. 2019;17(1):372–378. doi:10.3892/ol.2018.9628
20. Jiao Y, Zhang L, Li J, He Y, Zhang X, Li J. Exosomal miR-122-5p inhibits tumorigenicity of gastric cancer by downregulating *GIT1*. *Int J Biol Markers*. 2021;36(1):36–46. doi:10.1177/1724600821990677
21. Chang JS, Su CY, Yu WH, et al. GIT1 promotes lung cancer cell metastasis through modulating Rac1/Cdc42 activity and is associated with poor prognosis. *Oncotarget*. 2015;6(34):36278–36291. doi:10.18632/oncotarget.5531
22. Yang L, Zhang W, Li M, et al. Evaluation of the prognostic relevance of differential claudin gene expression highlights claudin-4 as being suppressed by TGFbeta1 inhibitor in colorectal cancer. *Front Genet*. 2022;13:783016. doi:10.3389/fgene.2022.783016
23. Ukey S, Ramteke A, Choudhury C, Purohit P, Sharma P. Differential expression of zinc-dependent HDAC subtypes and their involvement in unique pathways associated with carcinogenesis. *Asian Pac J Cancer Prev*. 2022;23(3):877–883. doi:10.31557/APJCP.2022.23.3.877
24. Guo Y, Shang A, Wang S, Wang M. Multidimensional analysis of CHMP family members in hepatocellular carcinoma. *Int J Gen Med*. 2022;15:2877–2894. doi:10.2147/IJGM.S350228
25. Wang Y, Zhang H. FAT10 is a prognostic biomarker and correlated with immune infiltrates in skin cutaneous melanoma. *Front Mol Biosci*. 2022;9:805887. doi:10.3389/fmolb.2022.805887
26. Wu H, Duan Y, Gong S, Zhu Q, Liu X, Liu Z. An integrative pan-cancer analysis of kinesin family member C1 (KIFC1) in human tumors. *Biomedicines*. 2022;10(3):637. doi:10.3390/biomedicines10030637
27. Wang Y, Song M, Gao B. EF-hand domain-containing protein D2 (EFHD2) correlates with immune infiltration and predicts the prognosis of patients: A pan-cancer analysis. *Comput Math Methods Med*. 2022;2022:4878378. doi:10.1155/2022/4878378
28. Ma J, Jin Y, Gong B, Li L, Zhao Q. Pan-cancer analysis of necroptosis-related gene signature for the identification of prognosis and immune significance. *Discov Onc*. 2022;13(1):17. doi:10.1007/s12672-022-00477-2
29. Liu Y, Lin L, Zou R, Wen C, Wang Z, Lin F. MSC-derived exosomes promote proliferation and inhibit apoptosis of chondrocytes via lncRNA-KLF3-AS1/miR-206/GIT1 axis in osteoarthritis. *Cell Cycle*. 2018;17(21–22):2411–2422. doi:10.1080/15384101.2018.1526603
30. Chen J, Yang P, Yang J, Wen Z, Zhang B, Zheng X. GIT1 is a novel prognostic biomarker and facilitates tumor progression via activating ERK/MMP9 signaling in hepatocellular carcinoma. *Onco Targets Ther*. 2015;8:3731–3742. doi:10.2147/OTT.S96715
31. Chen S, Hourwitz MJ, Campanello L, Fourkas JT, Losert W, Parent CA. Actin cytoskeleton and focal adhesions regulate the biased migration of breast cancer cells on nanoscale asymmetric sawteeth. *ACS Nano*. 2019;13(2):1454–1468. doi:10.1021/acsnano.8b07140
32. Mizutani Y, Kobayashi H, Iida T, et al. Meflin-positive cancer-associated fibroblasts inhibit pancreatic carcinogenesis. *Cancer Res*. 2019;79(20):5367-5381. doi:10.1158/0008-5472.CAN-19-0454
33. Ishibashi Y, Mochizuki S, Horiuchi K, et al. Periostin derived from cancer-associated fibroblasts promotes esophageal squamous cell carcinoma progression via ADAM17 activation. *Biochim Biophys Acta Mol Basis Dis*. 2023;1869(5):166669. doi:10.1016/j.bbadis.2023.166669
34. Dong J, Wang F, Gao X, et al. Integrated analysis of genome-wide DNA methylation and cancer-associated fibroblasts identified prognostic biomarkers and immune checkpoint blockade in lower grade gliomas. *Front Oncol*. 2023;12:977251. doi:10.3389/fonc.2022.977251

# The use of a loose seton as a definitive surgical treatment for anorectal abscesses and complex anal fistulas

Cemalettin Durgun[1,A–F], Abidin Tüzün[2,A–F]

[1] Clinic of General Surgery, Memorial Dicle Hospital, Diyarbakır, Turkey
[2] Department of General Surgery, Gazi Yaşargil Education and Research Hospital, Health Science University, Diyarbakır, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1149–1157

**Address for correspondence**
Cemalettin Durgun
E-mail: drcemal21@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on October 17, 2022
Reviewed on January 12, 2023
Accepted on February 12, 2023

Published online on March 15, 2023

## Abstract

**Background.** There is no consensus regarding the standard treatment method for anorectal abscesses accompanied by anal fistulas and complex anal fistulas. Simultaneous surgical treatment of the underlying anal fistula with anorectal abscess drainage is controversial due to incontinence problems.

**Objectives.** We aimed to investigate the effectiveness of the loose seton method for the treatment of chronic anal fistulas and acute anorectal abscesses accompanied by anal fistula.

**Materials and methods.** In this retrospective study, 114 patients who were operated on in our clinic due to chronic anal fistulas and anorectal abscesses with an applied loose seton between 2020 and 2022 were included in the study. The patients were divided into 2 groups: those with chronic complex anal fistula and those with anorectal abscess accompanied by anal fistula. The groups were compared in terms of their continence status, rate of recurrence, recurrent abscess formation, postoperative pain scores, duration of operation, and demographic characteristics.

**Results.** Of the patients included in the study, 78 had a complex chronic anal fistula, and 36 had an anorectal abscess accompanied by an anal fistula. There were no differences between the demographic characteristics of the 2 groups. The mean seton dissociation time was 6.8 (3–19) months. Gas or stool leakage was not observed in patients during the mean follow-up period of 18 (6–30) months. There was no difference in postoperative continence levels between the 2 groups. No recurrent fistulas were observed in patients during the follow-up period. Recurrent abscesses were observed in 5 (13.9%) patients in the anorectal abscess group. Abscesses due to insufficient drainage were observed in 2 (2.6%) patients in the chronic fistula group. There was no significant difference in operation time between the 2 groups.

**Conclusions.** A loose seton can be a safe and effective method for the treatment of abscesses. It is a painless surgical method that produces good results in the treatment of all types of abscesses.

**Key words:** fecal incontinence, anorectal abscess, anal fistula, loose seton

**Cite as**
Durgun C, Tüzün A. The use of a loose seton as a definitive surgical treatment for anorectal abscesses and complex anal fistulas. *Adv Clin Exp Med*. 2023;32(10):1149–1157. doi:10.17219/acem/161162

**DOI**
10.17219/acem/161162

# Background

Anal fistulas are tracts that usually develop due to cryptoglandular abscesses that extend from the anal canal or distal rectum to the skin.[1] Patients with anal fistulas present with complaints of intermittent perianal discharge, contamination, pain, and swelling. These fistulas occur at a rate of 1.2–2.8/10,000.[2] In addition to physical examinations, endoanal ultrasonography (USG) and pelvic magnetic resonance imaging (MRI) are used for diagnosis. Contrast-enhanced MRIs are accepted as the gold standard, as they allow for detailed visualization of the anal sphincter anatomy and fistula map.[3] Based on the normal muscular anatomy of the pelvic floor, fistulas are classified as intersphincteric, transsphincteric, suprasphincteric, or extrasphincteric.[4]

Loose setons are routinely used in surgical operations. The probability of regeneration after seton loss is high. Notably, the incidence of seton loss in patients is unknown. However, it has been reported that the rate of seton loss is high in patients with complex anal fistulas.[5] In a pilot study, the clinical advantages of using loose setons to prevent the recurrence of acute anorectal abscesses were demonstrated.[6] However, the treatment of patients with high anal fistulas remains challenging.

Short- and long-term benefits have been observed with the use of loose setons.[7] It has been reported that loose seton use is beneficial in patients with transsphincteric anal fistulas. The preservation of an anatomical loss of the external sphincter function is important with this technique.[8] In a study comparing the effectiveness of the traditional seton cutting method with the loose combined cutting method, it was reported that the loose combined cutting technique produced more successful and reliable results in the treatment of suprasphincteric anal fistulas.[9] The treatment of loosely located anal fistulas over a 3-year period has also been investigated retrospectively, and it was reported that this technique is the most appropriate, as it is both reliable and less costly.[10] The impact of the use of chronic loose and non-cutting seton types on the quality of life in patients with fistulized perianal Crohn's disease has also been investigated.[11] It has been reported that patients with this condition respond well to a seton technique combined with infliximab therapy, and outcomes are good no matter if early or late application is used.[12] Similarly, seton drainage in combination with infliximab treatment was observed to be an effective method for the closure of a fistula in 75% of patients with Crohn's disease.[13]

In surgical practice, fistulas are categorized as simple or complex based on their relationship with the anal sphincter complex. Fistulas where fistulotomy can safely be performed without the risk of incontinence are classified as simple fistulas, while others are classified as complex.[5] Complex anal fistulas include transsphincteric fistulas involving more than 30% of the external anal sphincter, suprasphincteric fistulas, extrasphincteric fistulas, horseshoe fistulas, fistulas with multiple tracts, and fistulas coexisting with an abscess.[14] Notably, 15–40% of complex anal fistulas are associated with abscesses.[15,16] Anal abscesses occur when an infection in the anal glands in the intersphincteric space spreads to the surrounding spaces,[1] and 40% of these turn into anal fistulas. Abscesses and fistulas should be considered acute and chronic stages of the same disease because of their pathophysiological, etiological and anatomical relationships.[17] Therefore, treatment strategies for these conditions should be evaluated similarly. In both diseases, the goal is to reduce the recurrence rate without affecting continence, while eliminating the painful condition and improving the quality of life.[18]

To date, many surgical methods have been described for both diseases. However, the reported success rates for most methods are controversial. Moreover, there is currently no standard treatment modality.[19]

# Objectives

We aimed to investigate the surgical results of the loose seton method for the treatment of acute anorectal abscesses accompanied by anal fistulas and chronic anal fistulas.

# Materials and methods

## Patient follow-up

A total of 114 patients who were operated on in our clinic for complex chronic anal fistulas and anorectal abscesses with the loose seton application between 2020 and 2022 were included in the study. Patient data were examined retrospectively using patient files. Of the 114 patients, 78 had a complex chronic anal fistula, and 36 had an anorectal abscess accompanied by an anal fistula. With the exception of 12 patients who were operated on for anorectal abscesses under emergency conditions, all patients were evaluated using contrast-enhanced pelvic MRI, in addition to a physical examination in the preoperative period. Patients with abscesses who underwent only incision and drainage for an anorectal abscess or those whose internal fistula os could not be detected during the operation were excluded from the study. Of the patients with complex fistulas included in the study, 3 were suprasphincteric, 2 were extrasphincteric, 2 were horseshoe fistulas, and the remaining were transsphincteric. Patients who underwent fistulotomy due to intersphincteric and anocutaneous fistulas in chronic anal fistula cases and patients with a history of inflammatory bowel disease were not included in the study.

A signed consent form was obtained from all patients. Our study was approved by the Ethics Committee of Memorial Şişli Hospital (Istanbul, Turkey) (approval No. July 5, 2022/004).

Table 1. Classification of fistulas

| Classifications | Parks | St James's University Hospital | Garg | Standard Practice Task Force |
|---|---|---|---|---|
| Grade I | intersphincteric | simple (linear) intersphincteric | LOW – linear intersphincteric or transsphincteric | simple (in which fistulotomy is possible without risk of incontinence), with fistulas involving less than 1/3 of sphincter |
| Grade II | transsphincteric | complex intersphincteric (intersphincteric with abscess, multiple tracts, or horseshoe tract) | LOW – intersphincteric or transsphincteric with abscess, multiple tracts or horseshoe tract | |
| Grade III | suprasphincteric | simple (linear) transsphincteric | HIGH – linear transsphincteric – anterior fistula in female patients or fistula with associated comorbidities (Crohn's disease, sphincter injury, post-radiation exposure) | complex (in which fistulotomy has high risk of incontinence); these include high fistula, supralevator fistula, fistula with horseshoe tracts, multiple tracts, anterior fistula in a female patient, fistula with associated abscess, existing continence disturbance, Crohn's disease, and malignancy |
| Grade IV | extrasphincteric | complex transsphincteric (transsphincteric with abscess or multiple tracts) | HIGH – transsphincteric fistula with multiple tracts, associated abscess or horseshoe tract | |
| Grade V | – | extrasphincteric or supralevator | extrasphincteric, suprasphincteric or supralevator | |

## Classification of fistulas

Fistulas are typically classified as described by Parks et al.,[4] Standard Practice Task Force (2005), St. James's University Hospital (2000), and Garg et al.[20] These classifications are summarized in Table 1. In the current study, the oldest and most widely used classification system (Parks et al.[4]) was utilized.

## Surgical technique

With the exception of 12 patients who were operated on for anal abscesses under emergency conditions, all patients were operated on under elective conditions. In the elective patients, a preoperative rectal enema was performed and bowel cleansing was achieved. Five patients were operated on under general anesthesia and the remaining patients were operated on under spinal anesthesia. Patients were operated on in the jackknife or lithotomy position, depending upon the location of the fistula.

In patients with a chronic fistula, diluted methylene blue was administered via the fistula tracts. After the internal os was detected, the fistula tract was passed with the help of a stylet. The external fistula os was excised all around up to the external sphincter border and the material was sent to the pathology laboratory. The loose seton was attached to the internal end of the stylet and passed through the tract. It was tied with silk sutures so that the sphincter was not compressed.

In patients with an anorectal abscess, an anoscope was inserted into the anal canal to check whether there was spontaneous abscess drainage from the internal os. With the help of electrocautery, an incision was made on the abscess pouch and the purulent fluid and debris were drained. Subsequently, an attempt was made to find the internal os by applying diluted hydrogen peroxide to the abscess pouch. In patients whose internal os could not be detected, the procedure was terminated and no additional procedures were performed. These patients were excluded from the study. In patients where the internal os could be found, it was passed with the help of a stylet. The same procedure was applied in fistula patients.

In addition to the vein strap used as a seton material, in several patients, a circular piece cut from the thickest part of a surgical glove sleeve was used. When adequate progression could not be observed, the vascular slings were replaced with the material prepared from surgical latex gloves. A seton was also applied in cases of low transsphincteric fistulas. Fistulotomy was not performed on any patient operated on for an anorectal abscess. Patients who underwent fistulotomy due to intersphincteric, subcutaneous-mucosal fistulas were excluded from the study. A seton was applied in all patients with an anorectal abscess who had an internal os to facilitate drainage of the abscess. Operation time was recorded for each patient.

## Postoperative patient follow-up

In the postoperative period, the patients received monthly follow-ups subsequent to 1$^{st}$- and 2$^{nd}$-week follow-ups. During the monthly follow-ups, seton materials that had loosened and sagged excessively were tied with silk sutures and shortened to prevent compression of the sphincter. Weakened seton materials were replaced end-to-end with new materials.

In patients whose setons had progressed to the anocutaneous level during follow-up, the seton was removed by fistulotomy under local anesthesia in an outpatient setting. In 8 patients, the seton spontaneously ruptured at the anocutaneous level. No interventions were performed on these patients. Patients were asked about their continence levels during follow-up. Postoperative continence levels were evaluated after the seton dissociation process was completed, using the Wexner incontinence score. This scoring system cross-tabulates frequencies and different anal incontinence presentations (solid/liquid/gas/wears pad/need for lifestyle alterations) and sums the returned score for a total of 0–20 (where 0 = perfect continence and 20 = complete incontinence; Table 2).[21]

Table 2. Wexner continence score

| Type of incontinence | Never | Rarely | Sometimes | Usually | Always |
|---|---|---|---|---|---|
| Solid | 0 | 1 | 2 | 3 | 4 |
| Liquid | 0 | 1 | 2 | 3 | 4 |
| Gas | 0 | 1 | 2 | 3 | 4 |
| Wears pad | 0 | 1 | 2 | 3 | 4 |
| Lifestyle alteration | 0 | 1 | 2 | 3 | 4 |

Rarely <1/month; sometimes <1/week; usually <1/day; always 1/day; 0 = perfect continence; 20 = complete incontinence.

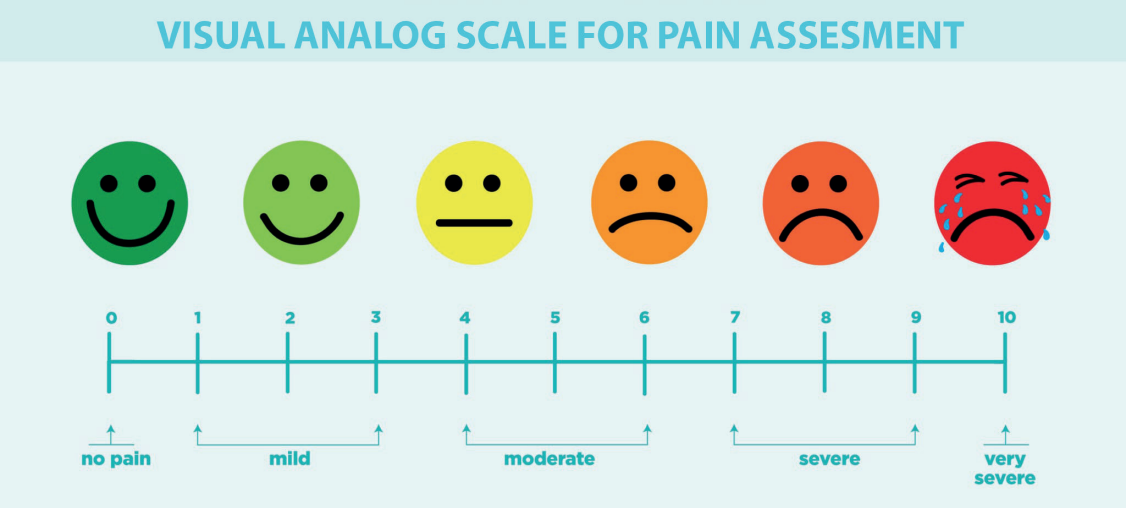



Fig. 1. The universal pain assessment tool (the pain assessment tool is intended to help patient care providers assess pain according to individual patient needs; a scale from 0 to 10 is used for patients' self-assessment)

The patients were assessed for recurrence during follow-up. Postoperative pain was assessed using the visual analogue scale (VAS) 24 h after the operation. On this scale, a score of 0 indicates no pain and a score of 10 indicates very severe pain. Scores <3 were interpreted as mild pain, 3–6 were considered to indicate mild–moderate pain, and >6 indicated severe pain (Fig. 1).

## Statistical analyses

The SPSS v. 11.5 (SPSS Inc., Chicago, USA) was used for data analyses. Mean ± standard deviation (M ±SD) and median (minimum–maximum) are used as descriptors for quantitative variables, and the number of patients (percentage) is presented for qualitative variables. The differences in quantitative variables between the 2 categories were examined using the Mann–Whitney U test because the assumption of a normal distribution was not met. Fisher's exact tests were used to examine the relationships between 2 qualitative variables. Results were considered statistically significant if the p-value was <0.05.

# Results

There was no statistically significant difference in patient gender between the 2 groups ($\chi^2$ = 1.380, p = 0.334). Mean age and operation times were significantly higher in the anorectal abscess group (z = –2.471, p = 0.013 and z = –3.224, p = 0.001, respectively; Table 3).

Of the 114 patients included in the study, 78 had complex chronic anal fistulas, and 36 had anorectal abscesses accompanied by anal fistulas. As a result of the MRIs performed in the anorectal abscess group, perianal abscesses were detected in 14 (58%) patients (Fig. 2A,B), ischiorectal abscesses in 6 (25%) patients (Fig. 3A,B) and intersphincteric abscesses in 4 (17%) patients (Fig. 4). Suprasphincteric fistulas were observed in 3 patients, extrasphincteric fistulas were found in 2 patients, horseshoe fistulas were present in 2 patients (Fig. 5A,B), and transsphincteric fistulas were observed in the remaining patients with complex fistulas. Thirteen patients had 2 external fistula ora and 4 patients had 3 external fistula ora. Eight patients in the fistula group had an abscess pouch associated with the fistula tract. These findings are consistent with the literature.

The mean time to seton removal was 6.8 (3–19) months. In patients whose setons progressed to the anocutaneous level during the follow-up period, the seton was removed by performing a fistulotomy under local anesthesia at the outpatient clinic. In 8 patients, the seton spontaneously ruptured and fell to the anocutaneous level. No interventions were performed on these patients.

In the early postoperative period, liquid stool and gas leakage from the seton area were observed in 3 patients. In these patients, continence improved in the 3rd month of follow-up after the fistula tract matured. Neither gas nor

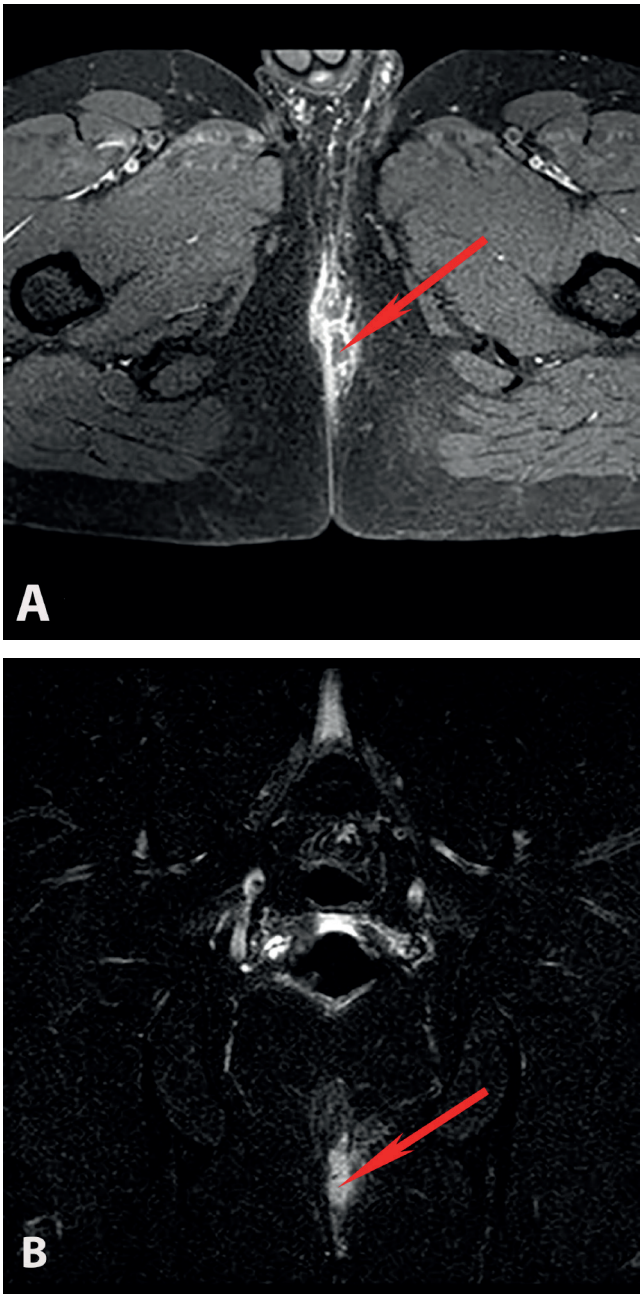



Fig. 2. A. Perianal abscess (axial section); B. Perianal abscess (sagittal section)

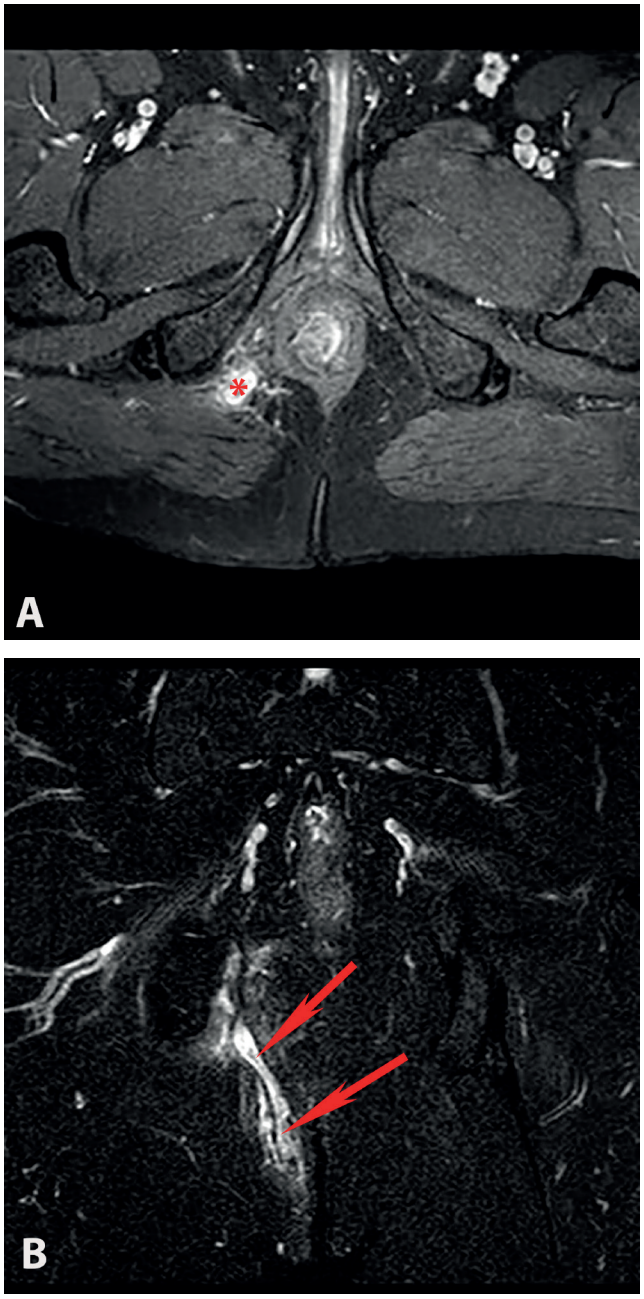



Fig. 3. A. Ischiorectal abscesses (axial section); B. Ischiorectal abscesses (sagittal section)

stool leakage was observed in any patient in the chronic fistula or anorectal abscess groups on the course of long-term follow-up after seton removal. There were no statistically significant differences in postoperative continence levels between the groups.

In our study, the median follow-up period was 24 (1–41) months. No fistula recurrence was observed in either the chronic fistula group or the abscess group during the follow-up period. Recurrent abscesses were observed in 5 (13.9%) patients in the anorectal abscess group. In addition, abscesses were observed in 2 (2.6%) patients in the chronic fistula group. These results were statistically significant ($\chi^2$ = 1.380, p = 0.031). Setons were renewed by applying drainage in these patients.

The anorectal abscess group had statistically significantly higher pain scores 24 h after the surgery (z = –8.235, p < 0.001). These findings are summarized in Table 3.

There was no significant difference in pre- and postoperative Wexner continence scores between the anorectal abscess and chronic anal fistula groups (0.03 ±0.17 and 0.03 ±0.16, respectively, p = 0.947). In the preoperative evaluation, a Wexner continence score above 0 was rarely seen, with only 1 (2.8%) patient with a gas leak in the anorectal abscess group and only 2 (2.6%) patients with gas leaks in the chronic anal fistula group. Postoperative evaluation results were the same as the preoperative evaluation results (Table 3).

**Table 3.** Comparisons of the patient characteristics and clinical parameters of both groups

| Variables | | Anorectal abscess group (n = 36) | Chronic anal fistula group (n = 78) | p-value | Test statistic |
|---|---|---|---|---|---|
| Gender, n (%) | male | 34 (94.4) | 68 (87.2) | 0.334[a] | $\chi^2 = 1.380$ df = 1 |
| | female | 2 (5.6) | 10 (12.8) | | |
| Age [years] | M ±SD | 45.92 ±13.25 | 39.87 ±10.55 | 0.013[b]* | Z = –2.471 |
| | median (min–max) | 43.00 (17.00–84.00) | 39.00 (17.00–73.00) | | |
| Mean operation time [min] | M ±SD | 19.44 ±2.97 | 17.44 ±2.47 | 0.001[b]* | Z = –3.224 |
| | median (min–max) | 20.00 (16.00–25.00) | 17.50 (13.00–21.00) | | |
| Visual analogue scale | M ±SD | 5.25 ±1.46 | 2.14 ±0.78 | <0.001[b] | Z = –8.235 |
| | median (min–max) | 6.00 (3.00–7.00) | 2.00 (1.00–3.00) | | |
| Recurrent abscesses, n (%) | yes | 5 (13.9) | 2 (2.6) | 0.031[b]* | $\chi^2 = 1.380$ df = 1 |
| Preop Wexner | M ±SD | 0.03 ±0.17 | 0.03 ±0.16 | 0.947[b] | Z = –0.066 |
| | median (min–max) | 0.00 (0.00–1.00) | 0.00 (0.00–1.00) | | |
| Postop Wexner | M ±SD | 0.03 ±0.17 | 0.03 ±0.16 | 0.947[b] | Z = –0.066 |
| | median (min–max) | 0.00 (0.00–1.00) | 0.00 (0.00–1.00) | | |

*statistically significant if the p-value <0.05. M ±SD – mean ± standard deviation; df – degrees of freedom; [a] Fisher's exact test; [b] Mann–Whitney U test; Preop Wexner – preoperative Wexner´s incontinence score; Postop Wexner – postoperative Wexner´s incontinence score.

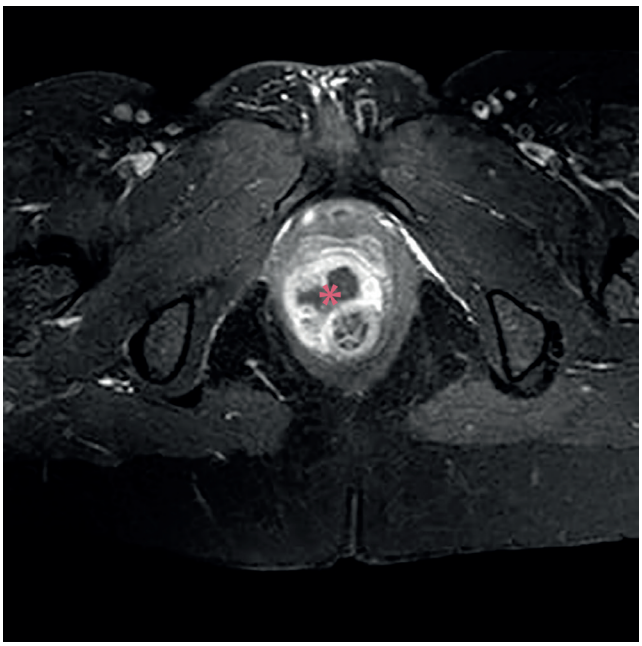


**Fig. 4.** Intersphincteric abscesses (axial section)

## Discussion

The most important factor for reducing recurrence is revealing the internal os. In the case of an anorectal abscess, it may be difficult to locate the internal os due to edema and debris in the tissues. It has been reported that the internal fistula os can be found in 83% of anorectal abscess

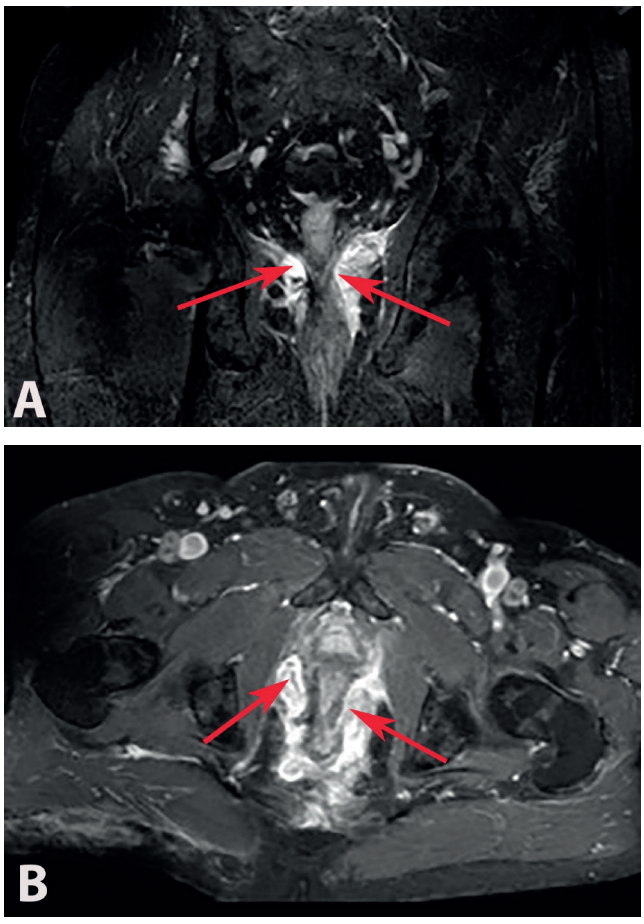



**Fig. 5.** A. Horseshoe fistula (axial section); B. Horseshoe fistula (sagittal section)

cases.[22] In our sample, the internal os was found in 45% of patients with anal abscesses. Patients in whom the internal os had not been found were excluded from the current study. Simultaneous fistula treatment carries a risk of incontinence at different rates depending on the surgical method chosen. The coexistence of anal fistulas is seen in approx. 1/3 of anorectal abscess cases.[23]

Garg et al. reported that an inaccurate identification of or an inability to find the internal opening is the most important cause of fistula recurrence.[24] An inability to determine the exact position of the internal os complicates the management of fistulas. A protocol to localize the internal os in patients where it cannot be clearly found was recently described by Garg et al.[24] According to this protocol, attempts are made to localize the internal os with preoperative clinical examination (maximum hardening point), MRI evaluation, intraoperative examination under anesthesia, and injection of povidone-iodine solution through the external opening. If the internal os of the fistula cannot be clearly found, a 3-step protocol is followed. First, MR images are re-evaluated by the surgical team. Second, it is assumed that the internal opening is at the location where the fistula is closest to the internal sphincter. Third, if there is a horseshoe fistula and the internal opening cannot be clearly found, the internal opening is assumed to be on the midline. If the horseshoe fistula is located posteriorly, the internal opening is assumed to be on the posterior midline, while in anterior horseshoe fistulas, it is assumed to be on the anterior midline, and it is recommended to manage fistulas accordingly.[25] Thus, the Garg protocol effectively helps to find the internal os of fistulas.

In patients receiving only incision and drainage of the abscess, the procedure can result in insufficient drainage in high abscesses, resulting in a recurrence rate of 44% and the formation of anal fistulas.[26] Along with drainage of the anorectal abscess, simultaneous surgery for the fistula is useful in preventing recurrent abscess formation and subsequent fistula surgery. Meta-analyses have shown that anal fistula surgery during abscess drainage reduces the presence of persistent abscesses and fistulas, recurrence, and repetitive surgery.[27] In our study, abscess recurrence was observed in 5 (13.38%) patients in the anorectal abscess group and 2 (2.56%) patients in the chronic fistula group. It seems that the recurrence was due to the insufficient drainage of deep abscesses. Setons were renewed by applying drainage to these patients. A low recurrence rate suggests that a seton is effective in reducing the rate of recurrent abscesses.

The 2017 German S3 guidelines recommend primary fistulotomy for superficial fistulas involving a small portion of the anal sphincter. The same guidelines recommend postponing definitive surgery to a later date in patients with high fistulas or fistulas where it is not clear how much of the sphincter is involved.[28] In contrast, in a meta-analysis that included 479 patients who had only abscess drainage or fistula surgery with abscess drainage, there was no statistically significant difference in incontinence in patients who underwent fistula surgery performed simultaneously with abscess drainage.[29] However, results vary according to the chosen surgical method. Recent studies have shown that even in fistulas with a high acute anorectal abscess, definitive fistula surgery can be carried out with excellent results by performing sphincter-sparing procedures.[30]

Many methods have been developed to prevent incontinence in patients with complex fistulas. In a study by Garg et al., in a series of 1250 patients, 4 different surgical techniques were applied and a 98.6% recovery rate was reported for simple fistulas that underwent fistulotomy without affecting continence. The healing rate was reported to be 90.6% in fistulas accompanied by abscess and 94.5% in fistulas without abscess, and the difference was not statistically significant ($p = 0.057$).[31] In this study, the same sphincter-sparing surgery (transanal opening of the intersphincteric space (TROPIS)) was applied to high fistulas with accompanying anorectal abscesses and chronic complex fistulas without abscesses. In the TROPIS procedure, the intersphincteric space is opened from the anal canal. The internal sphincter and mucosa are incised and the internal os of the fistula is deroofed. Thus, in this procedure, infected crypt glands and the internal opening of the fistula are destroyed and left open for secondary healing. Moreover, the external sphincter is preserved, minimizing the risk of incontinence. Healing rates in the acute anorectal abscess and chronic fistula groups were 87% (100 of 115) and 88% (168 of 191), respectively; thus, there was no difference in healing rate between the groups. However, the author emphasized that long-term results and a larger patient series are needed.[32]

In our surgical method, we used a loose seton in patients with a complex fistula or an anorectal abscess accompanied by a complex fistula. It is thought that setons facilitate the drainage of the associated abscess and create a local inflammatory reaction to provide resolution of the fistula pathway.[32] Setons used in the treatment of complex anal fistulas are divided into cutting and loose setons. Cutting setons create compression necrosis as periodic tightening is applied. Due to the painful tightening process, cutting setons have disadvantages, such as patient incompatibility and continence problems.[33,34]

In the literature, solid stool incontinence, liquid stool incontinence and gas incontinence were reported in 2.3%, 8.5% and 36% of patients, respectively, who underwent cutting seton due to complex fistulas.[35] Due to the disadvantages mentioned above, loose setons are often used instead of cutting setons. Loose setons also provide more effective drainage, facilitating drainage of the associated abscess and promoting resolution of the fistula tract by creating a local inflammatory reaction without causing compression necrosis.[36] Many materials are used as loose seton

material. Vascular slings, Penrose drains, rubber bands, or latex gloves are most commonly used.[37] In our study, we used a seton prepared by cutting a thick strip from the arm of a latex surgical glove. The prepared seton was formed by passing the material through the fistula area in a single layer, ensuring that the seton was not tight, and tying it with silk sutures. Using a similar material, Mentes et al. reported a 100% recovery rate after 3 months. In their study, incontinence worsening of 20% was reported in patients treated with an elastic seton compared to the initial scores, but this difference was not statistically significant.[37]

Seton application in a similar fashion has been reported in the literature but gas and stool incontinence was not monitored in any patient.[38] Similarly, in the study by Vrzgula et al., a loose seton was used in 14 out of 99 patients who were operated on for anal abscesses. In this study, no relapses were observed, and no solid or liquid stool leakage was reported in any patient.[39]

During the follow-up period, we did not observe gas or stool leakage in any of the patients in either group. The absence of clinical solid and liquid stool incontinence in any of the patients during the follow-up period in our series indicates that elastic seton application significantly contributes to the preservation of continence. However, a recurrent abscess was observed in 5 (13.9%) patients in the anorectal abscess group during the follow-up period. In addition, an abscess was observed in 2 (2.6%) patients in the chronic fistula group due to insufficient drainage. Drainage was applied to these patients and their setons were renewed.

We evaluated the patients' pre- and postoperative continence levels with the Wexner incontinence score. Gas or stool leakage was not observed in any patient in the chronic fistula or anorectal abscess groups during the follow-up period. There was no difference in pre- or postoperative continence scores between the groups. Our findings provide evidence that the seton method is a safe treatment option that can be used in both complex anal fistulas and perianal abscesses, providing a high recovery rate without affecting continence.

## Limitations

The current study has a number of limitations. First, the number of cases included in the study is relatively limited. In addition, this is a single-center study. The Wexner continence scale was used to evaluate incontinence. The definitive method for assessing the anal sphincter complex is to take preoperative and postoperative anal manometry measurements. However, preoperative anal manometry measurement is not possible in patients with acute anal abscess due to pain. Therefore, manometry was not used in our study. Despite these limitations, the results obtained in our study show that the loose seton method is safe.

# Conclusions

It is emerging that acute anorectal abscessed fistulas, including high complex fistulas, can be definitely treated by performing sphincter-sparing procedures at the initial surgery. We believe that using a loose seton is a safe and effective method for abscess treatment because, during patient follow-up, we observed a painless and continuous process with good results in the treatment of all types of abscesses.


## ORCID iDs

Cemalettin Durgun https://orcid.org/0000-0002-5797-7926
Abidin Tüzün https://orcid.org/0000-0002-4953-6602


## References


1. Parks AG. Pathogenesis and treatment of fistula-in-ano. *Br Med J*. 1961;1(5224):463–460. doi:10.1136/bmj.1.5224.463
2. Zanotti C, Martinez-Puente C, Pascual I, Pascual M, Herreros D, García-Olmo D. An assessment of the incidence of fistula-in-ano in four countries of the European Union. *Int J Colorectal Dis*. 2007;22(12):1459–1462. doi:10.1007/s00384-007-0334-7
3. Williams JG, Farrands PA, Williams AB, et al. The treatment of anal fistula: ACPGBI position statement. *Colorectal Dis*. 2007;9(Suppl 4):18–50. doi:10.1111/j.1463-1318.2007.01372.x
4. Parks AG, Gordon PH, Hardcastle JD. A classification of fistula-in-ano. *Br J Surg*. 1976;63(1):1–12. doi:10.1002/bjs.1800630102
5. Verkade C, Zimmerman DDE, Wasowicz DK, Polle SW, de Vries HS. Loss of seton in patients with complex anal fistula: A retrospective comparison of conventional knotted loose seton and knot-free seton. *Tech Coloproctol*. 2020;24(10):1043–1046. doi:10.1007/s10151-020-02254-1
6. Erol T, Mentes B, Bayri H, et al. Preventing the recurrence of acute anorectal abscesses utilizing a loose seton: A pilot study. *Pan Afr Med J*. 2020;35:18. doi:10.11604/pamj.2020.35.18.21029
7. Zhi C, Huang Z, Liu D, Zheng L. Long-term follow-up study of loose combined cutting seton surgery for patients with high anal fistula. *Ann Transl Med*. 2021;9(14):1160. doi:10.21037/atm-21-3242
8. Eitan A, Koliada M, Bickel A. The use of the loose seton technique as a definitive treatment for recurrent and persistent high transsphincteric anal fistulas: A long-term outcome. *J Gastrointest Surg*. 2009;13(6):1116–1119. doi:10.1007/s11605-009-0826-6
9. Cheng Y, Zheng L, Shi Y, et al. Loose combined cutting seton for patients with suprasphincteric anal fistula: A randomized clinical trial protocol. *Ann Palliat Med*. 2021;10(9):10022–10030. doi:10.21037/apm-21-2303
10. Kelly ME, Heneghan HM, McDermott FD, et al. The role of loose seton in the management of anal fistula: A multicenter study of 200 patients. *Tech Coloproctol*. 2014;18(10):915–919. doi:10.1007/s10151-014-1186-0
11. Gklavas A, Sotirova I, Karageorgou M, Kozonis T, Poulaki A, Papaconstantinou I. Is the quality of life of patients with fistulizing perianal Crohn's disease impaired by the presence of chronic loose, non-cutting seton? *J Gastrointest Surg*. 2021;25(10):2686–2689. doi:10.1007/s11605-021-04987-2
12. Jeon M, Song K, Koo J, Kim S. Evaluation of a seton procedure combined with infliximab therapy (early vs. late) in perianal fistula with Crohn disease. *Ann Coloproctol*. 2019;35(5):249–253. doi:10.3393/ac.2018.11.23.1
13. Haennig A, Staumont G, Lepage B, et al. The results of seton drainage combined with anti-TNFα therapy for anal fistula in Crohn's disease. *Colorectal Dis*. 2015;17(4):311–319. doi:10.1111/codi.12851
14. Garg P. Garg classification for anal fistulas: Is it better than existing classifications? A review. *Indian J Surg*. 2018;80(6):606–608. doi:10.1007/s12262-018-1788-2
15. Vogel JD, Johnson EK, Morris AM, et al. Clinical practice guideline for the management of anorectal abscess, fistula-in-ano, and rectovaginal fistula. *Dis Colon Rectum*. 2016;59(12):1117–1133. doi:10.1097/DCR.0000000000000733

16. Garg P. Comparison of preoperative and postoperative MRI after fistula-in-ano surgery: Lessons learnt from an audit of 1323 MRI at a single centre. *World J Surg*. 2019;43(6):1612–1622. doi:10.1007/s00268-019-04926-y
17. Garg P, Singh P, Kaur B. Magnetic resonance imaging (MRI): Operative findings correlation in 229 fistula-in-ano patients. *World J Surg*. 2017;41(6):1618–1624. doi:10.1007/s00268-017-3886-x
18. Abcarian H. Anorectal infection: Abscess-fistula. *Clin Colon Rectal Surg*. 2011;24(1):14–21. doi:10.1055/s-0031-1272819
19. Shi Y, Zheng L, Li X, et al. Clinical study on the effectiveness and safety of loose combined cutting seton in the treatment of high perianal abscess: A randomized controlled trial protocol. *Ann Transl Med*. 2022;10(2):108. doi:10.21037/atm-22-62
20. Garg P. Comparing existing classifications of fistula-in-ano in 440 operated patients: Is it time for a new classification? A retrospective cohort study. *Int J Surg*. 2017;42:34–40. doi:10.1016/j.ijsu.2017.04.019
21. Williams G, Williams A, Tozer P, et al. The treatment of anal fistula: Second ACPGBI Position Statement – 2018. *Colorectal Dis*. 2018;20(Suppl 3):5–31. doi:10.1111/codi.14054
22. Jorge MJN, Wexner SD. Etiology and management of fecal incontinence. *Dis Colon Rectum*. 1993;36(1):77–97. doi:10.1007/BF02050307
23. Oliver I, Lacueva F, Pérez Vicente F, et al. Randomized clinical trial comparing simple drainage of anorectal abscess with and without fistula track treatment. *Int J Colorectal Dis*. 2003;18(2):107–110. doi:10.1007/s00384-002-0429-0
24. Garg P, Kaur B, Singla K, Menon GR, Yagnik VD. A simple protocol to effectively manage anal fistulas with no obvious internal opening. *Clin Exp Gastroenterol*. 2021;14:33–44. doi:10.2147/CEG.S291909
25. Yagnik VD, Kaur B, Dawka S, Sohal A, Menon GR, Garg P. Non-locatable internal opening in anal fistula associated with acute abscess and its definitive management by Garg protocol. *Clin Exp Gastroenterol*. 2022;15:189–198. doi:10.2147/CEG.S374848
26. Nelson R. Anorectal abscess fistula: What do we know? *Surg Clin North Am*. 2002;82(6):1139–1151. doi:10.1016/S0039-6109(02)00063-4
27. Wang Y, Tian GB, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: An epidemiological and clinical study. *Lancet Infect Dis*. 2017;17(4):390–399. doi:10.1016/S1473-3099(16)30527-8
28. Quah HM, Tang CL, Eu KW, Chan SYE, Samuel M. Meta-analysis of randomized clinical trials comparing drainage alone vs primary sphincter-cutting procedures for anorectal abscess–fistula. *Int J Colorectal Dis*. 2006;21(6):602–609. doi:10.1007/s00384-005-0060-y
29. Ommer A, Herold A, Berg E, et al. German S3 guidelines: Anal abscess and fistula (second revised version). *Langenbecks Arch Surg*. 2017;402(2):191–201. doi:10.1007/s00423-017-1563-z
30. Malik AI, Nelson RL, Tou S. Incision and drainage of perianal abscess with or without treatment of anal fistula. *Cochrane Database Syst Rev*. 2010;7:CD006827. doi:10.1002/14651858.CD006827.pub2
31. Garg P, Kaur B, Goyal A, Yagnik VD, Dawka S, Menon GR. Lessons learned from an audit of 1250 anal fistula patients operated at a single center: A retrospective review. *World J Gastrointest Surg*. 2021;13(4):340–354. doi:10.4240/wjgs.v13.i4.340
32. Garg P, Kaur B, Menon GR. Transanal opening of the intersphincteric space: A novel sphincter-sparing procedure to treat 325 high complex anal fistulas with long-term follow-up. *Colorectal Dis*. 2021;23(5):1213–1224. doi:10.1111/codi.15555
33. Memon AA, Murtaza G, Azami R, Zafar H, Chawla T, Laghari AA. Treatment of complex fistula in ano with cable-tie seton: A prospective case series. *ISRN Surg*. 2011;2011:636952. doi:10.5402/2011/636952
34. McCourtney JS, Finlay IG. Setons in the surgical management of fistula in ano. *Br J Surg*. 1995;82(4):448–452. doi:10.1002/bjs.1800820406
35. García-Aguilar J, Belmonte C, Wong W, Goldberg DW, Madoff RD. Cutting seton versus two-stage seton fistulotomy in the surgical management of high anal fistula. *Br J Surg*. 1998;85(2):243–245. doi:10.1046/j.1365-2168.1998.02877.x
36. Isbister WH, Al Sanea N. The cutting seton: An experience at King Faisal Specialist Hospital. *Dis Colon Rectum*. 2001;44(5):722–727. doi:10.1007/BF02234574
37. Mentes BB, Öktemer S, Tezcaner T, Azılı C, Leventoğlu S, Oğuz M. Elastic one-stage cutting seton for the treatment of high anal fistulas: Preliminary results. *Tech Coloproctol*. 2004;8(3):159–162. doi:10.1007/s10151-004-0080-6
38. Bektaşoğlu HK, Kunduz E. Elastic seton placement in treatment of complex anal fistula: Analysis of 44 patients. *Turk J Colorectal Dis*. 2018;28(1):18–21. doi:10.4274/tjcd.04834
39. Vrzgula A, Bober J, Val'ko M. Simultaneous treatment of an anal abscess and fistula [in Slovak]. *Rozhl Chir*. 1996;75(4):219–221. PMID:8768998.

# Indicators of integrated care for patients with chronic cardiovascular disease in ambulatory care

Elżbieta Szlenk-Czyczerska[1,A–F], Donata Kurpas[2,A–F]

[1] Institute of Health Sciences, University of Opole, Poland
[2] Department of Family Medicine, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1159–1166

**Address for correspondence**
Elżbieta Szlenk-Czyczerska
E-mail: ela6035@onet.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on December 8, 2022
Reviewed on February 1, 2023
Accepted on February 20, 2023

Published online on March 15, 2023

## Abstract

**Background.** Patients with cardiovascular disease (CVD) have an increased need for medical care and a high risk of hospitalization. It is necessary to improve the integration between healthcare, long-term care and social care for these individuals, as poor integration limits the full potential of care.

**Objectives.** This study aims to identify effective indicators of CVD management, including variables that promote the horizontal and vertical integration of planned interventions.

**Materials and methods.** Patients with chronic CVD managed by a general practitioner (GP) or a primary care cardiologist will be enrolled in the study. The study will use the World Health Organization Quality of Life Questionnaire (WHOQOL)-BREF, the Health Behavior Inventory (HBI) questionnaire, the Camberwell Assessment of Need (CAN) Short Appraisal Schedule, the Hospital Anxiety and Depression Scale-Modified Version (HADS-M), a Self-Description Questionnaire, and the authors' self-prepared questionnaire to collect data.

**Results.** The main results will allow for the identification of the variables that influence the effectiveness of healthcare (understood as the synergy of high quality of life, intensification of health behaviors and high satisfaction of needs) for patients with CVD. In addition, an examination of the relationships between quality of life and health behaviors, assessment of needs (health and social), level of religiosity and spirituality, expectations, and variables affecting anxiety and depressive symptoms will allow for the identification of indicators that favor the integration of care both horizontally and vertically.

**Conclusions.** The results of this study will support the development of systems aimed at identifying CVD patients at risk for lower effectiveness of care in integrated care. In addition, the results may help to develop clinical information and decision support systems aimed at designing personalized care models for patients with CVD. They may also help to develop coordinated care plans and patient education programs, and obtain data useful for implementing system changes.

**Key words:** patients, cardiovascular disease, primary healthcare, integrated care

**Cite as**
Szlenk-Czyczerska E, Kurpas D. Indicators of integrated care for patients with chronic cardiovascular disease in ambulatory care. *Adv Clin Exp Med*. 2023;32(10):1159–1166. doi:10.17219/acem/161462

**DOI**
10.17219/acem/161462

## Introduction

Research has identified poor coordination between healthcare, long-term care and social care, and has shown that such a weak integration limits the full capacity of patient care.[1] With a paradigm change in service delivery, healthcare systems will be more consistent and potentially even more financially efficient and cost-effective. As a result, the integration of various health services is increasingly seen to provide more coordinated care and stem the rapid cost inflation that the treatment sector is causing.[2,3]

It has been pointed out that healthcare systems should be more people-centered and able to provide integrated, high-quality care.[4,5] Interventions to ensure coordination and continuity of care when people move from one place to another or from one level of care to another in the same location are advisable for particular patient groups, such as the elderly.[3,6]

The growing emphasis on population health and chronic disease management requires the intervention of many providers within a continuum of care.[5] Horizontal and vertical integration is the answer to this challenge. Horizontal integration relates to tactics that combine similar levels of care and address the provision of varied complementary health services to meet the diverse health needs of patients. Vertical integration, on the other hand, refers to strategies that combine different levels of care (from primary care clinics to general tertiary hospitals).[5,3] The call for integrated care has already been taken up in many countries, resulting in several experimental programs and innovations in health policy.[3,5,7–11]

Undoubtedly, patients with cardiovascular disease (CVD) have a high risk of hospitalization and an elevated need for medical care.[12] As a result, they often require multiple contacts with different healthcare providers, increasing the likelihood of receiving poor and fragmented care.[4] Given the expected incidence of CVD and demographic changes, a more effective and targeted model of care for patients with chronic CVD needs to be developed.[2,12–15] In addition, the Polish healthcare system is characterized by fragmented healthcare administration (which significantly complicates coordination at the systemic level) and relatively low healthcare spending.[16] These features make it necessary to study the requirements, practices and challenges of integrating care for people with CVD in the country. Improving the integration of CVD care will effectively impact patient functioning, and reduce maintenance costs and needless hospitalizations.[12]

It should be noted that, in recent years, there has been a return to the promotion of holistic care, defined as a caring approach that is more concerned about the patient and takes into account all of their needs, goals and values.[12–15] In addition, available research emphasizes that integration of care should focus on a comprehensive approach to care and multidisciplinary collaboration.[3,17] There is also evidence that the social dimension needs to be integrated into health service delivery to meet the population health needs. Community support and interpersonal ties are key to health and wellbeing.[3]

Cardiovascular disease is known to negatively affect mental and physical health, social components of life activities, and emotional state. All of the above consequences associated with CVD can be measured and detected by assessing patients' quality of life indicators.[15,18] Significantly, assessing the quality of life affects the efficiency of medical treatment and makes therapeutic options more accessible and acceptable to patients. Quality of life is also perceived as an indicator of the effectiveness of current political and social support systems.[15,18–21] A correlation between the quality of life in patients with CVD and point of healthcare has been demonstrated.[15]

Another critical topic is the health-promoting behaviors of CVD patients. Lifestyle, including health-promoting behaviors, is an important determinant of health.[22] Unhealthy behaviors lead to, for example, an increased incidence of heart failure, poorer survival rates, an aging population, and an obesity epidemic.[23,24] Health behaviors are considered a key element in the prevention of CVD. Notably, lower levels of health-promoting behaviors have been shown to increase the risk of anxiety disorders in patients with CVD.[14] Health-promoting behaviors also delay patient disability and thus the need for social support.[25,26] Data analyses have shown that older people with healthier lifestyles can expect to spend about 1.7 years less with disability at the end of their lives compared to their peers with unhealthy lifestyles.[27] The possibility of delaying death by fostering health-promoting behaviors has also been demonstrated.[27–29] Health behavior assessment of patients with CVD is a significant indicator of health care effectiveness.[14] The cost-effectiveness of encouraging health-promoting behaviors has also been confirmed. For example, behavioral change has been shown to reduce both direct and indirect medical costs.[30,31] It has also been noted that health-promoting behaviors should be fostered, particularly at the primary care level. Identifying risk factors for CVD and education about these factors should be a regular part of primary care visits.[12,32,33]

However, the issue of health still needs to be addressed in primary health care (PHC) analyses of the CVD delivery system. Health needs are seen as the product of a specific clinical condition, resulting in health behaviors, quality of life and the evaluation of health services. The PHC design is premised on identifying patients' individual biopsychosocial needs. This also facilitates the determination of further clinical management and patient care.[34] It is considered that identifying a need is equivalent to specifying a problem and allows for appropriate intervention.[34,35] It has also been stressed that the identification of needs, especially unmet needs, is essential, as they are related to health conditions and, thus, to quality of life and medical costs. The identification of needs is an explanatory

variable that has a greater impact on quality of life than clinical or sociodemographic factors and, in addition, is indirectly related to the level of quality of care. It has also been found that, when comparing patients' and health professionals' assessments of needs, patients' estimates are more accurate, and the extent of unmet needs remains related to patients' level of functioning.[35]

Spiritual beliefs and religious practices affect coping mechanisms for various chronic diseases, including CVD.[36–39] For many CVD patients, religious practices and beliefs are highly personal and noteworthy features of their disease, and provide critical management strategies.[40] Cardiovascular disease impairs mobility and limits social activities and work, thus becoming a cause of isolation for patients. The condition can lead to chronic anxiety and a depressed mood, resulting in the development or exacerbation of anxiety-depression syndrome. As mental state significantly affects prognosis and treatment, the necessity for better detection tactics is becoming more recognized. Mental disorders should be treated accordingly and detected earlier.[14] In addition, a PHC efficacy study indicates that future research should also consider the relationships between spirituality and religiosity, quality of life, and anxiety and depression in patients with CVD.[12]

In sum, the basis of person-centered care is a holistic understanding of an individual's health and wellbeing, the ability and capacity to care for themselves, and their needs, preferences and environment.[41] It has also been noted that self-care should be tailored to a person's baseline condition.[12] In addition, the patient's motivation and knowledge are essential for effective self-management.[42] Therefore, when it comes to the need for care integration, it is essential that healthcare providers have a holistic understanding of the frail patient in order to individually assess self-care options and provide support and encouragement as needed.[6,12] In addition, some studies suggest that the disappointing results observed in many existing integration models are often due to an inadequate assessment of patients' needs and preferences,[6] and an underestimation of community-based care.[6]

Regarding the integrated care of CVD patients, there is still little research on this topic.[12–15] There are some studies, but they focus on clinically homogeneous populations.[40,43,44] The central role of PHC is evident in almost all integration models studied so far.[3] Given the role that PHC plays in improving the healthcare system, it is essential to identify indicators that can be used to assess the process maturity of integrated CVD care.

# Objectives

In light of these considerations, this study aims to identify effective indicators of healthcare delivery in patients with CVD, including variables that promote the horizontal and vertical integration of planned interventions.

# Materials and methods

## Study design and setting

This observational and cross-sectional project will be conducted with the approval of the University of Opole Research Ethics Committee and will be carried out in accordance with the requirements of the 1975 Declaration of Helsinki (as amended in 2000). The project will be performed among Polish patients with CVD in Opole (Poland) who are receiving outpatient care. The PHC physicians and cardiologists will follow these patients to determine specific indicators of integrated care.

## Participants

An invitation to participate will be sent to the chief executive officer (CEO) of the company which comprises the 4 municipal primary care clinics. Patients will be selected by general practitioners (GPs)/cardiologists in accordance with the inclusion criteria. Chronic CVD will be defined on the basis of primary medical history (diagnosis by the GP). Patients with CVD under the care of a GP (group A) and, to identify variables specific to ambulatory care, patients with CVD under the supervision of a cardiologist (group B) will be invited to participate. They will be encouraged to take part in the study by their GP/cardiologist during scheduled PHC visits. The doctor will interview the patient to outline the aim and methods of the study and to obtain preliminary verbal consent. Patients will receive a set of questionnaires and an informed consent form to participate in the study. The inclusion criteria are being over 18 years of age, a diagnosis of CVD (International Classification of Diseases 10$^{th}$ Revision (ICD-10) codes) at least 1 year before the start of the study, and receiving care from a GP/cardiologist at a primary care center. The criteria for exclusion are cognitive disorders and other severe mental illnesses and/or other difficulties that prevent active participation. Participation in the study will be voluntary and anonymous. Before taking part in the study, each patient will be informed of the purpose, methods and the possibility of withdrawal at any stage of the study. As mentioned above, the purpose and procedures will be explained at the selection stage, and only those who voluntarily consent will be accepted. Candidates will complete the questionnaire in person (paper-and-pencil method) at the PHC.

## Variables and data collection

### WHOQOL-BREF

Numerous studies have shown that the assessment of the quality of life is as important as physical, laboratory or clinical findings in the CVD patient population.[15] Although current treatment strategies for patients with CVD

aim to reduce morbidity and prolong survival, integrated treatment should also focus on improving the patient's quality of life by reducing symptoms, optimizing daily living and improving overall wellbeing.[15]

With this in mind, the current study will assess variables related to the quality of life in people with CVD using a short version of the World Health Organization Quality of Life Questionnaire (WHOQOL).[45] Aspects of quality of life assessed through this questionnaire include physical status (mobility and independence), emotional status (depressive symptoms, anxiety, anger, mood swings, feelings of shame, helplessness, and future expectations), social relationships (social, sexual and family activities, marital satisfaction), economic status (income and employment), intellectual abilities (memory, ability to concentrate, ability to learn), and a self-assessment of health status (self-assessment of the severity of symptoms and degree of disability).[46,47] This questionnaire assesses the quality of life of people with CVD in 4 main domains: physical health, psychological health, social relationships, and environment. The test also includes scored questions on individual perception of quality of life (question 1) and health status (question 2). Responses are recorded on a 5-point Likert scale. The reliability of the Polish version of WHOQOL-BREF was checked using the Cronbach's α coefficient, which was 0.81 for physicality, 0.78 for psychology, 0.69 for social relationships, and 0.77 for the environment. The internal consistency for the whole questionnaire was 0.90.[46,47]

## Health Behavior Inventory questionnaire

Another critical issue is the health-promoting behaviors of CVD patients, which are considered an essential component of CVD prevention.[14] Assessing the degree of health-promoting behaviors allows for the identification of CVD patients who may need stimulation of these behaviors. With this in mind, we will assess the overall level of health-promoting behaviors and 4 categories of these behaviors in people with CVD, including proper eating habits, preventive measures and appropriate health attitude practices. The measurement of proper eating habits will involve an assessment of the types of foods consumed (e.g., whole grain breads, vegetables and fruits). Statements describing preventative behaviors will refer to following health recommendations and obtaining information about health and disease. Assessed healthy habits will include physical activity, and daily sleep and rest habits. The positive mental attitude category will assess the extent of avoidance of excessive emotions, tension and stress, depressing situations, and positive perceptions.[48]

The Juczyński Health Behavior Inventory (HBI) questionnaire will be used to assess the level of health-seeking behaviors in CVD patients. This questionnaire consists of 24 statements that consider the categories outlined above. Respondents rate each statement on a scale from 1 to 5, where 1 represents almost never, 2 represents rarely, 3 represents sometimes, 4 represents often, and 5 represents most often/almost always. The scores are then added to calculate the overall intensity of health activities, ranging from 24 to 120 points. The higher the score, the higher the intensity of health-promoting behaviors. In addition, the intensity of each category is assessed separately.[48]

## Camberwell Assessment of Need Short Appraisal Schedule

The poor performance of many existing integration models may be due to a lack of or inadequate assessment of patient needs and preferences.[6] Therefore, the modified Camberwell Assessment of Need (CAN), which focuses on 22 problem areas (Table 1) and can be used with chronically ill patients, will be utilized for this study.[35] A comprehensive identification of needs allows integrated care to identify the problem and take appropriate action to eliminate or reduce the need, including in the social dimension.[15]

Table 1. CAN problem areas[40]

| CAN problem areas |
|---|
| 1. Housing conditions |
| 2. Ability to prepare meals and do the shopping themselves |
| 3. Ability to take care of the home |
| 4. Self-care – ability to maintain hygiene |
| 5. Daily activities |
| 6. Physical health |
| 7. Mental health |
| 8. Health information and treatment |
| 9. Psychological stress |
| 10. Drinking alcohol and related problems (health, work, family) |
| 11. Drug use |
| 12. Taking medication not recommended by a doctor |
| 13. Social contacts |
| 14. Need for intimate relationships |
| 15. Satisfaction with intimate relationships |
| 16. Satisfaction with sex life |
| 17. Need for children |
| 18. Satisfaction with relationships with children |
| 19. Ability to communicate by telephone |
| 20. Ability to use public transport |
| 21. Ability to manage own money |
| 22. Receiving all money due (benefits) |

CAN – Camberwell Assessment of Need.

For the current study, the Camberwell Needs Index will be calculated. This calculation involves determining the number (N) of satisfied (1) and unmet (0) needs of the patient based on 24 questions. In turn, the number (M) of satisfied needs (1) is determined within the number (N) of needs indicated by the respondent. The M/N formula is used to calculate the Camberwell Index. The above method also allows for a calculation of the Camberwell Index for unmet needs according to the formula: 1 – M/N. The consistency of the modified version of the CAN questionnaire was 0.82 (Cronbach's α).[35]

### Hospital Anxiety and Depression Scale-Modified Version

Studies have shown that the prevalence of anxiety disorders and depression in patients with CVD is twice as high as in the general population and leads to a worse medical prognosis for these patients. Moreover, anxiety and depression symptoms reduce the motivation to change one's lifestyle and seek social interaction, and can cause patients to even psychologically disengage from central problems. Therefore, anxiety and depressive disorders should be recognized as early as possible and treated accordingly. Patients with anxiety and depression without social support whose symptoms do not progress may have an unfavorable attitude toward the disease and a sense of helplessness.[14]

Given this, in the context of examining integrated care in the outpatient setting, it is crucial to carefully determine the epidemiology of anxiety and depressive disorders and their risk factors in patients with CVD. A modified version of the Hospital Anxiety and Depression Scale (HADS) questionnaire, the HADS-M, will be used to assess anxiety and depression. This instrument uses 7 items to measure anxiety, 7 items to evaluate the level of depression and 2 items to measure nervousness and aggression. The questionnaire is helpful in assessing anxiety, depression and aggression in both inpatients and outpatients. It contains 16 test questions scored from 0 to 3. The test score is the sum of all scores in each category. The maximum score for anxiety and depression is 21 and for aggression it is 6. The subscales for anxiety and depression are interpreted as follows: scores of 0–7 correspond to normal behavior, 8–10 are borderline and indicate mild anxiety, and 11–21 are pathological and indicate an anxiety syndrome/disorder. Validation studies of the original and modified versions of the HADS scale demonstrate its reliability and accuracy.[49,50]

### Self-Description Questionnaire

Studies have shown that higher levels of religiosity and spirituality may be associated with a better quality of life in CVD patients.[36] Moreover, further research in this area is suggested.[12] With this in mind, we decided to investigate this aspect and use it in the context of integrated care. The instrument used to examine the level of spirituality in CVD patients is the Self-Description Questionnaire by Heszen-Niejodek, Gruszczyńska and Metlak. It consists of 20 statements that the respondent is asked to rate on a 5-point scale. The instrument contains 3 subscales with a Cronbach's α between 0.90 and 0.81: religiosity – 7 statements, ethical sensitivity – 7 statements, and harmony – 6 statements. The reliability of the questionnaire, differentiated by gender, age and education was 0.88 for the total index, 0.94 for religious attitudes, 0.77 for ethical sensitivity, and 0.80 for harmony. The results are obtained by summing the scale choices within each factor and, finally, the total index of spirituality is obtained from the sum of the sub-factors.[51]

### Authors' self-prepared questionnaire

A self-prepared interview questionnaire will also be used to collect data on relevant sociodemographic characteristics (gender, age, marital status, educational level, financial circumstances, place of residence) and the number and type of healthcare services provided (number of hospitalizations in the last 3 years, number of visits to GP and cardiology clinic in the last 12 months, among others).

## Statistical analyses

For quantitative variables, the arithmetic mean, standard deviation, 1$^{st}$ quartile (Q.25%), median (Q.50%), 3$^{rd}$ quartile (Q.75%), minimum, and maximum will be calculated. For nominal variables, the frequency (i.e., percentage) will be determined. The normality of the distribution of the variables will be checked using the Shapiro–Wilk test. For numerical variables with a normal distribution, the Student's t-test and Pearson's correlation will be used. For numerical variables that deviate from a normal distribution, the Wilcoxon test and Spearman's rank correlation will be used. Relationships between categorical variables will be analyzed using the $\chi^2$ test or Fisher's exact test (when the subclass size is small). An $\alpha$ value of $p \leq 0.05$ will be considered statistically significant.

# Analysis of the obtained results

The main results obtained in this study will relate to indicators of healthcare effectiveness, namely the quality of life, health behaviors, and health and social needs. To identify variables specific to health behaviors, ambulatory care, quality of life, and needs assessment will be compared between CVD patients under the supervision of a GP (group A) and those under the care of a cardiologist (group B).

Several additional analyses will be included to flesh out indicators favoring integration of care (both horizontally and vertically), including whether there is an association between CVD patients' health-related quality of life and their health behaviors, needs, levels of religiosity and spirituality, expectations, and whether there are variables affecting anxiety and depressive symptoms in this patient group. Groups A and B will be compared with each other in regard to the number of GP visits and cardiology outpatient clinic visits over the past year. Additional tests will include any relevant variables, such as, for example, comorbidities and length of hospitalization for CVD in the previous 12 months.

Based on data on higher levels of healthcare effectiveness (understood as a synergy of high quality of life, intensification of health behaviors and high levels of needs met), we will select a group of patients from those who we believe will most likely experience poorer healthcare

effectiveness. The analysis of the results will allow us to isolate the variables that determine the greatest extent of the improvement of healthcare efficiency in people with CVD, including variables that favor the horizontal and vertical integration of the planned interventions. The results obtained in this study will also be compared with those obtained in earlier studies on healthcare effectiveness in CVD patients.[12–15]

## Advantages and limitations of the study

The strength of this study is that it will undoubtedly be the first to identify indicators of healthcare effectiveness favoring the horizontal and vertical integration of planned interventions in Polish CVD patients. Furthermore, the obtained results can be used in the future, allowing for the monitoring of changes regarding the efficiency of care within integrated care (nationally and internationally). The study will also have the advantage of being conducted in different groups (patients under the supervision of a GP and patients under the care of a cardiologist), increasing the study's reliability and objectivity. A limitation of the study is that the sample size is relatively small and the number of centers from which patients will be drawn is too few. In the future, it is recommended to conduct a similar study with a greater number of patients at a larger number of centers and to compare the results obtained in Poland with those obtained at foreign research centers.

## Clinical implications

The fragmentation of healthcare services poses a significant challenge to implementing comprehensive, coordinated and continuous care.[3] This is especially important for the growing population of people with CVD, who typically have complex medical and non-medical needs. The results of our study may help overcome the barriers created by the roles and characteristics of primary and specialty healthcare, so that all providers can collaborate to provide seamless services for CVD patients. In addition, it has been suggested that taking a holistic approach is essential for integrated care.[3,6,17] The results of the present study can provide valuable information about the holistic health picture of patients with CVD. In addition, care teams and coordinators can use the findings to develop systems aimed at identifying groups at risk for lower effectiveness of care in integrated care system (both vertically and horizontally), because there is evidence that intensive follow-up, the involvement of informal caregivers and shared decision-making increase the success of transitional care in particular patient populations, such as the elderly.[52] The results of our study may also be useful in developing transitional care systems for patients with CVD. In addition, our results can be used to create online databases containing all documented patient information to support professional communication and the care process.

The results of the present study can be a valuable source of data that can be used to focus on the full spectrum of healthcare services for people with CVD, including prevention, treatment, promotion, and rehabilitation. In addition, they may be helpful in developing coordinated care plans, creating patient education programs and developing strategies to help implement systemic changes in order to recognize individuals and support families who care for patients with CVD.

Because integrated care services should be monitored, regularly evaluated and improved,[6] the results of our study may help create clinical information and decision support systems for designing personalized care models for patients with CVD. Moreover, the obtained results can provide information for the development of tools to assess the quality of care in CVD patients by analyzing their psychological, somatic, environmental, and social needs and expectations.

### ORCID iDs

Elżbieta Szlenk-Czyczerska https://orcid.org/0000-0002-8145-6768
Donata Kurpas https://orcid.org/0000-0002-6996-8920

### References

1. Organisation for Economic Co-operation and Development (OECD). *Realising the Potential of Primary Health Care*. Paris, France: Organisation for Economic Co-operation and Development (OECD); 2020. doi:10.1787/a92adee4-en
2. Kurpas D. Challenges in implementing integrated care in Central and Eastern Europe: Experience of Poland. *Int J Integr Care*. 2020;20(2):7. doi:10.5334/ijic.5533
3. He AJ, Tang VFY. Integration of health services for the elderly in Asia: A scoping review of Hong Kong, Singapore, Malaysia, Indonesia. *Health Policy*. 2021;125(3):351–362. doi:10.1016/j.healthpol.2020.12.020
4. Barrenho E, Haywood P, Kendir C, Klazinga N. *International Comparisons of the Quality and Outcomes of Integrated Care: Findings of the OECD Pilot on Stroke and Chronic Heart Failure*. Vol 142. Paris, France: Organisation for Economic Co-operation and Development (OECD); 2022. doi:10.1787/480cf8a0-en
5. Heeringa J, Mutti A, Furukawa MF, Lechner A, Maurer KA, Rich E. Horizontal and vertical integration of health care providers: A framework for understanding various provider organizational structures. *Int J Integr Care*. 2020;20(1):2. doi:10.5334/ijic.4635
6. Leithaus M, Beaulen A, De Vries E, et al. Integrated care components in transitional care models from hospital to home for frail older adults: A systematic review. *Int J Integr Care*. 2022;22(2):28. doi:10.5334/ijic.6447
7. Glendinning C. Breaking down barriers: Integrating health and care services for older people in England. *Health Policy*. 2003;65(2):139–151. doi:10.1016/S0168-8510(02)00205-1
8. Nolte E, Knai C, Hofmarcher M, et al. Overcoming fragmentation in health care: Chronic care in Austria, Germany and the Netherlands. *Health Econ Policy Law*. 2012;7(1):125–146. doi:10.1017/S1744133111000338
9. Somme D, de Stampa M. Ten years of integrated care for the older in France. *Int J Integr Care*. 2011;11(Special 10th Anniversary Edition): e141. doi:10.5334/ijic.668
10. Hébert R, Dubois MF, Raïche M, Dubuc N. The effectiveness of the PRISMA integrated service delivery network: Preliminary report on methods and baseline data. *Int J Integr Care*. 2008;8:e03. doi:10.5334/ijic.229
11. Kodner DL, Kyriacou CK. Fully integrated care for frail elderly: Two American models. *Int J Integr Care*. 2000;1:e08. doi:10.5334/ijic.11

12. Szlenk-Czyczerska E, Guzek M, Bielska DE, Ławnik A, Polański P, Kurpas D. Variables determining higher home care effectiveness in patients with chronic cardiovascular disease. *Int J Environ Res Public Health*. 2022;19(9):5170. doi:10.3390/ijerph19095170
13. Szlenk-Czyczerska E, Guzek M, Bielska DE, Ławnik A, Polański P, Kurpas D. Needs, aggravation, and degree of burnout in informal caregivers of patients with chronic cardiovascular disease. *Int J Environ Res Public Health*. 2020;17(17):6427. doi:10.3390/ijerph17176427
14. Szlenk-Czyczerska E, Guzek M, Bielska DE, Ławnik A, Polański P, Kurpas D. Factors differentiating rural and urban population in determining anxiety and depression in patients with chronic cardiovascular disease: A pilot study. *Int J Environ Res Public Health*. 2021;18(6):3231. doi:10.3390/ijerph18063231
15. Szlenk-Czyczerska E, Guzek M, Bielska DE, Ławnik A, Polański P, Kurpas D. The analysis of the relationship between the quality of life level and expectations of patients with cardiovascular diseases under the home care of primary care nurses. *Int J Environ Res Public Health*. 2022;19(6):3300. doi:10.3390/ijerph19063300
16. Organisation for Economic Co-operation and Development (OECD), European Observatory on Health Systems and Policies. *Polska: Profil systemu ochrony zdrowia 2021*. Paris, France: Organisation for Economic Co-operation and Development (OECD); 2022. doi:10.1787/b562ffe2-pl
17. Leijten FRM, Struckmann V, van Ginneken E, et al. The SELFIE framework for integrated care for multi-morbidity: Development and description. *Health Policy*. 2018;122(1):12–22. doi:10.1016/j.healthpol.2017.06.002
18. Kalininskaya A, Lazarev A, Abebo T, Kizeev M, Rassoha D. The medical social characteristics and evaluation of life quality of patients with diseases of blood circulation system [in Russian]. *Probl Sotsialnoi Gig Zdravookhranenniiai Istor Med*. 2021;29(3):456–461. doi:10.32687/0869-866X-2021-29-3-456-461
19. Szyguła-Jurkiewicz B, Kowalska M, Mościński M. Jakość życia jako element stanu zdrowia i zdrowia leczenia chorych ze schorzeniami układu sercowo-naczyniowego. *Fol Kardiol Fragment*. 2011;6(1):62–71. https://journals.viamedica.pl/folia_cardiologica/article/view/23617/18803. Accessed October 12, 2022.
20. Wnuk M, Marcinkowski J. Quality of life in chronic diseases [in Polish]. *Hygeia Publ Health*. 2013;48(3):274–278. http://www.h-ph.pl/pdf/hyg-2013/hyg-2013-3-274.pdf. Accessed October 12, 2022.
21. Cieślik B. Influence of the heart diseases on quality of life: Literature review [in Polish]. *Inż Biomed*. 2014;20(2):101–118. http://yadda.icm.edu.pl/baztech/element/bwmeta1.element.baztech-5dfaac5d-4bd2-4c59-adbb-96158c099aa2?q=bwmeta1.element.baztech-1bef df11-d585-4643-8b04-67d25eea73f4;3&qt=CHILDREN-STATELESS. Accessed October 12, 2022.
22. Szlenk-Czyczerska E, Semla W, Jenczura A. Assessment of health behaviors of patients with heart failure treated in a cardiology department. *Health Prob Civil*. 2021;15(2):79–86. doi:10.5114/hpc.2021.104625
23. Arora S, Patel P, Lahewala S, et al. Etiologies, trends, and predictors of 30-day readmission in patients with heart failure. *Am J Cardiol*. 2017;119(5):760–769. doi:10.1016/j.amjcard.2016.11.022
24. Chamberlain AM, Dunlay SM, Gerber Y, et al. Burden and timing of hospitalizations in heart failure: A community study. *Mayo Clin Proc*. 2017;92(2):184–192. doi:10.1016/j.mayocp.2016.11.009
25. Liao WC, Li CR, Lin YC, et al. Healthy behaviors and onset of functional disability in older adults: Results of a national longitudinal study. *J Am Geriatr Soc*. 2011;59(2):200–206. doi:10.1111/j.1532-5415.2010.03272.x
26. Artaud F, Dugravot A, Sabia S, Singh-Manoux A, Tzourio C, Elbaz A. Unhealthy behaviours and disability in older adults: Three-City Dijon cohort study. *BMJ*. 2013;347:f4240. doi:10.1136/bmj.f4240
27. Jacob ME, Yee LM, Diehr PH, et al. Can a healthy lifestyle compress the disabled period in older adults? *J Am Geriatr Soc*. 2016;64(10):1952–1961. doi:10.1111/jgs.14314
28. Shaw BA, Agahi N. A prospective cohort study of health behavior profiles after age 50 and mortality risk. *BMC Public Health*. 2012;12(1):803. doi:10.1186/1471-2458-12-803
29. Abbasi J. As walking movement grows, neighborhood walkability gains attention. *JAMA*. 2016;316(4):382–383. doi:10.1001/jama.2016.7755
30. Eriksson MK, Hagberg L, Lindholm L, Malmgren-Olsson EB, Österlind J, Eliasson M. Quality of life and cost-effectiveness of a 3-year trial of lifestyle intervention in primary health care. *Arch Intern Med*. 2010;170(16):1470–1479. doi:10.1001/archinternmed.2010.301
31. Saha S, Carlsson KS, Gerdtham UG, et al. Are lifestyle interventions in primary care cost-effective? An analysis based on a Markov model, differences-in-differences approach and the Swedish Björknäs Study. *PLoS One*. 2013;8(11):e80672. doi:10.1371/journal.pone.0080672
32. Fernald DH, Dickinson LM, Froshaug DB, et al. Improving multiple health risk behaviors in primary care: Lessons from the Prescription for Health COmmon Measures, Better Outcomes (COMBO) Study. *J Am Board Fam Med*. 2012;25(5):701–711. doi:10.3122/jabfm.2012.03.110057
33. Lewis KH, Gillman MW, Greaney ML, Puleo E, Bennett GG, Emmons KM. Relationships between social resources and healthful behaviors across the age spectrum. *J Aging Res*. 2012;2012:501072. doi:10.1155/2012/501072
34. Mazurek J, Rymaszewska J. Needs assessment of elderly patients based on the Camberwell Assessment of Need for the Elderly: Literature review [in Polish]. *Gerontol Pol*. 2012;20(1):1–7. https://gerontologia.org.pl/wp-content/uploads/2016/05/2012-01-1.pdf. Accessed October 15, 2022.
35. Kurpas D. *Paradygmat opieki nad chorymi przewlekle w ramach podstawowej opieki zdrowotnej*. Wrocław, Poland: Wroclaw Medical University; 2013. https://ppm.umed.wroc.pl/docstore/download/@UMW5de4d88174d34218a13ad443744c5db8/Kurpas%20Donata-wcag.pdf. Accessed October 15, 2022.
36. Abu HO, Ulbricht C, Ding E, et al. Association of religiosity and spirituality with quality of life in patients with cardiovascular disease: A systematic review. *Qual Life Res*. 2018;27(11):2777–2797. doi:10.1007/s11136-018-1906-4
37. van Groenestijn AC, Kruitwagen-van Reenen ET, Visser-Meily JMA, van den Berg LH, Schröder CD. Associations between psychological factors and health-related quality of life and global quality of life in patients with ALS: A systematic review. *Health Qual Life Outcomes*. 2016;14:107. doi:10.1186/s12955-016-0507-6
38. Yamout B, Issa Z, Herlopian A, et al. Predictors of quality of life among multiple sclerosis patients: A comprehensive analysis. *Eur J Neurol*. 2013;20(5):756–764. doi:10.1111/ene.12046
39. Paiva CE, Paiva BSR, de Castro RA, et al. A pilot study addressing the impact of religious practice on quality of life of breast cancer patients during chemotherapy. *J Relig Health*. 2013;52(1):184–193. doi:10.1007/s10943-011-9468-6
40. Gok Ugur H. The effect of home care for stroke patients and education of caregivers on the caregiver burden and quality of life. *Acta Clin Croat*. 2019;58(2):321–332. doi:10.20471/acc.2019.58.02.16
41. Leijten FRM, Struckmann V, van Ginneken E, et al. The SELFIE framework for integrated care for multi-morbidity: Development and description. *Health Policy*. 2018;122(1):12–22. doi:10.1016/j.healthpol.2017.06.002
42. Huckfeldt PJ, Reyes B, Engstrom G, et al. Evaluation of a multicomponent care transitions program for high-risk hospitalized older adults. *J Am Geriatr Soc*. 2019;67(12):2634–2642. doi:10.1111/jgs.16189
43. Sevilla-Cazes J, Ahmad FS, Bowles KH, et al. Heart failure home management challenges and reasons for readmission: A qualitative study to understand the patient's perspective. *J Gen Intern Med*. 2018;33(10):1700–1707. doi:10.1007/s11606-018-4542-3
44. Lang CC, Smith K, Wingham J, et al. A randomised controlled trial of a facilitated home-based rehabilitation intervention in patients with heart failure with preserved ejection fraction and their caregivers: The REACH-HFpEF pilot study. *BMJ Open*. 2018;8(4):e019649. doi:10.1136/bmjopen-2017-019649
45. World Health Organization (WHO). Tools and toolkits/WHOQOL/WHOQOL-BREF. https://www.who.int/tools/whoqol/whoqol-bref. Accessed October 18, 2022.
46. Wołowicka L, Jaracz K. Polska wersja WHOQOL 100 i WHOQOL BREF. In: Wołowicka L, ed. *Jakość życia w naukach medycznych*. Poznań, Poland: Dział Wydawnictw Uczelnianych Akademii Medycznej im. Karola Marcinkowskiego. ISBN:838873265X.
47. Jaracz K, Kalfoss M, Górna K, et al. Quality of life in Polish respondents: psychometric properties of the Polish WHOQOL – Bref. *Scand J Caring Sci*. 2006;20:251–260. doi:10.1111/j.1471-6712.2006.00401.x
48. Juczyński Z. *Narzędzia pomiaru w promocji i psychologii zdrowia*. 2nd ed. Warszawa, Poland: Pracownia Testów Psychologicznych; 2012. ISBN:9788360733431.

49. Stachowiak-Andrysiak M, Stelcer B, Mikstacki A, Kuliński D, Tamowicz B. Assessment of mental well-being of patients with chronic kidney disease (CKD) and their adaptation to stress caused by the disease [in Polish]. *Nowiny Lek*. 2012;81(6):636–640. https://jms.ump.edu.pl/uploads/2012/6/636_6_81_2012.pdf. Accessed November 15, 2022.
50. Kozera E. Zależność między akceptacją choroby a lękiem i depresją u pacjentek z nowotworem gruczołu piersiowego. *Współcz Pielęg Ochr Zdr*. 2015;4:85–88.
51. Heszen-Niejodek I, Gruszczyńska E. Spirituality as a human dimension, its importance in health psychology, and its measurement [in Polish]. *Przegl Psychol*. 2004;47(1):15–31. https://www.kul.pl/files/714/media/1.47.2004.art.1.pdf..pdf. Accessed November 16, 2022.
52. Shakib S, Dundon BK, Maddison J, et al. Effect of a multidisciplinary outpatient model of care on health outcomes in older patients with multimorbidity: A retrospective case control study. *PLoS One*. 2016;11(8):e0161382. doi:10.1371/journal.pone.0161382

# Assessment and therapeutic management of acute asthma: The approaches of nursing staff in patient care

*Dan Sun[1,B,D], *Ping Sun[2,A,C], Zhengyan Wang[3,E,F]

[1] Department of Hepatology, Yantai Qishan Hospital, China
[2] Department of Nursing, Yantai Qishan Hospital, China
[3] Endoscopic Center, The Fourth Hospital of Baotou, China

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1167–1178

**Address for correspondence**
Zhengyan Wang
E-mail: zhengyanwang01@outlook.com

**Funding sources**
None declared

**Conflict of interest**
None declared

*Dan Sun and Ping Sun contributed equally to this work.

Received on June 13, 2022
Reviewed on December 21, 2022
Accepted on February 12, 2023

Published online on May 4, 2023

## Abstract

Acute severe asthma describes serious asthmatic attacks, which remain a major treatment challenge and a significant source of morbidity in adults. It places the patient in danger of developing respiratory failure, a condition known as status asthmaticus. It is often fatal if not recognized and treated early. Many patients are at risk for numerous reasons; thus, the key issues are early detection, assessment and management. A multidisciplinary and collaborative approach is needed to effectively treat acute respiratory failure (ARF). Considerable research has investigated the range of opportunities available for treating asthma. Current treatment options include conventional agents, such as inhalational corticosteroids, β-agonists, leukotriene modulators, monoclonal antibodies, and oral corticosteroids (OCS). Nurses are in a perfect position to assess patients' risk of developing respiratory failure, monitor them, evaluate their care, and coordinate a multidisciplinary approach. In this review, we discuss acute asthma and the role of the nursing officer (NO) in the management of the illness. The review will also emphasize various current treatment approaches available for the NO that can effectively target and prevent respiratory failure. This review provides nurses and other healthcare workers with updated information on timely, effective and safe supportive management of patients with asthma.

**Key words:** assessment, drugs, nursing officer, status asthmaticus, therapeutic management

**Cite as**
Sun D, Sun P, Wang Z. Assessment and therapeutic management of acute asthma: The approaches of nursing staff in patient care. *Adv Clin Exp Med*. 2023;32(10):1167–1178. doi:10.17219/acem/161156

**DOI**
10.17219/acem/161156

## Introduction

Asthma is a common and serious inflammatory illness of the airways characterized by fluctuating expiratory flow, respiratory symptoms and exacerbations (flare-ups) that can lead to hospitalization and even death.[1] In contrast to other respiratory conditions, airway obstruction in asthma is usually reversible.[2] An exacerbation is a change in the patient's prior state, which involves diminished respiratory function and a gradual rise in other related symptoms associated with an asthma episode. Asthma has significant morbidity, mortality and financial impact. It is generally a chronic condition; understanding the typical development and recurrence of symptoms in the past is critical.[3] The history of current illness and complications show a recurrent pattern of wheezing, chest tightness, dyspnea, and cough. Symptoms of asthma are mostly aggravated during nighttime, after the awakening or due to the exposure to various asthma triggers (Fig. 1). Asthma is a complicated genetic condition according to both family-based and twin research. A variety of genetic and environmental variables influence how the condition manifests clinically, including the phenotypes of bronchial hyperresponsiveness, atopy and elevated immunoglobin E (IgE). Most experts agree that gene–environment interactions play a role in the development of asthma. Several well-known risk factors for developing asthma include pollution, obesity, microbial exposure, occupation, nutritional factors, genetic predisposition, and stress.[4,5]

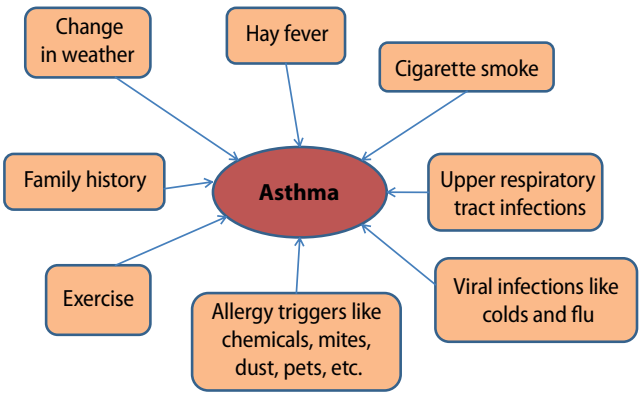



Fig. 1. Common triggers of asthma

Airway inflammation is the primary issue in asthma. Multiple inflammatory cells, mediators and tissues in the airways interact intricately to trigger asthma (Fig. 2). Most cases of asthma that become clinically noticeable are treated either outside hospitals or in emergency departments (EDs). Asthma exacerbations can also be severe enough to require hospital admission. Approximately 25,000–50,000 of the 2 million adult asthma patients treated in EDs require intensive care unit (ICU) treatment; however, the majority do not require intubation or mechanical ventilation.[6]

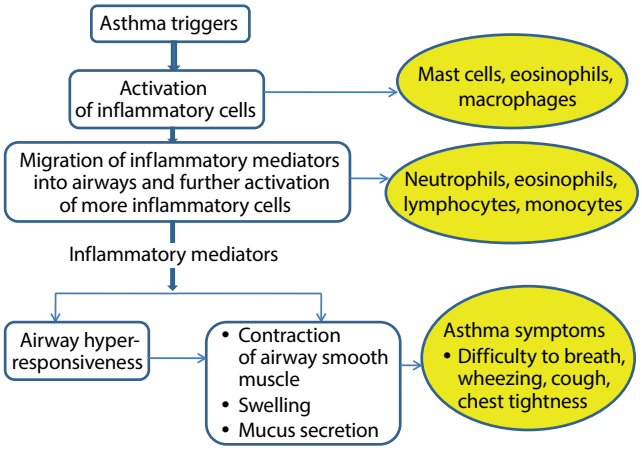



Fig. 2. Various inflammatory mediators, tissues and cell interactions in the airways

## Objectives

This study aimed to provide a report on the clinical approaches and therapeutic management of nursing interventions in alleviating acute asthma. This review also gathered information regarding various pharmacotherapeutic agents that have been used to control asthma episodes. Moreover, the article focuses on the role of nursing officers (NOs) in the management of asthma.

## Methodology

Data for this review were collected from different electronic scientific databases, including PubMed, Scopus, Science Direct, and Google Scholar. Various recent research and review papers were studied in order to gain insight into the therapeutic management of asthma and the role of NOs regarding various aspects of acute asthma management, such as intensive care providers and educators in self-management, as well as inhaler use during an asthmatic episode. After rigorous and thorough study, we identified significant information regarding various triggers, assessments, therapeutic agents, and role of NOs in the management of asthma. The gathered data were divided into several sections in accordance with the paper's objective. To find relevant articles, various keywords, including "nursing officer", "acute asthma", "status asthmaticus", "respiratory failure", "drugs", and "asthma management" were used. Of all 94 studies, 25 focused on the introduction, assessment, diagnosis, and benefits of NOs as educators. Forty-five studies presented a brief introduction to the management of asthma, 7 studies highlighted the role of NOs in the ICU and in asthma self-management, and 17 studies investigated the role of NOs in inhaler education. The methodology adopted for conducting the structured literature review is presented in Fig. 3.

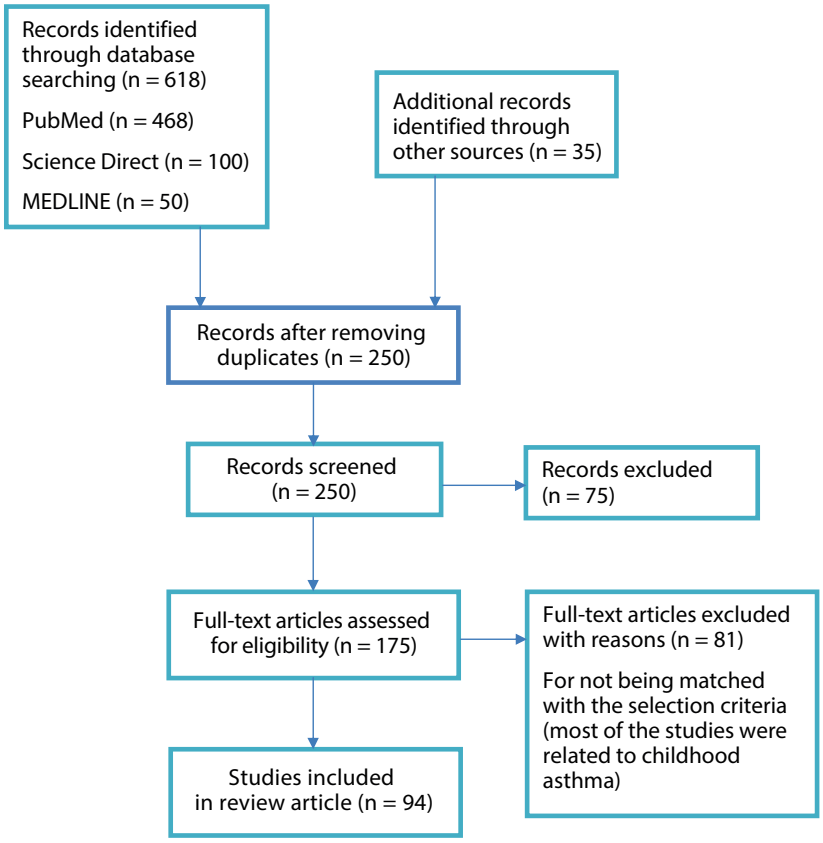



Fig. 3. Methodology adopted for conducting the review

## Assessment

The Global Initiative for Asthma (GINA) has established various guidelines for assessing, preventing and managing asthma attacks.[7] According to these recommendations, a patient with well-controlled asthma should not have any activity restrictions, no nocturnal or early-morning symptoms, and only sometimes require relief medication (less than twice per week). Inquiries on symptom frequency and intensity, exacerbation episodes, oral corticosteroid usage, and emergency room visits should be straightforward and precise. The Asthma Control Test (ACT) (QualityMetric/GlaxoSmithKline) and Asthma Control Questionnaire (www.qoltech.co.uk/acq.html) are validated questionnaires that are readily available and easy for patients to complete. They offer a useful framework that is objective and can be utilized to gauge therapy response over time.[8] Asthma control refers to the extent to which asthma symptoms in a patient are observed, have decreased or been eradicated as a result of treatment.[8] Before starting treatment, nurses must assess whether the patient's asthma is controlled (Fig. 4).

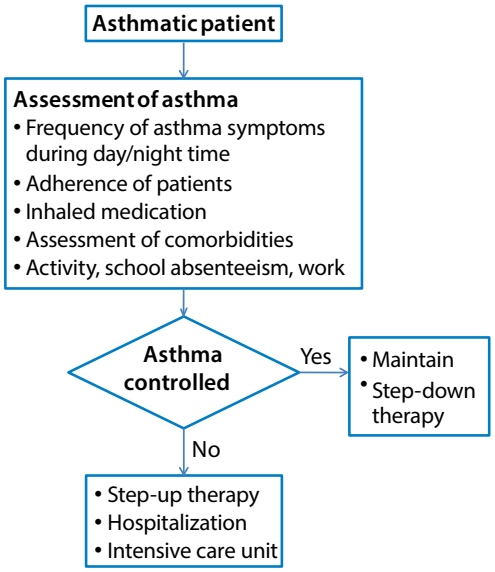



Fig. 4. Assessment of asthma

## Diagnosis of asthma

In order to accurately diagnose asthma in teenagers and determine the severity of their condition, it is necessary to combine rigorous history-taking, clinical examination, objective measurements of airway obstruction, airway hyperresponsiveness, and atopy. Basic diagnostic assessments include clinical history and examination, lung function tests (spirometry), exhaled nitric oxide tests (to assess airway inflammation), assessments of airway hyperresponsiveness, sputum eosinophil levels, blood eosinophil levels, and chest X-rays.[9]

Spirometry may be the most accessible test that can be performed to confirm an asthma diagnosis. Asthma

is consistent with an obstructive picture, a forced expiratory volume in 1 s/forced vital capacity ($FEV_1$/FVC) ratio of less than 70%, and related reversibility after bronchodilator treatment. This test is included as a "must perform" objective test in the National Institute for Health and Care Excellence (NICE) recommendations, which were released in the UK for all patients over the age of 5 years with a probable diagnosis of asthma (https://www.nice.org.uk/guidance/ng80). All individuals aged ≥17 years should undergo a bronchodilator reversibility test if an obstructive spirometry result is present; an improvement of ≥12% in $FEV_1$ and an elevation of ≥200 mL is considered positive of reversible airflow obstruction. However, one of the main problems with using spirometry to diagnose asthma is that it must always be used and interpreted correctly.[10] If this is not accomplished, there is a significant risk of both underdiagnosis and overdiagnosis.[11] Lack of consistency in terms of the definitions for the lower limit of "normal" can cause the interpretation of spirometry findings to vary even among specialized lung function laboratories.[12] The fact that the values may be normal when measured in the clinic during stable illness has an impact on how spirometry results are interpreted in the context of an asthma diagnosis. Adults who are examined in primary care often have minimal illness and well-preserved lung function.[13]

The exhaled nitric oxide test is another diagnostic test for asthma. This test is used to assess airway inflammation in asthma patients. Exhaled air can be used to track elevations in nitric oxide caused by inflammatory cytokines in the central and peripheral airways. This test for an increased percentage of exhaled nitric oxide (FeNO) reflects relatively good steroid responsiveness and possibly eosinophilic-mediated inflammatory pathways in asthma.[14] The FeNO testing is considered a supplement to measurements of lung function as an indirect marker of airway inflammation, since the core pathophysiology underlying asthma includes eosinophilic airway inflammation as well as reversible airflow restriction. However, other variables, such as atopy, smoking and current steroid therapy, affect FeNO assessment. As a result, although considered helpful in assisting with an asthma diagnosis, it is clear that its clinical value and accuracy are highest for individuals who have never used steroids and who do not smoke. It is important to keep in mind that even though a high level is supportive of an asthma diagnosis, a level below 40 ppb does not eliminate the possibility of asthma because of the unpredictability of FeNO.[15]

Currently, measuring airway eosinophils is not necessary to diagnose mild to moderate asthma. Patients who are believed to have severe illness are more likely to benefit from induced sputum inflammation.[16] The time and skill needed for the induction, processing and analysis of the sample make the use of sputum eosinophil levels to diagnose asthma difficult to put into practice. This is why it is preferable to utilize less intrusive biomarkers that might indicate airway eosinophilia.[17] Since a blood test is easier and can be conducted frequently in all clinical settings, it is more appealing to use peripheral eosinophils to diagnose asthma in both adults and children compared to airway eosinophils.[18]

For the usual evaluation or diagnosis of asthma, chest X-rays are not advised. An X-ray of the chest is often unremarkable or may only show hyperinflation, and is only advised when other illnesses beyond asthma, such as cardiovascular disorders in older persons, are suspected.[19]

## The benefits of nursing officers as educators

Being on the front lines of patient care, NOs have a unique opportunity to examine patients at risk and provide them with additional care and knowledge.[20] Nursing officers have been recognized as important care providers and educators in the supervision of chronic illnesses, including asthma.[21] The management of chronic illnesses is in the hands of nurses, who are frequently primary caregivers. In the majority of care programs for patients with asthma, both expert and nonspecialist NOs play a critical role in the delivery of care at the primary, secondary and tertiary levels. In certain contexts, prescribing nurses also make treatment choices and adjustments.[21] According to a cross-sectional study, more than half of the patients favored nurse practitioners over general practitioners for the educational aspects of their treatment and were happy with the NO for the supportive components of care for themselves and their families.[22] On the other hand, the patients preferred that their physician handle the medical aspects of their care. According to a qualitative interview study, specialist nurses' input aided practice nurses in identifying, monitoring and auditing the care of high-risk asthma patients.[23] Pediatric NOs have been found to be good care supervisors and educators in the inpatient setting. These results highlight the positive skill set provided when NOs and physicians collaborate for the benefit of their patients, demonstrating that this strategy may better address the desires of patients than the care provided by a physician alone. Consistent asthma management necessitates strong cooperation between the patient and the NO.[24] The NO can forge an active partnership with patients by fostering open communication, identifying and addressing patient and family concerns about asthma and asthma treatment, creating treatment objectives, choosing medications in conjunction with the patient, the patient's family and the patient's doctor, and promoting self-monitoring and treatment. It has been proven that self-management education in particular improves the status and quality of life of asthma patients.[25] A hospital-based investigation revealed that asthma-related discussions, specifically with an expert NO, increased patient self-management activities, which resulted in fewer symptoms, better lung function and fewer missed workdays.[24]

## Therapeutic management

The major goals in treating asthma patients are to alleviate symptoms, prevent exacerbations and allow them to continue their daily activities.[26] Nurses play a critical role in managing the course of asthma, and they should broaden their knowledge and abilities in order to provide appropriate treatment advice. Nurses' responsibilities include diagnosing asthma, managing acute exacerbations, supervising self-management programs, discontinuing therapy, adjusting medication dosage, and educating patients on inhaler technique.[27] The GINA established a number of guidelines for the diagnosis, prevention and treatment of asthma. Asthma control refers to how well a patient's asthma symptoms are controlled and whether they are visible or have been reduced or eliminated as a result of treatment.

The principal aim of asthma management is to control the disease (Fig. 5). By initiating treatment at the level most likely to produce peak flow, the stepwise strategy seeks to eliminate symptoms as quickly as possible. Patients should begin the treatment at the step that corresponds to the severity of their asthma. The goal is to achieve early control and maintain it by increasing medications as needed and decreasing treatment when control is satisfactory.[28] The most common classes of medications used to treat asthma are discussed below.

### $\beta_2$-adrenergic receptor agonists

Bronchodilators, especially short-acting beta agonists (SABAs), such as salbutamol (albuterol), terbutaline, levalbuterol, and pirbuterol, are the first-line medications and the cornerstone of acute asthma medications. The SABAs should be given repeatedly or continuously to people with acute severe asthma. The $\beta_2$-adrenoreceptors ($\beta_2$-ARs), which are predominantly found on smooth airway muscle cells but also on certain airway cells, including inflammatory cells, are activated by these substances. Their most distinguishing feature is that they begin to work quickly while still being easily tolerated, even at large doses. The $\beta_2$-AR agonists have been known for decades, but there is still much that can be done to improve their selectivity, so that they can have the desired impact with fewer side effects. All current asthma recommendations support SABAs as first-line therapy for acute severe asthma. Patients are encouraged to optimize their use as required in the early stages of increasing therapy during an exacerbation. Additionally, in the primary care environment and in the emergency room, it is recommended that SABAs be repeatedly provided by inhalation. In studies comparing the efficacy of nebulizers compared to metered-dose inhalers (MDIs), nebulized administration was shown to be ineffective. In a recent study, nebulized delivery had no effect on hospital admission, ED length of stay or pulmonary function.[29] The GINA 2021 states that a pressurized MDI (pMDI) with a spacer is the preferred mode of administration, with solid evidence (evidence A).[30] When discussing severe and nearly deadly cases of asthma, this evidence becomes less compelling. Several trials and meta-analyses have been unsuccessful in providing significant research in favor of continuous use of nebulized SABAs for acute asthma, despite the fact that it was initially a highly promising approach. There was no change in respiratory function evaluated in the first hours of administration or the risk of hospital admissions, according to a systematic review and meta-analysis.[31] A Cochrane systematic review on the subject, which included a few more studies, found a remarkable variation equally in respiratory function and hospital admissions in favor of long-term use of SABAs, as well as demonstrated a high level of tolerance among patients who had no adverse effects with this

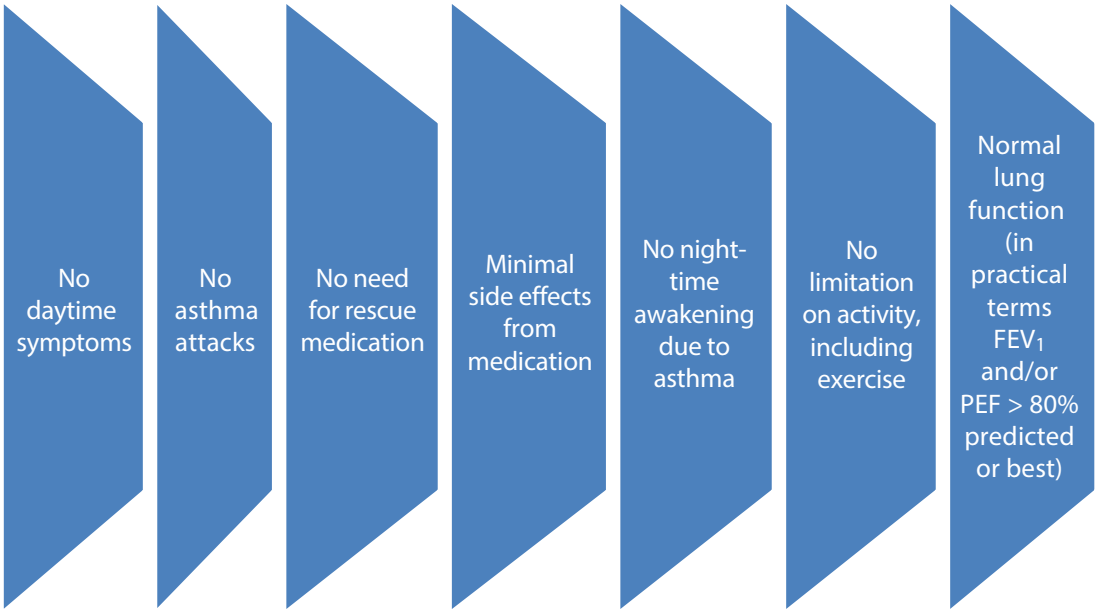



**Fig. 5.** The aims of asthma control

$FEV_1$ – forced expiratory volume in 1 s; PEF – peak expiratory flow.

technique of administration.[32] Salbutamol (i.e., albuterol) is the most often prescribed SABA, with an onset of action of less than 10 min and a duration of approx. 6 h. Levalbuterol is a relatively new addition to the list of SABAs, having the advantage of a lower dose that has a similar effect to salbutamol. There is currently no confirmation of its potential in treating acute severe asthma as an intermittent regimen; however, there is evidence of its usefulness as a continuous nebulization method.[33,34] Another alternative for treatment, particularly for those who did not benefit from considerable bronchodilation, has been continuous intravenous infusions of $\beta_2$-agonists. The use of intravenous $\beta_2$-agonists[35,36] or the technique of continuous subcutaneous infusions of terbutaline is not supported by any data.[37] Epinephrine has been considered in subcutaneous, nebulized, intravenous, and intramuscular forms, but its usage is now limited to acute asthma, according to current guidelines.

## Anticholinergics

Anticholinergic drugs block acetylcholine from binding to the muscarinic cholinergic receptor. As a result, they suppress parasympathetic nerve impulses and cause airway smooth muscle relaxation, which is advantageous in acute asthma. Furthermore, they prolong the bronchodilator action of $\beta_2$-agonist-mediated bronchodilation through intracellular mechanisms.[38] Ipratropium bromide is the most commonly used anticholinergic drug because of its specificity for airway smooth muscle receptors, which minimizes systemic side effects. According to current recommendations, patients with moderate to severe acute and critical asthma, as well as those who did not respond well to SABA therapy, should use anticholinergics.[39] However, they are not suggested as the sole treatment for acute asthma. Adding inhaled ipratropium bromide to SABA treatment has been proven to improve hospitalization rates and lung function while reducing relapse rates.[40,41] The benefit of combination treatment is stronger for people with acute asthma who are at greater risk of hospitalization. A rise in the number of mild adverse effects, such as tremor and mouth dryness, has been observed.

## Corticosteroids

Inhaled corticosteroids (ICS) have been shown to decrease the rates of hospitalization and mortality in asthma patients.[42] Beclomethasone, prednisone, fluticasone, mometasone, and formoterol are some of the commonly used ICS. Current recommendations suggest that large dosages of ICS administered during the first hour of a patient's presentation to the ED lower the probability of hospital admissions for patients who are not receiving systemic corticosteroid therapy.[30] Recent research concerning the rate of hospitalization or changes in lung function without the use of systemic corticosteroids appears to be contradictory.[43] Owing to their potent anti-inflammatory effects, systemic corticosteroids play a vital function in the treatment of acute asthma, mainly in patients who are experiencing exacerbations while taking oral corticosteroids (OCS) or those having a history of exacerbations requiring OCS. They are also indicated for people who have not had a long-term response to SABA therapy. They appear to control the activity and migration of eosinophils and other inflammatory cells, as well as increase the number and sensitivity of β-ARs, in addition to their role in preventing asthma-related inflammation. On the other hand, their lack of bronchodilatory effects precludes their usage as monotherapy for acute asthma.[44] Despite the unambiguous recommendations of current guidelines, a recent multicenter study found that a significant number of patients who are admitted to hospitals do not receive systemic corticosteroids.[45] In terms of the route of administration, intravenous injection does not appear to be more effective than oral medication.[46] In reducing the risk of recurrence, intramuscular regimens are equally effective as oral ones.[47] Because it is faster and less expensive, the oral route is more accepted and favored. Intravenous administration must be explored for individuals who are not able to swallow because of dyspnea, or who cannot absorb medication adequately due to gastrointestinal problems, such as vomiting, according to guidelines.[30]

## Magnesium sulfate

Magnesium sulfate (MS) has been found to be a key cofactor in enzymatic activities, and alterations in its content can cause smooth muscles to respond differently. Hypomagnesemia can produce smooth muscle contraction, whereas hypermagnesemia can cause smooth muscle relaxation and bronchodilation, presumably by inhibiting the influx of calcium into the muscles. Magnesium sulfate has recently been suggested as a second-line therapy for acute severe asthma exacerbations at a dosage of 2 g over 20 min.[48] It has been demonstrated to lower the hospitalization rate in patients with an $FEV_1$ of 25–30% upon presentation, as well as in those who are refractory to early treatment. It is also correlated with improved lung function.[49] Its administration has not been linked to serious side effects; however, it is contraindicated in patients with hypermagnesemia and renal insufficiency. Magnesium sulfate has also been tested in a nebulized form for cases of asthma exacerbation, although there is little evidence to support it. A recent systematic evaluation of the effectiveness and safety of magnesium inhalation found that while safe, it does not provide remarkable benefits when evaluated in comparison to first-line inhaled medications; hence, it is not regularly recommended.[50] The existing literature is hesitant to completely support the usage of magnesium, owing to the wide range of severity in asthma episodes seen in trials, particularly in the setting of optimal first-line therapy with corticosteroids and $\beta_2$-agonists.[51]

## Methylxanthines

Aminophylline and theophylline (methylxanthines) are key therapies used for acute asthma because of their anti-inflammatory characteristics. They have been excluded from current guidelines due to their reduced safety profile, which comprises considerable harmful effects, as well as their failure to demonstrate improved results, in terms of pulmonary function or hospitalization rates, when being used for severe acute asthma.[51] However, a review and meta-analysis found data to support the effectiveness of aminophylline when used in conjunction with other bronchodilators, but additional research is required in this area.[52,53]

## Leukotriene modulators

Leukotrienes are naturally occurring inflammatory substances that tighten airway muscles and produce mucus. Leukotriene modifiers help regulate asthma by inhibiting leukotrienes. According to a previous study, these drugs improve airflow and reduce asthma symptoms.[55] Montelukast, zafirlukast and zileuton are some of the widely used leukotriene modulators. Although leukotriene receptor antagonists are used to evaluate asthma treatment, research on the efficacy of antileukotriene medications given intravenously or orally in acute asthma is limited. Zafirlukast and montelukast have been examined in cases of patients with acute asthma and have been shown to improve lung function.[54,55]

## Oxygen supply

Although severe hypoxemia is not frequently associated with asthma exacerbations, arterial $PO_2$ derangements are ordinary in acute severe asthma because of a substantial ventilation/perfusion (V/Q) mismatch. Oxygen should be delivered using a nasal cannula or mask, with a target arterial oxygen saturation of 93–95%, or to patients who do not have access to saturation monitoring.[29] Although not all guidelines agree on the desired target saturation level, various studies have revealed that in severe acute asthma, treatment with controlled low-flow oxygen administration and a target $SpO_2$ is associated with better outcomes than high-flow 100% oxygen delivery, as it has been linked to increases in $PaCO_2$ and lower peak expiratory flow (PEF) values.[56,57] Because of the pulmonary vasodilation caused by $\beta_2$-agonists, which causes an increased perfusion of poorly ventilated areas, there is some evidence that using oxygen-driven nebulization with SABAs can worsen V/Q mismatches.[58] A V/Q mismatch occurs when part of the lung receives oxygen without blood flow or blood flow without oxygen. This occurs because of an obstructed airway or obstructed blood vessels in the lung.

## Heliox

Heliox gas is composed of helium (70–80%) and oxygen (20–30%). It can be utilized to treat severe asthma exacerbations resistant to normal treatment or in individuals who have blockages in the upper airways. Heliox has a lower density than air, and its Reynolds number is lower, which means that it is less resistant to airflow in turbulent flow. This impact has the potential to reduce breathing effort and increase ventilation. Heliox will not enhance airflow in smaller airways, which are most impacted during an asthma episode, since they are frequently laminar and dependent on gas viscosity rather than density. Despite its potential advantages, some studies have recommended that it may help with acute asthma; however, no human trials have shown that it is superior to normal oxygen therapy. Heliox has not shown consistent efficacy in asthma exacerbations, either with or without intubation.[59] It has been demonstrated to be the most effective in reducing symptoms when utilized as a nebulizing gas for $\beta_2$-agonist medications. The benefits of therapy are usually visible within minutes.[60] According to another study that applied a mechanical model, using heliox as a carrier gas increased gas delivery by up to 50% for both MDIs and nebulizers. Because its efficacy is dependent on the percentage of helium present, it should not be given to patients who require $FiO_2$ levels greater than 40%.[61]

## Ketamine

Ketamine is a well-recognized medication that has been used in the USA since the 1960s. It is a dissociative anesthetic that can have a variety of effects, depending on the dosage. Ketamine can act as a strong analgesic and anesthetic drug, but also as a bronchodilator. In asthma patients undergoing rapid sequence intubation, a dosage of 1–2 mg/kg has been shown to be an inductive agent.[61] It has no sedative effects at dosages lower than this, but it can produce laryngospasm and apnea at greater doses. Its psychotropic properties make it even less popular as a recreational drug. There are no large randomized studies to investigate its effects on asthma patients. There is some indication of its bronchodilatory action, particularly in mild and moderate asthma exacerbations and at dosages lower than 1 mg/kg, but larger studies are needed to confirm its role in asthma.[62,63]

## Antibiotics

Unless the clinical evaluation and patient history suggest that there is an infection, there is no evidence to support the use of antibiotics for severe acute asthma. In a retrospective cohort analysis, it was discovered that antibiotic usage was related to longer hospital stay and greater hospital expenses in patients hospitalized with acute asthma

and receiving OCS, despite a similar probability of therapy failure.[64] In prior research conducted in the USA, 60% of patients brought to the hospital with asthma exacerbations were given antibiotics without any explanation.[65] Current recommendations advise against antibiotic use and recommend that they be used only when all treatment options have been exhausted and there is definitive evidence of infection.

### Add-on therapies

Three distinct monoclonal antibodies, omalizumab (an anti-IgE antibody), mepolizumab (an interleukin 5 (IL-5) antagonist monoclonal antibody (IgG1 kappa)), and reslizumab (an IL-5 antagonist monoclonal antibody (IgG4 kappa)), are additional treatments for uncontrolled severe asthma. Due to the need for injections and the possibility of major side effects, these medications are usually only used by experts. Some common side effects of the drugs[66,67] used in asthma treatment are mentioned in Table 1.

## Noninvasive ventilation in asthma

Noninvasive ventilation (NIV) is a well-established treatment for acute respiratory failure (ARF), congestive heart failure and immunocompromised individuals to avoid intubation. The goal of mechanical NIV is to reduce the effort required to breathe. If clinical improvement is not seen with conservative therapy, endotracheal intubation and mechanical ventilation should not be postponed. According to a new study, NIV can be used quite safely in certain patients with asthma exacerbations who are being well-monitored in order to shorten their hospital stays and avoid the difficulties that come with invasive mechanical breathing.[68,69] According to research, younger individuals with asthma exacerbations are more likely than older patients to require invasive mechanical breathing. Patients receiving NIV treatment had considerably shorter hospital stay than patients who did not receive it.[70]

## Role of nursing officers in guiding self-management of asthma

Nursing staff can help patients control their asthma on their own. Effective self-management, according to the GINA recommendations, can significantly reduce asthma mortality. Self-monitoring of symptoms, a written asthma action plan (WAAP) to detect symptoms and respond to them, as well as frequent assessment of asthma control and treatment by healthcare practitioners are considered critical components of asthma management.[71] Without any take-home booklets, patient education is ineffective; therefore, the absence of a WAAP is even less helpful. To assist in reducing symptoms, each WAAP should increase communication between helthcare professionals and patients.[72] Patients with chronic illnesses who use smartphones or tablets have reported an improvement in their quality of life as this technology can provide clearer, visual explanations.[73] Consequently, Thammasat University's Center of Excellence for Allergy, Asthma and Pulmonary Diseases created the Asthma Care Application (ACA). An asthma action plan with videos demonstrating inhaler device use, emergency numbers for ambulances, basic asthma knowledge, the ACT, a record of exacerbations, reminders for inhaler usage/medications, and future appointments are the main features of this application. In a recent study, Hirunyanitiwattana et al. discovered that, when compared to the present WAAP, ACA has a high potential for enhancing a patient's asthma knowledge and plan satisfaction.[74]

## Role of nursing officers in the management of asthma patients admitted to the ICU

The level of care required must be carefully determined when a patient having an asthma exacerbation is admitted to the hospital. Patients should be hospitalized for

**Table 1.** Drugs used in asthma – indications and side effects

| Medication | Side effects |
|---|---|
| β-agonist | headache, tremor, nausea, increased insomnia and nervousness in children, bronchospasm, pain, dizziness, cough, allergic reaction, dry mouth, fever, chills, sweating, dyspepsia |
| Inhaled corticosteroids | nasopharyngitis, pharyngitis, bronchitis, respiratory tract infection, sinusitis, influenza, oropharyngeal pain, back pain, viral gastroenteritis, abdominal pain, oropharyngeal candidiasis, dysphonia, rhinitis |
| Magnesium sulphate | respiratory failure, epigastric warmth, pain at the site of infusion, numbness and tingling |
| Methylxanthine | seizure, skeletal muscle tremors, urinary retention, tachycardia, myocardial infarction, cardiac flutter |
| Leukotriene modulators | dyspepsia, cholestatic hepatitis, urticaria, allergic granulomatous angiitis, eczema, dizziness, bronchitis, sinusitis and rarely aggressive behavior |
| Omalizumab | upper respiratory tract infection, dermatitis, pyrexia, streptococcal pharyngitis, alopecia, bronchitis, arthralgia, sinusitis, pain, otitis, pharyngitis, fatigue and nasopharyngitis |
| Mepolizumab | injection site reactions, eczema, headache, systemic allergic reactions, fatigue, systemic hypersensitivity reactions, influenza, muscle spasms, upper abdominal pain, pruritus, urinary tract infection |

examination and additional therapy if their pretreatment FEV or PEF is below 25% of anticipated or personal best, or if their post-treatment values are below 40% after ED treatment, according to recommendations. Patients should be admitted to the ICU if they have a poor response to treatment (10% increases in PEF/FEV), chronic or unresponsive hypercapnia, altered mental status, hypotension, or major concomitant diseases (myocardial ischemia, tachyarrhythmias or pneumonia). High levels of inspired oxygen, constant nebulization of β-agonists, intravenous corticosteroids, and respiratory support should all be included in the ICU management of poorly responding asthmatic patients.[75] Clinicians must be aware of the need to maintain optimal oxygenation while avoiding dehydration and hypokalemia. In contrast to patients with chronic obstructive pulmonary disease who require regulated limiting oxygen, unrestricted high quantities of oxygen (60–100%) must be provided to overcome hypoxemia. Hypokalemia is common, and fluid resuscitation as well as the use of β-agonist bronchodilators can exacerbate it. Potassium chloride infusions may be necessary on a regular basis, with careful monitoring of serum levels and continuous electrocardiogram (ECG) monitoring. On admission to the ICU, a fast assessment of previous asthma therapy should be performed to identify aspects that may be intensified or deficiencies corrected. The β-blockers, aspirin, nonsteroidal anti-inflammatory medications, and adenosine are all contraindicated in asthma.[76,77]

## Role of nursing officers in inhaler education

The mainstay of asthma treatment, inhalers, deliver inhaled medication directly to the lungs during asthma exacerbations with minimal adverse systemic effects.[78] This method can be used to deliver all of the most frequently used treatments, including corticosteroids, anticholinergic medications, and short- and long-acting $\beta_2$-agonists. The efficacy of therapy and medical outcomes, however, are contingent on a patient's ability to follow their dose regimen and use the device correctly.[79] The most popular devices on the market can be grouped into 4 categories: pMDIs, dry-powder inhalers (DPIs), nebulizers, and soft mist inhalers, each of which have its own set of benefits. There are some typical mistakes made by patients when using these products.[80,81] Preparation, preinhalation, expiration, speed and/or depth of inhalation, and postinhalation breath hold are all common faults with the majority of prescribed inhalers.[82,83] The pMDIs are the most commonly recommended devices for asthma, but they can be challenging to operate because of the high level of harmonization needed to activate the device while inhaling slowly and deeply.[84] A study in almost 3,000 patients found that more than half of those tested made at least one mistake when using their inhaler.[83] Spacers, valved holding chambers and nebulizers are commonly utilized for young children who are unable to self-manage their inhaler use. However, the practicality of these systems and convenience difficulties limit their attractiveness to a wider range of patients. Some inhalers are linked to a high number of patient mistakes. For many patients, it is difficult to breathe deeply and forcefully, which is a key requirement of the DPI procedure and has an impact on particle size, which affects deposition of the drug and its usefulness.[80] Patient mistakes can lead to poor disease control.[85] Despite advances in inhaler manufacturing and instructions, the high frequency of erroneous inhaler techniques has remained consistent over the last 40 years.[83] Care should be given in matching each unique patient with the appropriate medicine for efficient asthma management. The 3 critical components of this decision – patient, device and medication – form a triangle, and it is critical that they all work together. Each patient should be given the best prescription, be knowledgeable about how to use their device properly to enhance its efficiency, and be satisfied with using it.[80] Finding the right inhaler for a patient, whether a toddler or an adult, is difficult, and many criteria must be taken into account, including age, physical dexterity, cognitive disability, personal choice, convenience of use, inspiratory flow rate, and medication drug required.[86] The 3W-H strategy, suggested by Dekhuijzen et al., is an extremely useful way for prescribing inhalers. It evaluates 4 simple questions: Who? What? Where? How?[87] The comorbidities and inspiratory ability that may impair medication administration, as well as obtaining the correct diagnosis and determining illness severity are crucial patient factors to consider. Furthermore, the type of drug and the location in the lungs where it should have its effect must be determined. When choosing the device and delivery method to utilize for each patient, the answers to these questions can be applied. These questions should be revisited on a regular basis, as the answers could change over time, and drugs may need to be adjusted. The administration strategy for optimal inhaler choice for treatment of adult asthma patients was recently devised by a specialist panel that included a respiratory specialist, a general practitioner, a nurse, and a pharmacist. This dual-action approach includes determining a patient's inspiratory capability, which can help select a suitable device, as well as successful patient involvement, which includes observing inhaler technique closely.[88] If a patient's prescription is altered, it is important to realize that not all inhalers are created equal, and devices are not interchangeable, necessitating additional teaching.[89] Nurses are in a unique position to better supervise asthma patients due to frequent nurse/patient/caregiver communication at various phases of disease management. They can identify patients who are unsuitable for a given medication or inhaler by following the steps indicated above. Furthermore, nurse-led inhaler instructions for patients or caregivers can have a favorable impact on the decision triangle's critical "technique" part.[90]

In asthma, nurse-led patient evaluation and inhaler teaching are linked to better technique, adherence and patient confidence, with long-term effects.[91] A comprehensive review of randomized controlled trials in asthma found that nurse-led educational interventions significantly improved self-management and self-efficacy.[92] Research has found that nurse-led patient education remarkably improved inhaler proficiency and reduced noncompliance behaviors in asthma patients.[93] Such advancements should improve clinical results while also lowering morbidity and healthcare utilization. The patient's inhaler technique must be observed regularly, since poor habits and technique can develop over time, necessitating a review and adjustment of technique at each visit, and anytime a new inhaler is implemented. The guidelines also indicate that inhaler technique should be observed whenever asthma control deteriorates, particularly after an asthma episode and whenever a patient needs a check-up.[94,95]

## Limitations

This review article provides information regarding clinical approaches and therapeutic management of nursing interventions in alleviating acute asthma by studying various reviews and research articles. Due to the inability to examine electronic records, the authors were unable to include recommendations for patients given by NOs for self-management of asthma. Due to prevailing pandemic conditions, we were also unable to visit the hospital and conduct personal interviews with patients and NOs to make this article more informative.

# Conclusions

Health education by nurses can significantly improve a patient's physical activity and efficiency. Practice nurses and respiratory nurse specialists play a significant role in the care of asthma patients, and they are ideally positioned to identify and assist people who have severe asthma. To reduce the risk of disease exacerbation, the NO should counsel the patient on the techniques of eliminating allergens from their environment and educate them about stress management, as these are 2 main problems that can lead to exacerbations. Aside from OCS, a variety of specialty treatments are available exclusively through specialist asthma services; thus, prompt referral is critical for all patients with poorly controlled asthma. This ensures that patients receive the best therapy available, with guidance from clinical trials, in order to reduce the number of acute deadly and near-fatal exacerbations, enhance symptom management, and improve the patient's quality of life. The nurse monitors and controls the patient's medication intake frequency as part of their treatment responsibilities. The goal of this task is to guarantee that the therapy has the best possible results.

## ORCID iDs

Dan Sun https://orcid.org/0000-0002-5626-0886
Ping Sun https://orcid.org/0000-0002-8022-2820
Zhengyan Wang https://orcid.org/0000-0001-9418-9542

## References

1. Kostakou E, Kaniaris E, Filiou E, et al. Acute severe asthma in adolescent and adult patients: Current perspectives on assessment and management. *J Clin Med*. 2019;8(9):1283. doi:10.3390/jcm8091283
2. So JY, Albert AJ, Shenoy K. Asthma: Diagnosis and treatment. *Eur Med J*. 2018;3(4):111–121. doi:10.33590/emj/10313763
3. Wang L, Foer D, Bates DW, Boyce JA, Zhou L. Risk factors for hospitalization, intensive care, and mortality among patients with asthma and COVID-19. *J Allergy Clin Immunol*. 2020;146(4):808–812. doi:10.1016/j.jaci.2020.07.018
4. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: Is prevention possible? *Lancet*. 2015;386(9998):1075–1085. doi:10.1016/S0140-6736(15)00156-7
5. Miller RL, Grayson MH, Strothman K. Advances in asthma: New understandings of asthma's natural history, risk factors, underlying mechanisms, and clinical management. *J Allergy Clin Immunol*. 2021;148(6):1430–1441. doi:10.1016/j.jaci.2021.10.001
6. Louie S, Morrissey BM, Kenyon NJ, Albertson TE, Avdalovic M. The critically ill asthmatic: From ICU to discharge. *Clinic Rev Allerg Immunol*. 2012;43(1–2):30–44. doi:10.1007/s12016-011-8274-y
7. Global Initiative for Asthma (GINA). GINA Report, Global Strategy for Asthma Management and Prevention, 2017. 2017. https://ginasthma.org/wp-content/uploads/2019/04/wmsGINA-2017-main-report-final_V2.pdf. Accessed November 18, 2021.
8. David-Wang A, Price D, Cho SH, et al. Time for a new language for asthma control: Results from REALISE Asia. *J Asthma Allergy*. 2015;9:93–103. doi:10.2147/JAA.S82633
9. Bakritas A. Diagnostic challenges of childhood asthma. *Curr Opin Pulm Med*. 2017;23(1):27–33. doi:110.1097/MCP.0000000000000338
10. Garcia-Marcos L, Edwards J, Kennington E, et al. Priorities for future research into asthma diagnostic tools: A PAN-EU consensus exercise from the European asthma research innovation partnership (EARIP). *Clin Exp Allergy*. 2018;48(2):104–120. doi:10.1111/cea.13080
11. Gershon AS, Victor JC, Guan J, Aaron SD, To T. Pulmonary function testing in the diagnosis of asthma. *Chest*. 2012;141(5):1190–1196. doi:10.1378/chest.11-0831
12. Holt NR, Thompson BR, Miller B, Borg BM. Substantial variation exists in spirometry interpretation practices for airflow obstruction in accredited lung function laboratories across Australia and New Zealand: Variation in spirometry interpretation. *Intern Med J*. 2019;49(1):41–47. doi:10.1111/imj.14047
13. Lusuardi M, De Benedetto F, Paggiaro P, et al. A randomized controlled trial on office spirometry in asthma and COPD in standard general practice. *Chest*. 2006;129(4):844–852. doi:10.1378/chest.129.4.844
14. Guo Z, Wang Y, Xing G, Wang X. Diagnostic accuracy of fractional exhaled nitric oxide in asthma: A systematic review and meta-analysis of prospective studies. *J Asthma*. 2016;53(4):404–412. doi:10.3109/02770903.2015.1101132
15. Apter AJ. Advances in adult asthma diagnosis and treatment in 2014. *J Allergy Clin Immunol*. 2015;1;46–53. doi:10.1016/j.jaci.2014.10.050
16. Yancey SW, Keene ON, Albers FC, et al. Biomarkers for severe eosinophilic asthma. *J Allergy Clin Immunol*. 2017;140(6):1509–1518. doi:10.1016/j.jaci.2017.10.005
17. Korevaar DA, Westerhof GA, Wang J, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: A systematic review and meta-analysis. *Lancet Respir Med*. 2015;3(4):290–300. doi:10.1016/S2213-2600(15)00050-8
18. Saglani S, Menzie-Gow AN. Approaches to asthma diagnosis in children and adults. *Front Pediatr*. 2019;7:148. doi:10.3389/fped.2019.00148
19. Durham CO, Fowler T, Smith W, Sterrett J. *Nurse Pract*. 2017;42(11):16–24. doi:10.1097/01.NPR.0000525716.32405.eb
20. Panlilio AL, Foy DR, Edwards JR, et al. Blood contacts during surgical procedures. *JAMA*. 1991;265(12):1533–1537. PMID:1999903.

21. Litaker D, Mion LC, Planavsky L, Kippes C, Mehta N, Frolkis J. Physician–nurse practitioner teams in chronic disease management: The impact on costs, clinical effectiveness, and patients' perception of care. *J Interprof Care*. 2003;17(3):223–237. doi:10.1080/1356182031000122852
22. Laurant MG, Hermens RP, Braspenning JC, Akkermans RP, Sibbald B, Grol RP. An overview of patients' preference for, and satisfaction with, care provided by general practitioners and nurse practitioners. *J Clin Nurs*. 2008;17(20):2690–2698. doi:10.1111/j.1365-2702.2008.02288.x
23. Foster G, Gantley M, Feder G, Griffiths C. How do clinical nurse specialists influence primary care management of asthma? A qualitative study. *Prim Care Respir J*. 2005;14(3):154–160. doi:10.1016/j.pcrj.2005.02.004
24. Borgmeyer A, Gyr PM, Jamerson PA, Henry LD. Evaluation of the role of the pediatric nurse practitioner in an inpatient asthma program. *J Pediatr Health Care*. 2008;22(5):273–281. doi:10.1016/j.pedhc.2007.07.004
25. National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. 2007. https://www.ncbi.nlm.nih.gov/books/NBK7232/. Accessed December 20, 2021.
26. National Institute for Health and Care Excellence. Asthma. Quality standard [QS25]. 2017. https://www.nice.org.uk/guidance/qs25. Accessed December 28, 2021.
27. Peters SP, Jones CA, Haselkorn T, Mink DR, Valacer DJ, Weiss ST. Real-world Evaluation of Asthma Control and Treatment (REACT): Findings from a national Web-based survey. *J Allergy Clin Immunol*. 2007;119(6):1454–1461. doi:10.1016/j.jaci.2007.03.022
28. British Thoracic Society, Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma: A national clinical guideline. 2014. https://www.brit-thoracic.org.uk/document-library/guidelines/asthma/btssign-asthma-guideline-2014/. Accessed January 15, 2022.
29. Cates CJ, Welsh EJ, Rowe BH. Holding chambers (spacers) versus nebulisers for beta-agonist treatment of acute asthma. *Cochrane Database Syst Rev*. 2013;2013(9):CD000052. doi:10.1002/14651858.CD000052.pub3
30. Reddel HK, Bacharier LB, Bateman ED, et al. Global Initiative for Asthma Strategy 2021: Executive summary and rationale for key changes. *Am J Respir Crit Care Med*. 2022;205(1):17–35. doi:10.1164/rccm.202109-2205PP
31. Rodrigo GJ, Rodrigo C. Continuous vs intermittent β-agonists in the treatment of acute adult asthma. *Chest*. 2002;122(1):160–165. doi:10.1378/chest.122.1.160
32. Camargo CA Jr, Spooner C, Rowe BH. Continuous versus intermittent beta-agonists for acute asthma. *Cochrane Database Syst Rev*. 2003;2003(4):CD001115. doi:10.1002/14651858.CD001115
33. Wilkinson M, Bulloch B, Garcia-Filion P, Keahey L. Efficacy of racemic albuterol versus levalbuterol used as a continuous nebulization for the treatment of acute asthma exacerbations: A randomized, double-blind, clinical trial. *J Asthma*. 2011;48(2):188–193. doi:10.3109/02770903.2011.554939
34. Nowak R, Emerman C, Hanrahan JP, et al. A comparison of levalbuterol with racemic albuterol in the treatment of acute severe asthma exacerbations in adults. *Am J Emerg Med*. 2006;24(3):259–267. doi:10.1016/j.ajem.2006.01.027
35. Tobin A. Intravenous salbutamol: Too much of a good thing? *Crit Care Resusc*. 2005;7(2):119–127. PMID:16548805.
36. Travers AH, Milan SJ, Jones AP, Camargo CA Jr, Rowe BH. Addition of intravenous beta$_2$-agonists to inhaled beta$_2$-agonists for acute asthma. *Cochrane Database Syst Rev*. 2012;12:CD010179. doi:10.1002/14651858.CD010179
37. Jones GH, Scott SJ. Continuous infusions of terbutaline in asthma: A review. *J Asthma*. 2011;48(8):753–756. doi:10.3109/02770903.2011.613509
38. Vogelberg C. Anticholinergics in asthma: Are we utilizing asthma therapies effectively? *Ther Clin Risk Manag*. 2019;15:405–408. doi:10.2147/TCRM.S190363
39. Teach SJ, Gill MA, Togias A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol*. 2015;136(6):1476–1485. doi:10.1016/j.jaci.2015.09.008
40. Gottlieb M, Kuhns MJ. Do inhaled anticholinergic agents in addition to β-agonists improve outcomes in acute asthma exacerbations? *Ann Emerg Med*. 2017;70(3):421–422. doi:10.1016/j.annemergmed.2017.02.026
41. Bobrovitz N, Heneghan C, Onakpoya I, et al. Medications that reduce emergency hospital admissions: An overview of systematic reviews and prioritisation of treatments. *BMC Med*. 2018;16(1):115. doi:10.1186/s12916-018-1104-9
42. Edmonds ML, Milan SJ, Camargo CA, Pollack CV, Rowe BH. Early use of inhaled corticosteroids in the emergency department treatment of acute asthma. *Cochrane Database Syst Rev*. 2012;12(12):CD002308. doi:10.1002/14651858.CD002308.pub2
43. Craig S, Kuan WS, Kelly A, et al. Treatment and outcome of adult patients with acute asthma in emergency departments in Australasia, South East Asia and Europe: Are guidelines followed? AANZDEM/EuroDEM study. *Emerg Med Australas*. 2019;31(5):756–762. doi:10.1111/1742-6723.13242
44. Hancox RJ, Cowan JO, Flannery EM, Herbison GP, Mclachlan CR, Taylor DR. Bronchodilator tolerance and rebound bronchoconstriction during regular inhaled β-agonist treatment. *Respir Med*. 2000;94(8):767–771. doi:10.1053/rmed.2000.0820
45. Alangari A. Corticosteroids in the treatment of acute asthma. *Ann Thorac Med*. 2014;9(4):187–192. doi:10.4103/1817-1737.140120
46. Kirkland SW, Cross E, Campbell S, Villa-Roel C, Rowe BH. Intramuscular versus oral corticosteroids to reduce relapses following discharge from the emergency department for acute asthma. *Cochrane Database Syst Rev*. 2018;6(6):CD012629. doi:10.1002/14651858.CD012629.pub2
47. Normansell R, Kew KM, Mansour G. Different oral corticosteroid regimens for acute asthma. *Cochrane Database Syst Rev*. 2016;2016(5):CD011801. doi:10.1002/14651858.CD011801.pub2
48. Kew KM, Kirtchuk L, Michell CI, Griffiths B. Intravenous magnesium sulfate for treating adults with acute asthma in the emergency department. *Cochrane Database Syst Rev*. 2014;5:CD010909. doi:10.1002/14651858.CD010909
49. Knightly R, Milan SJ, Hughes R, et al. Inhaled magnesium sulfate in the treatment of acute asthma. *Cochrane Database Syst Rev*. 2017;11(11):CD003898. doi:10.1002/14651858.CD003898.pub6
50. Javor E, Grle SP. Limitations of the results from randomized clinical trials involving intravenous and nebulised magnesium sulphate in adults with severe acute asthma. *Pulm Pharmacol Ther*. 2019;55:31–37. doi:10.1016/j.pupt.2019.01.005
51. Goodacre S, Cohen J, Bradburn M, et al. The 3Mg trial: A randomised controlled trial of intravenous or nebulised magnesium sulphate versus placebo in adults with acute severe asthma. *Health Technol Assess*. 2014;18(22):1–168. doi:10.3310/hta18220
52. Nair P, Milan SJ, Rowe BH. Addition of intravenous aminophylline to inhaled beta$_2$-agonists in adults with acute asthma. *Cochrane Database Syst Rev*. 2012;12(12):CD002742. doi:10.1002/14651858.CD002742.pub2
53. Mahemuti G, Zhang H, Li J, Tieliwaerdi N, Ren L. Efficacy and side effects of intravenous theophylline in acute asthma: A systematic review and meta-analysis. *Drug Des Devel Ther*. 2018;12:99–120. doi:10.2147/DDDT.S156509
54. Silverman RA, Nowak RM, Korenblat PE, et al. Zafirlukast treatment for acute asthma. *Chest*. 2004;126(5):1480–1489. doi:10.1378/chest.126.5.1480
55. Watts K, Chavasse RJ. Leukotriene receptor antagonists in addition to usual care for acute asthma in adults and children. *Cochrane Database Syst Rev*. 2012;2012(5):CD006100. doi:10.1002/14651858.CD006100.pub2
56. Perrin K, Wijesinghe M, Healy B, et al. Randomised controlled trial of high concentration versus titrated oxygen therapy in severe exacerbations of asthma. *Thorax*. 2011;66(11):937–941. doi:10.1136/thx.2010.155259
57. Rodrigo GJ, Verde MR, Peregalli V, Rodrigo C. Effects of short-term 28% and 100% oxygen on Paco2 and peak expiratory flow rate in acute asthma. *Chest*. 2003;124(4):1312–1317. doi:10.1378/chest.124.4.1312
58. Rodrigo GJ, Castro-Rodriguez JA. Heliox-driven β2-agonists nebulization for children and adults with acute asthma: A systematic review with meta-analysis. *Ann Allergy Asthma Immunol*. 2014;112(1):29–34. doi:10.1016/j.anai.2013.09.024

59. El-Khatib MF, Jamaleddine G, Kanj N, et al. Effect of heliox- and air-driven nebulized bronchodilator therapy on lung function in patients with asthma. *Lung*. 2014;192(3):377–383. doi:10.1007/s00408-014-9570-0
60. Rodrigo GJ, Rodrigo C, Pollack CV, Rowe B. Use of helium-oxygen mixtures in the treatment of acute asthma. *Chest*. 2003;123(3):891–896. doi:10.1378/chest.123.3.891
61. Godwin HT, Fix ML, Baker O, Madsen T, Walls RM, Brown CA. Emergency department airway management for status asthmaticus with respiratory failure. *Respir Care*. 2020;65(12):1904–1907. doi:10.4187/respcare.07723
62. Agrawal A, Goyal S. Ketamine in status asthmaticus: A review. *Indian J Crit Care Med*. 2013;17(3):154–161. doi:10.4103/0972-5229.117048
63. Petite SE, Murphy JA. Systemic corticosteroid and antibiotic use in hospitalized patients with chronic obstructive pulmonary disease exacerbation. *Ann Pharmacother*. 2019;53(2):144–150. doi:10.1177/1060028018799696
64. Lindenauer PK, Stefan MS, Feemster LC, et al. Use of antibiotics among patients hospitalized for exacerbations of asthma. *JAMA Intern Med*. 2016;176(9):1397–1400. doi:10.1001/jamainternmed.2016.4050
65. Stefan MS, Nathanson BH, Lagu T, et al. Outcomes of noninvasive and invasive ventilation in patients hospitalized with asthma exacerbation. *Annals Am Thorac Soc*. 2016;13(7):1096–1104. doi:10.1513/AnnalsATS.201510-701OC
66. Sharma S, Hasmi M, Chakraborty R. Asthma medications. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2022. https://www.ncbi.nlm.nih.gov/books/NBK531455/.
67. Irazuzta JE, Chiriboga N. Magnesium sulfate infusion for acute asthma in the emergency department. *J Pediatria*. 2017;93:19–25. doi:10.1016/j.jped.2017.06.002
68. Cappiello JL, Hocker MB. Noninvasive ventilation in severe acute asthma. *Respir Care*. 2014;59(10):e149–e152. doi:10.4187/respcare.02730
69. Garner O, Ramey JS, Hanania NA. Management of life-threatening asthma. *Chest*. 2022;162(4):747–756. doi:10.1016/j.chest.2022.02.029
70. Manglani R, Landaeta M, Maldonado M, Hoge G, Basir R, Menon V. The use of non-invasive ventilation in asthma exacerbation: A two year retrospective analysis of outcomes. *J Community Hosp Int Med Perspect*. 2021;11(5):727–732. doi:10.1080/20009666.2021.1955448
71. Horak F, Doberer D, Eber E, et al. Diagnosis and management of asthma: Statement on the 2015 GINA Guidelines. *Wien Klin Wochenschr*. 2016;128:541–554. doi:10.1007/s00508-016-1019-4
72. Gillette C, Rockich-Winston N, Shepherd M, Flesher S. Children with asthma and their caregivers help improve written asthma action plans: A pilot mixed-method study. *J Asthma*. 2018;55(6):609–614. doi:10.1080/02770903.2017.1355379
73. Kim BY, Lee J. Smart devices for older adults managing chronic disease: A scoping review. *JMIR Mhealth Uhealth*. 2017;5(5):e69. doi:10.2196/mhealth.7141
74. Hirunyanitiwattana T, Pirmpanich P, Boonya-Anuchit P, Maitree T, Poachanukoon O. Effectiveness of asthma education for nurses using an asthma care application compared to written asthma action plan in Thailand. *Respirology*. 2018;23(2):5–89. doi:10.1111/resp.13419_138
75. Garrard C, Nolan K. Intensive care management of asthma. In: Webb AR, ed. *Oxford Textbook of Critical Care*. Oxford Medical Publications. Oxford, UK-New York, USA: Oxford University Press; 1999. ISBN:978-0-19-262737-7.
76. Garrard C, Denham S. Bronchodilators in intensive care. In: Webb AR, ed. *Oxford Textbook of Critical Care*. Oxford Medical Publications. Oxford, UK-New York, USA: Oxford University Press; 1999. ISBN:978-0-19-262737-7.
77. Lipworth BJ, Jackson CM, Ziyaie D, Winter JH, Dhillon PD, Clark RA. An audit of acute asthma admissions to a respiratory unit. *Health Bull (Edinb)*. 1992;50(5):389–398. PMID:1399586.
78. Usmani OS, Hickey AJ, Guranlioglu D, et al. The impact of inhaler device regimen in patients with asthma or COPD. *J Allergy Clin Immunol Pract*. 2021;9(8):3033–3040.e1. doi:10.1016/j.jaip.2021.04.024
79. Price D, Bosnic-Anticevich S, Briggs A, et al. Inhaler competence in asthma: Common errors, barriers to use and recommended solutions. *Respir Med*. 2013;107(1):37–46. doi:10.1016/j.rmed.2012.09.017
80. Crompton GK, Barnes PJ, Broeders M, et al. The need to improve inhalation technique in Europe: A report from the Aerosol Drug Management Improvement Team. *Respir Med*. 2006;100(9):1479–1494. doi:10.1016/j.rmed.2006.01.008
81. Lavorini F. The challenge of delivering therapeutic aerosols to asthma patients. *ISRN Allergy*. 2013;2013:102418. doi:10.1155/2013/102418
82. Molimard M, Raherison C, Lignot S, et al. Chronic obstructive pulmonary disease exacerbation and inhaler device handling: Real-life assessment of 2935 patients. *Eur Respir J*. 2017;49(2):1601794. doi:10.1183/13993003.01794-2016
83. Sanchis J, Gich I, Pedersen S. Systematic review of errors in inhaler use. *Chest*. 2016;150(2):394–406. doi:10.1016/j.chest.2016.03.041
84. Capstick TG, Clifton IJ. Inhaler technique and training in people with chronic obstructive pulmonary disease and asthma. *Exp Rev Respir Med*. 2012;6(1):91–103. doi:10.1586/ers.11.89
85. Melani AS, Bonavia M, Cilenti V, et al. Inhaler mishandling remains common in real life and is associated with reduced disease control. *Respir Med*. 2011;105(6):930–938. doi:10.1016/j.rmed.2011.01.005
86. Cataldo D, Hanon S, Peché RV, et al. How to choose the right inhaler using a patient-centric approach? *Adv Ther*. 2022;39(3):1149–1163. doi:10.1007/s12325-021-02034-9
87. Dekhuijzen PNR, Vincken W, Virchow JC, et al. Prescription of inhalers in asthma and COPD: Towards a rational, rapid and effective approach. *Respir Med*. 2013;107(12):1817–1821. doi:10.1016/j.rmed.2013.09.013
88. Usmani OS. Choosing the right inhaler for your asthma or COPD patient. *Ther Clin Risk Manag*. 2019;15:461–472. doi:10.2147/TCRM.S160365
89. Scullion J. The nurse practitioners' perspective on inhaler education in asthma and chronic obstructive pulmonary disease. *Can Respir J*. 2018;2018:2525319. doi:10.1155/2018/2525319
90. Spencer P, Hanania N. Optimizing safety of COPD treatments: Role of the nurse practitioner. *J Multidiscip Healthc*. 2013;6:53–63. doi:10.2147/JMDH.S35711
91. Mac Hale E, Costello RW, Cowman S. A nurse-led intervention study: Promoting compliance with Diskus Inhaler use in asthma patients. *Nurs Open*. 2014;1(1):42–52. doi:10.1002/nop2.10
92. Alsumaily MA. A systematic review of randomized controlled trials of nurse-led educational interventions for adults with asthma. *J Med Med Sci*. 2015;6(4):72–81. doi:10.14303/jmms.2015.027
93. Al-Kalaldeh M, El-Rahman MA, El-Ata A. Effectiveness of nurse-driven inhaler education on inhaler proficiency and compliance among obstructive lung disease patients: A quasi-experimental study. *Can J Nurs Res*. 2016;48(2):48–55. doi:10.1177/0844562116676119
94. National Institute for Health and Care Excellence (NICE). Asthma: Diagnosis, monitoring and chronic asthma management. NICE Guideline, No. 80. 2021. https://www.ncbi.nlm.nih.gov/books/NBK560178/. Accessed January 19, 2022.
95. Institute for Quality and Efficiency in Health Care (IQWiG). Asthma: Symptoms and diagnosis. In: *InformedHealth.Org*. Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG); 2017. https://www.ncbi.nlm.nih.gov/books/NBK279521/.

# The natural origins of cytostatic compounds used in rhabdomyosarcoma therapy

Damian Lewandowski[1,A–F], Anna Szewczyk[2,B–D,F], Justyna Radzka[1,B–D,F], Magda Dubińska-Magiera[1,A–F], Weronika Kazimierczak[1,B–D,F], Małgorzata Daczewska[1,A–F], Marta Migocka-Patrzałek[1,A–F]

[1] Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wrocław, Poland
[2] Department of Molecular and Cellular Biology, Faculty of Pharmacy, Wroclaw Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1179–1191

**Address for correspondence**
Marta Migocka-Patrzałek
E-mail: marta.migocka-patrzalek@uwr.edu.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on November 30, 2022
Reviewed on January 28, 2023
Accepted on February 12, 2023

Published online on March 15, 2023

## Abstract

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and represents a high-grade neoplasm of skeletal myoblast-like cells. About 40% of all registered soft tissue tumors are RMSs. This paper describes our current understanding of the RMS subtypes (alveolar (ARMS), embryonic (ERMS), pleomorphic (PRMS), and spindle cell/sclerosing (s/scRMS)), diagnostic methods, molecular bases, and characteristics. We also present the currently used treatment methods and the potential use of natural substances in the treatment of this type of cancer. Natural cytotoxic substances are compounds that have been the subject of numerous studies and discussions in recent years. Since anti-cancer therapies are often limited by a low therapeutic index and cancer resistance to pharmacotherapy, it is very important to search for new, effective compounds. Additionally, compounds of a natural origin are usually readily available and have a reduced cytotoxicity. Thus, the undiscovered potential of natural anti-cancer compounds makes this field of research a very important area. The introduction of model species into research examining the use of natural cytostatic therapies for RMS will allow for further assessment of the effects of these compounds on cancerous and healthy tissues.

**Key words:** rhabdomyosarcoma, natural compounds, anti-cancer therapy, muscle cells

**Cite as**
Lewandowski D, Szewczyk A, Radzka J, et al. The natural origins of cytostatic compounds used in rhabdomyosarcoma therapy. *Adv Clin Exp Med*. 2023;32(10):1179–1191. doi:10.17219/acem/161165

**DOI**
10.17219/acem/161165

# Introduction

Rhabdomyosarcomas (RMSs) are a heterogeneous group of malignant myogenous tumors. They are one of the most common soft tissue sarcomas considered a "childhood disease." Soft tissue sarcoma in children represents a large group of malignant tumors, including RMS (approx. 40%).[1–4] The importance of the problem is manifested by the establishment of global organizations that deal with rare diseases of soft tissue sarcomas, mainly in children. The 2 organizations in North America – the Intergroup Rhabdomyosarcoma Study Group (IRSG) and the Children's Oncology Group (COG) – and the International Society for Pediatric Oncology (SIOP) in Europe strive to improve and support RMS treatment.[2,5–7] They have developed a risk stratification system based on the primary localization of the tumor, pathology staging (occurrence of tumor-node-metastasis and anatomical localization), disease status after tumor resection, and histological assessment. These factors facilitate grouping cases into several subgroups, including low, standard, high, and very high-risk groups, and finally enable the selection of adequate treatment.[7–9] According to the literature, RMS can develop in children with no family cancer history. However, the incidence of RMS is higher in children with parents or siblings who have had cancer, especially at a young age. Nevertheless, RMS is not considered a hereditary disease.[10]

Four main RMS subtypes are distinguished: alveolar (ARMS), embryonic (ERMS), pleomorphic (PRMS), and spindle cell/sclerosing (s/scRMS; Table 1[9,11–31]). Based on microscopic observations, each RMS subtype is characterized by specific features. However, some researchers have suggested the use of a new molecular rather than histopathological RMS classification based on *PAX3/FOXO1* fusion oncogene expression. According to this system, RMSs can be classified as fusion-positive (FPRMS) and fusion-negative (FNRMS) tumors.[11] There are no genomic markers currently available (except *PAX/FOXO1* fusion) that can be used in RMS risk stratification. However, mutant *MYOD1* and *TP53* genes have recently been nominated as indicators of poor prognosis in FNRMS.[12]

The most frequent histological types of RMS observed in children and young people are ERMS and ARMS.[13] Changes in ERMS patients are observed in the head and neck region, and in the genitourinary system. In contrast, ARMS occurs in the large muscles of the trunk, arms and legs, and has a high capacity for invasive growth and metastasis during the early stages of the disease.[9,13,14] The PRMS is a rare tumor with aggressive behavior that occurs mainly in adulthood and very rarely in children. The most common primary sites of PRMS are the extremities, trunk wall and the genitourinary system. The PRMS may also give rise to cardiac metastasis.[15] Clinical studies have also revealed PRMS in the liver with a hepatic cyst and in the pancreas.[16,17] The s/scRMSs have a predilection for paratesticular, head, neck, and limb sites in children and adults (Table 1).[18,19]

Recent studies have revealed some genetic factors (age and biological sex) that can increase the risk for RMS. Studies have shown differences in the incidence of RMS by age, with peaks occurring during early childhood (children aged ≤4 years).[20] Additionally, a 2nd peak has been noticed during adolescence. [10] It has also been shown that RMS is predominant in males; however, this discrepancy is consistent with most other pediatric cancers.[21,22] More than half of RMS cases occur before 10 years of age, which indicates that in utero and early-life environmental exposures may play a large role in RMS etiology.[20] Martin-Giacalone et al. reviewed non-genetic factors increasing the risk of RMS, including parental age, recreational drug use by parents, prenatal diagnostic radiation, birth weight, allergies, hives, incomplete immunization, and breastfeeding for less than 12 months.[10]

Currently used cytostatics (i.e., doxorubicin, vinorelbine, vincristine, dactinomycin, or cyclophosphamide) cause numerous side effects. Therefore, it is important to find novel solutions to improve patient comfort. Natural origin compounds are shown to be as effective as standard cytostatics; however, they do not cause severe side effects. In this paper, we summarize the current knowledge on natural cytostatic compounds used in the treatment of RMS.

# Signaling pathways in RMS

The effects of RMS therapies have been investigated in numerous areas of tumor cell activity considering various targets, including receptor tyrosine kinases (RTKs) and associated downstream signaling pathways, such as the Hedgehog (HH) signaling pathway, apoptosis, DNA damage response (DDR), cell cycle regulation, fusion proteins, and epigenetic modifications.[23]

## RTK signaling

Receptor tyrosine kinases are membrane-bound proteins involved in several physiological (e.g., embryonic development and wound healing) and pathological (signal transduction to tumor cells) processes.[23,24] Since cancer cells produce and use growth factors, several RTKs, such as insulin-like growth factors (IGFs) and vascular endothelial growth factors (VEGFs), have been proposed as potential targets to treat RMS.[23,25]

The IGFs 1 and 2 and insulin play crucial roles during skeletal muscle growth and differentiation, and act through the IGF 1 receptor (IGF1R). Furthermore, they are involved in the maintenance of adult muscle homeostasis. It has been found that IGF1R and its potent ligand IGF2 are widely overexpressed in childhood sarcomas, including RMS.[26–31] The activity of IGF1R can be inhibited (via blocking its phosphorylation and downstream signaling)

Table 1. Comparison of 4 rhabdomyosarcoma subtypes: embryonic (ERMS), alveolar (ARMS), pleomorphic (PRMS), and spindle cell/sclerosing (s/scRMS)

| RMS subtype | Characteristic features | Localization of neoplastic changes | Diagnosis methods | Molecular basis | Patients' age | References |
|---|---|---|---|---|---|---|
| ERMS | composed of primitive mesenchymal cells that show variable degrees of skeletal muscle differentiation; they are moderately cellular but in the typical pattern often contain both hypo- and hypercellular areas with a loose, myxoid stroma; perivascular condensations of tumor cells in the less cellular regions are common; sheets of small, stellate, spindled, or round cells with scant or deeply eosinophilic cytoplasm and eccentric, small oval nuclei with a light chromatin pattern and inconspicuous nucleoli | head and neck region including the nasal and oral cavities, as well as the middle ear and genitourinary system | immunodetection of MyoG; genotyping; vimentin in all cells (even most primitive); actin, myoglobin, myosin, and creatine kinase M staining in more differentiated cells | trisomy of chromosome 8 and loss of heterozygosity in the 11p15 chromosome region; inactivating mutations of TP53 and CDKN2A and activating mutations of RAS family genes | adolescents | 9, 12, 18, 19, 20, 21 |
| ARMS | cells form aggregates interrupted by fibrovascular septa that resemble alveoli of the lung with numerous aggregates and small, round scant in the cytoplasm of undifferentiated cells; nuclei of the cells are round with normal, dull, chromatin structures | large muscles of the trunk, arms and legs; genitourinary system | immunodetection of MyoG (>75% tumor cells), AP2β and NOS1; genotyping; strong IHC staining | fusion in frame between undisrupted *PAX3* or *PAX7* gene on chromosome 2 or FOXO1 on chromosome 13; chromosomal translocations, including a frequent t(2;13)(q35;q14) or a variant t(1;13)(p36;q14); N-myc amplification is seen in 50% of cases (more aggressive cases) | children | 9, 12, 18, 22, 23 |
| PRMS | polymorphic, spindle-shaped, multinucleated, large cells with acidophilic cytoplasm | limbs, trunk wall, genitourinary system, cysts in the liver and pancreas, cardiac metastasis | positive immunocytochemical reaction for myoglobin, MyoD1, MyoG, fast skeletal muscle myosin, desmin; MSA and SMA | amplification of oncogenes JUN, MYC, CCND1, INT2, MDM2, and MALT; CGH reveals 8 highly amplified regions at 1p36.1-p36.2, 1p31-p32, 1q21-q31, 8q12-q21, 8q24-qter, 11q12-q13, 12q13-q14 and 18q12-q22 | adults, very rarely in children | 13, 14, 15, 18, 24, 25, 26, 27 |
| s/scRMS | cells situated in sclerotic submucosa can be ribbon-shaped; neoplastic cells are arranged into microalveoli or lobules | head, neck, limbs | immunohistochemical diagnosis demonstrates positivity for desmin and vimentin; expression of myogenin (Myf-4), MyoD1 (Myf-3), myoglobin, SMA, and MSA | PAX fusion negative; recurrent *MyoD1* mutations; specific *MDM2/HMGA2* amplification at 12q13-15; loss of 10q22, loss of chromosome Y and gain of 18; in an adult cases: gain of chromosome 11 and loss of chromosome 22 | children, adults | 11, 16, 17, 18, 28, 29, 30, 31 |

MyoG – MoD1 myogenin; MSA – muscle-specific actin; SMA – smooth muscle actin; CGH – comparative genomic in situ hybridization; IHC – immunohistochemistry.

by picropodophyllin (PPP), which is a cyclolignan isolated from *Podophyllum* species.[32,33] In vitro studies have revealed that PPP significantly blocks ERMS cell activity, especially migration and proliferation. Furthermore, RMSs increase their sensitivity to chemotherapy (e.g., vincristine and cisplatin) after PPP exposure. Additionally, the volume of the tumor is decreased after 2 weeks of PPP treatment in the human RMS xenograft model.[34] Importantly, PPP does not interfere with the insulin receptor (which is characterized by a high similarity to RTK) or RTKs.[35] Another advantage of PPP treatment is that it induces apoptosis and tumor regression, and interferes with microtubule assembly.[33,35] The main side effect of vincristine is neurotoxicity. Other side effects include a syndrome of inappropriate anti-diuretic hormone secretion, myelosuppression and alopecia.[36]

The RAS/MEK/ERK and PI3K/AKT are signaling pathways promoting transcription, cell growth, motility, metabolism, and invasion. It has been shown that in RMSs, both pathways are characterized by abnormally increased activity. Therefore, they are frequent targets in ERMS treatment using doxorubicin, irinotecan, temozolomide, vinblastine, cyclophosphamide, or topotecan.[23,35,37,38] Some in vitro studies have revealed positive effects when combining ERMS therapy with buparlisib (PI3K inhibitor), AEW541 (IGF1R inhibitor) and rapamycin (mTOR

inhibitor).[38] Similar effects have been observed for ERMS treatment with multiple PI3K/mTOR and MEK inhibitors (either trametinib or selumetinib).[39–41] The combination of mTOR inhibitors and chemotherapy seems to be promising because it is well tolerated by pediatric, adolescent and young adult patients suffering from RMS.[35,37]

## HH signaling

The HH pathway is considered a key regulator of embryonic development and plays a crucial role in the adult organism in stem cell maintenance and tissue repair/regeneration.[42] In humans, 3 HH proteins have been identified: Sonic (SHH), Indian (IHH) and Desert (DHH).[43] All of these proteins are ligands of Patched receptors (PTCH1, PTCH2). The binding between PTCH receptors and one of its ligands leads to the activation of smoothened (SMO) and prevents proteomic processing of GLI family zinc finger proteins (GLI1, GLI2 and GLI3). As a result, GLIs translocate to the nucleus, where they can act as transcription factors.[44] The HH signaling is necessary for many tumors, including ERMS, for their self-renewal and initiation. Gene mutation or deregulation results in alterations in this pathway.[44–47] Since the HH pathway is constitutively activated in ERMS, blocking this pathway seems to have therapeutic potential. The inhibition of GLI1/GLI2 by GANT-61 causes a significant reduction in cell growth in RMS xenograft models, and this effect is increased by combined therapy with temsirolimus, rapamycin or vincristine.[42,48]

## Cell cycle and DDR

As inhibition of the cell cycle and DDR affects cell viability, targeted therapies could have potential anti-RMS applications.

### Cell cycle

The cell cycle is a process strictly regulated by kinases. For example, cyclin-dependent kinases (CDKs), polo-like kinase 1 (PLK1) and Wee1 kinase are all involved in the regulation of cell cycle.[49]

The CDKs play a crucial role during the whole cell cycle, and their activation is necessary for the progression of this process. Induced by Wee1 kinase, CDK1 phosphorylation leads to G2/M phase arrest and DNA repair. Therefore, CDK inhibitors may be useful in the treatment of RMSs when combined with other therapeutic agents. Indeed, promising results for ARMS have been observed with combined therapy using CDK, IGF1R or Wee1 inhibitors. However, combined treatment with palbociclib and doxorubicin has shown antagonistic effects in ERMS cells.[50,51]

The PLK1 is involved in the G2/M phase transition. Since Wee1 kinase negatively regulates entry to the M phase, PLK1 is responsible for its phosphorylation leading to Wee1 degradation. Therefore, PLK1 inhibition can lead to mitotic arrest and induce cell death.[49] In RMS cells, a higher level of PLK1 is observed, and ERMS is sensitive to PLK1 inhibition because it is involved in the activation of PAX3/FOXO1 expression.[52–54] Interestingly, positive effects in RMS therapy have been observed with PLK1 inhibitors combined with anti-microtubule agents. Additionally, polo-box domain (a PLK1 catalytic domain) inhibitors combined with vincristine show a positive effect. However, the observed anti-cancer effects were less pronounced compared with monotherapies.[52,54]

As mentioned above, Wee1 kinase is responsible for CDK1 phosphorylation leading to DNA repair. In vitro studies have shown that AZD1775 (Wee1 inhibitor)-combined therapy with cabozantinib or bortezomib is characterized by the highest efficiency in ERMS.[55] Moreover, patient-derived xenograft RMS models show a sensitivity to AZD1775 therapy, especially when combined with irinotecan and vincristine.[56]

### DDR

Poly (ADP-ribose) polymerase 1 (PARP1) is involved in single-strand break repair and is crucial for DNA protection.[57,58] The PARP1 binds to damaged DNA, and this binding leads to the synthesis of poly (ADP-ribose) (pADPr) chains and recruits repair proteins. Since pADPr is involved in the release of PARP1 from DNA, the repair proteins can be attached (e.g., tyrosyl-DNA phosphodiesterase (TPD1)) to form a DNA repair complex with PARP. In vitro studies have revealed that olaparib treatment (PARP1 inhibitor) affects ERMS; however, this impact is indirect. On the other hand, combined therapy with irinotecan, melphalan, doxorubicin, and temozolomide increases the mortality of cancer cells.[35,59,60] Additionally, irinotecan or rucaparib (PARP1 inhibitor) therapy combined with *TDP1* knockdown shows enhanced anticancer effects.[59]

### Apoptosis pathway

In anti-cancer treatment, therapies are mostly focused on inducing apoptosis, which leads to cell death. Some treatments are designed to activate apoptosis by extrinsic (through the death receptor) or intrinsic (through mitochondria) pathways. Apoptosis is induced by the caspase cascade activation that occurs after membrane-bound tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptors (TRAILR1 or TRAILR2) connect with their ligands, namely TRAIL. The intrinsic mitochondrial pathway requires the release of mitochondrial cytochrome c and Smac to the cytoplasm. Released cytochrome c induces caspase-9 activation. On the other hand, Smac antagonizes survivin, which is one of the inhibitors of apoptosis protein (IAP).[61]

In vitro studies have revealed that TRAIL1 agonistic antibodies used in monotherapy or in combination with

IAP inhibitors do not affect RMS cells. However, TRAIL2 monotherapy shows dose-dependent cell viability. This treatment is more effective in combination with IAP inhibitors in ERMS cells.[61] Additionally, ERMS in vitro therapy with YM155 (survivin inhibitor) reduces cell viability. Similarly, combined in vitro and in vivo therapies with cisplatin influence ERMS cells.[62]

## Current RMS therapies

Over 90% of patients with low-risk localized disease can be cured with multi-modal therapy, but the overall survival rates of patients with metastatic or recurrent disease remain dismal at 21% and 30%, respectively.[63,64] There is no evidence of an improvement in the survival outcomes for metastatic or recurrent RMS in the past 30 years. Therefore, there is a need to develop new treatment strategies.

Currently, all RMS risk groups are treated in a multimodal method, with the use of chemotherapy, surgical resection and/or radiation therapy (RT). In North American countries, chemotherapy includes treatment with natural origin substances such as vincristine, actinomycin D and cyclophosphamide (VAC).[65–67]

Vincristine belongs to a group of drugs known as the vinca alkaloids. The source of these alkaloids is the Madagascar periwinkle (*Catharanthus roseus*). Vincristine acts by binding tubulin and inhibiting the formation of microtubules. Vinca alkaloids depolymerize microtubules and disrupt mitotic spindles, leading to cell cycle arrest.[68] Another mechanism of action of vincristine includes interfering with nucleic acid and protein synthesis by blocking glutamic acid utilization.[69]

Actinomycin D is one of the oldest anti-cancer drugs and the first antibiotic to show anti-cancer activity.[70] It was isolated from *Actinomyces* species bacteria. This substance can inhibit transcription by binding DNA at the transcription initiation complex and preventing elongation of the RNA chain by the polymerase.[71]

In European countries, chemotherapy is carried out with the use of ifosfamide, vincristine and actinomycin D (IVA).[72] The mechanism of action of ifosfamide is based on its reaction with DNA, with which it forms cross-links. This reaction leads to a blockage of the cell cycle. For this to occur, the biologically inactive form of ifosfamide must be metabolized into the active alkylating drug. This is done with the help of oxidases contained in the liver's cytochrome P-450.[73]

Current research has shown that children with low-risk ERMS treated with multi-modal therapy have very promising results (90% of patients do not have relapses).[74] Primary tumor elimination is accomplished by surgery and/or RT with chemotherapy. Radiation therapy is part of the first-line treatment of virtually all ERMS patients.[75] According to the European Pediatric Soft Tissue Sarcoma Study Group (EpSSG), in patients with high-risk ERMS, the use of cyclophosphamide/vinorelbine (maintenance chemotherapy) improves overall survival.[76] Since the EpSSG publication on treatment, maintenance chemotherapy has become the standard treatment for high-risk ERMS patients.[76] The long-term use of RT may cause toxicity.[75] The approach used to reduce toxicity proposes to include intensity-modulated RT (IMRT) or proton beam RT. In addition, it is recommended to use brachytherapy in areas that are assumed to reduce late toxicity (skeletal muscles, soft tissues), such as the vagina or bladder.[77–80]

In the case of patients with ERMS metastatic disease, treatment with the multi-modal method does not tend to bring about the expected results. This method does not consider local therapy in the treatment of metastatic sites, such as in bone marrow. It has also been shown that high-dose chemotherapy, characterized by using very high doses of cytostatic drugs, does not improve patient outcomes in the same way as standard chemotherapy.[81] Patients with low-risk ERMS who have relapsed are treated with chemotherapy drugs such as vincristine or irinotecan.[82]

The identification and targeting of pathways responsible for ERMS invasion and metastasis are crucial for effective therapy. Our current knowledge on ERMS treatment failures caused by either metastatic disease, inadequate treatment or local tumor invasion forced us to plan different therapies. Given the limited number of patients with this disease, it is important to prioritize treatments that will bring about the greatest clinical benefits. The marked differences between RMS subtypes require more personalized treatments. In this context, one can mention the PAX3/FOXO1 oncogene, which is associated with enhanced FPRMSs metastasis, as an example.[83,84] It has been shown that PAX3/FOXO1 fusion oncogene expression can be decreased by siRNA-mediated gene silencing.[85] Currently, preclinical and clinical trials are focused on small molecule inhibitors such as JQ1, which selectively disrupt the interaction between PAX3/FOXO1 and BRD4, disintegrating the fusion gene.[86] This approach has allowed for clinical success in some types of cancers, including lung carcinoma (inhibition of EML4-ALK1) or leukemia (inhibition of BCR-ABL).[87,88] Another approach is to target the regulatory networks of PAX3/FOXO1 by targeting the regulatory kinases that are responsible for the stability or activity of the fusion protein. A recent study has highlighted RTKs as the target for small molecule inhibitors or immunotherapy, such as mAb CAR-T. A multi-track approach for RTK inhibition has proven to be an efficient strategy for refractory RMS.[89] A combination of ganitumab (an IGF1R monoclonal antibody) and dasatinib (a SRC family kinase inhibitor) is effective in blocking pathway substitutions and reducing tumor progression.[90,91] A powerful but still limited treatment method is cancer vaccines based on human cytotoxic lymphocytes T that are capable of lysing HLA-B7+ RMS tumor cells.[92] Biomarkers must be identified so that the most effective treatment method can be selected for each patient. One such factor could be *miR-486-5p*, which is increased in exosomes derived

from FPRMS patients.[93] Another interesting candidate as a possible therapeutic target is CD147, which is considered a cancer metastasis indicator expressed within FPRMS- and FNRMS-derived exosomes.[94–96]

## Limitations in current RMS treatment

As outlined above, the standard treatment for RMS is a combination of chemotherapy, radiotherapy and surgical resection of the tumor, adjusted to the stage of the disease. Commonly used drugs (e.g., vinca alkaloids) cause alkylation, leading to single- or double-stranded breaks in the DNA helix, preventing the cell from correct replication. However, the activity of alkylating compounds is not related to the cell cycle. Alkylating drugs target cancer cells that divide more often compared to other cells. As a result, the target of these substances is also normal cells that are characterized by frequent division (e.g., bone marrow).[97–99] The other group of anti-cancer chemicals, antimetabolite drugs, are highly toxic to cancer cells, but also to normal cells. These drugs can damage the bone marrow and gastrointestinal mucosa and, in high doses, can be nephrotoxic and neurotoxic.[100]

Although anti-cancer agents and RT have demonstrated many benefits in patients, these therapies in RMSs have many disadvantages, including the induction of multidrug resistance protein activity and the appearance of toxic side effects. It is also noteworthy that almost all anti-cancer agents affect not only cancer but also healthy cells.[89,101] Moreover, most cytostatic compounds are only approved for the treatment of adults and not for pediatric cancers. Despite this, chemotherapy is still one of the most widely used treatments for all kinds of RMS at every stage of cancer progression.[102]

## Natural cytostatic substances in cancer therapies

Natural cytostatic substances are a group of compounds that have been under intense research for many years. The sources of anti-cancer natural compounds are arthropods, marine invertebrates, higher vertebrates, plants, and fungi. Numerous natural products exhibit anti-cancer activities, including anti-proliferative, pro-apoptotic, anti-metastatic, and anti-angiogenic effects, and also regulate autophagy, balance immunity, and enhance chemotherapy both in vitro and in vivo.[103,104] Since anti-cancer therapies are often limited by a low therapeutic index and cancer resistance to pharmacotherapy, it is very important to search for new, effective compounds. The most common mechanisms for the anti-cancer activity of these compounds include, but are not limited to, migration, proliferation and cell death pathways, such as apoptosis and autophagy. Their influences on the embryonic developmental signaling pathways (Notch, Wnt and HH) are especially advantageous for childhood cancers, such as ERMS.[105,106] More than half of the anti-cancer drugs in use today have their origins in natural substances. In addition, naturally occurring chemicals and molecules often serve as a model for designing more active or more specific synthetic analogs.[107,108] In many cases, a natural compound's absorption, distribution, metabolism, and excretion parameters can be improved by additional chemical modifications. Natural products, such as phytochemicals, minerals and vitamins, are also used in combination with anti-cancer drugs to facilitate treatment efficiency and minimize side effects.[105,106,108–111] Moreover, natural compounds are usually easily accessible and have a reduced cytotoxicity (Table 2). The use of natural resources also offers a chance to find multi-target active compounds, allowing for a more effective way to treat cancer. The potential existing in as yet undiscovered natural anti-cancer compounds makes this field of research a very important area.

### Tubulin-binding agents

An important group of natural anti-cancer therapeutics is comprised of tubulin-binding agents. Tubulin is the building block of microtubules, an essential part of the cytoskeleton that plays a vital role in the cell cycle. The mechanism of action of these chemicals is based on interference of the mitosis process and influence on the interphase, directing cells to the apoptotic pathway.[112] The main classes of drugs influencing the microtubules include the vinca alkaloids and taxanes.[110]

Alkaloids, such as vincristine, vinblastine and vindesine, are commonly used to treat hematological and lymphatic neoplasms, as well as several solid tumors. Structural modifications of these compounds led to the synthesis of vinorelbine, which is used to treat several cancers.[113] The vinca alkaloids are effective drugs; however, they cause some side effects, such as myelosuppression and neurotoxicity.[110,114]

The most common taxanes, paclitaxel and docetaxel, are obtained through semi-synthesis of a chemical compound (10-deacetylbaccatin III) obtained from the needles of the European yew tree (*Taxus baccata*). Several new drugs with improved toxicity and efficiency profiles have been tested (e.g., abazitaxel, paclitaxel poliglumex, paclitaxel+endotag, and polymeric-micellar paclitaxel).[115] Taxanes are used in the treatment of metastatic breast cancer and as an adjuvant in chemotherapy.[116]

Other microtubule-destabilizing agents acquired from different natural sources, such as cyanobacteria (cryptophycins), marine mollusks (dolastatins) and Japanese sponge (halicondrins), have also been characterized. Their modified derivatives are undergoing clinical trials, and some of them show promising anti-cancer potential and good safety profiles.[117] On the other hand,

**Table 2.** List of drugs/drug candidates (at different stages of development) used/assessed (in alphabetical order) for the use in the treatment for rhabdomyosarcoma and mentioned in the article

| Drug | Origin | Mode of action | Compound ID number |
|---|---|---|---|
| Actinomycin D | antibiotic from *Streptomyces parvulus* spp. | DNA helix intercalating agent | 457193 |
| AEW541 | pyrrolo[2,3-d]pyrimidine | IGF1R inhibitor | 11476171 |
| AZD1775 (adavosertib) | piperazine | Wee1 inhibitor | 24856436 |
| Beta-lapachone | benzochromenone from pink lapacho (*Handroanthus impetiginosus*) | redox disrupting agent | 3885 |
| Buparlisib | synthesis, aminopyridine | PI3K inhibitor | 16654980 |
| Camptothecin | alkaloid from the Chinese tree (*Camptotheca acuminata*) | topoisomerase inhibitor | 24360 |
| Cisplatin | synthesis, platinum coordination complex | alkylating agent | 5460033 |
| Combretastatin A4 | combretastatin from the South African bushwillow tree (*Combretum caffrum)* | tubulin-binding agent | 5351344 |
| Cyclophosphamide | synthesis, nitrogen mustard-originated compound | alkylating agent | 2907 |
| Daunorubicin | anthracycline antibiotic from the bacterium *Streptomyces peucetius* var. caesius | topoisomerase inhibitor | 30323 |
| Docetaxel | tetracyclic diterpenoid from Pacific yew tree (*Taxus brevifolia*) | tubulin-binding agent | 148124 |
| Doxorubicin | anthracycline antibiotic from the bacterium *Streptomyces peucetius* var. caesius | DNA helix intercalating agent | 31703 |
| Epipodophyllotoxin | lignan from the Indian wild mandrake plant (*Podophyllum peltatum*) | topoisomerase inhibitor | 105111 |
| Etoposide | derivative of podophyllotoxin from the wild mandrake plant (*Podophyllum peltatum*) | topoisomerase inhibitor | 36462 |
| Exatecan mesylate | agent related to camptothecin (*Camptotheca acuminata*) | topoisomerase inhibitor | 6918249 |
| Flavopiridol | flavonoid from the pithraj tree (*Aphanamixis polystachya*) | CDK inhibitor | 5287969 |
| GANT-61 | hexahydropyrimidine | GLI1/GLI2 inhibitor | 421610 |
| Geldanamycin | rapamycin analogue (macrolide lactam from *Streptomyces hygroscopicus* spp.) | mTOR inhibitor | 5288382 |
| Gimatecan | analogue of camptothecin (*Camptotheca acuminata*) | topoisomerase inhibitor | 9577124 |
| Homoharringtonine | cephalotaxine-derived alkaloid from Japanese plum yew (*Cephalotaxus harringtonia*) | 80S ribosome-binding agent | 285033 |
| Irinotecan | pyranoindolizinoquinoline (carbamate ester) from camptothecin (*Camptotheca acuminata*) | topoisomerase inhibitor | 60838 |
| Isofasfamide | synthesis, nitrogen mustard-originated compound | alkylating agent | 3690 |
| Karenitecin | agent related to camptothecin (*Camptotheca acuminata*) | topoisomerase inhibitor | 148202 |
| Lurtotecan | analogue of camptothecin (*Camptotheca acuminata*) | topoisomerase inhibitor | 60956 |
| Paclitaxel | tetracyclic diterpenoid from Pacific yew tree (*Taxus brevifolia*) | tubulin-binding agent | 36314 |
| Palbociclib | synthesis, pyridopyrimidine | CDK inhibitor | 5330286 |
| Picropodophyllin (PPP) | cyclolignan alkaloid from mayapple plant family (*Podophyllum peltatum*) | IGF1R inhibitor | 72435 |
| Rapamycin | macrolide lactam from *Streptomyces hygroscopicus* spp. | mTOR inhibitor | 5284616 |
| Rucaparib | synthesis, tricyclic indole | PARP1 inhibitor | 9931954 |
| Selumetinib | synthesis, 1-methyl-1H-benzimidazole | PI3K/mTOR and MEK inhibitor | 10127622 |
| Temozolomide | synthesis, triazene analog of dacarbazine | alkylating agent | 5394 |
| Temsirolimus | ester analog of rapamycin (macrolide lactam from *Streptomyces hygroscopicus* spp.) | mTOR inhibitor | 6918289 |
| Teniposide | derivative of podophyllotoxin from the Indian podophyllum plant (*Podophyllum peltatum*) | topoisomerase inhibitor | 452548 |
| Topotecan | quinoline-based alkaloid extracted from the Asian tree (*Camptotheca acuminata*) | topoisomerase inhibitor | 60700 |
| Trametinib | synthesis, pyridopyrimidine | MEK inhibitor | 11707110 |
| Vinblastine | vinca alkaloid from Madagascar periwinkle (*Catharanthus roseus*) | tubulin-binding agent | 13342 |
| Vincristine | vinca alkaloid from Madagascar periwinkle (*Catharanthus roseus*) | tubulin-binding agent | 5978 |
| Vindesine | vinca alkaloid from Madagascar periwinkle (*Catharanthus roseus*) | tubulin-binding agent | 40839 |
| Vinflunine | semisynthetic vinca alkaloid from Madagascar periwinkle (*Catharanthus roseus*) | tubulin-binding agent | 10629256 |
| Vinorelbine | semisynthetic vinca alkaloid from Madagascar periwinkle (*Catharanthus roseus*) | tubulin-binding agent | 5311497 |
| YM155 (sepantronium bromide) | synthesis, organic bromide salt | surviving inhibitor | 11178236 |

CDK – cyclin-dependent kinase. The table includes compound identifiers (CIDs) to facilitate access to the chemical molecule database, PubChem (https://pubchem.ncbi.nlm.nih.gov), which provides information on the structure and characteristics of the compounds.

microtubule-stabilizing compounds can also be effective against cancer. Some substances obtained from natural sources can bind microtubules and preserve them from dynamic reorganization. One example is epothilones obtained from myxobacterium (*Sorangium cellulosum*) and their chemical derivatives, such as ixabepilone.[110,118]

## Topoisomerase inhibitors

A 2nd group of important, natural anti-cancer chemicals are the topoisomerase inhibitors. Topoisomerase is a nuclear enzyme responsible for proper DNA replication and cell division. Its activity is highly increased in intensively dividing cancer cells. The DNA topoisomerases are well-known targets for anti-cancer therapies relying on enzyme poisoning. Such an approach leads to replication arrest and double-strand break formation. This mechanism of action is potentially dangerous since it brings the risk of therapy-related cancer and cardiotoxicity.[119] Among the topoisomerase inhibitors, we can distinguish the camptothecins and their synthetic analogs. Camptothecin is an active compound present in an extract from the Chinese tree *Camptotheca acuminata*, and its derivatives include lurtotecan, exatecan mesylate, karenitecin, and gimatecan. These compounds are currently undergoing different phases of clinical trials.[107,120]

A 2nd important group of topoisomerase inhibitors are the epipodophyllotoxins extracted from the wild mandrake plant (*Podophyllum peltatum*). Among the synthetic chemical derivatives, 2 compounds have been found to be active, namely etoposide and teniposide. Several side effects of both etoposide and teniposide have been observed, including hypersensitivity. However, some of these side effects have resulted from the use of adjuvants.[107]

The 3rd prominent group of inhibitors specific to topoisomerase includes the anthracyclines. These compounds are derived from the *Streptomyces peucetius* bacterium. The most commonly used anthracyclines in clinical practice are doxorubicin and daunorubicin. This class of compounds exhibits a wide spectrum of anti-tumor activity, but at the same time, severe toxic side effects, such as cardiomyopathy and the induction of secondary cancers, can occur. The mentioned drugs and their analogs are effectively used in approaches combining immunotherapy and chemotherapy. The anthracyclines have been shown to induce immunological response.[107,121]

## Other natural anti-cancer compounds

Other natural anti-cancer compounds include active substances found in plants, microorganisms and marine organisms. At present, traditional plant-based medicines (e.g., flavopiridol, homoharringtonine, β-lapachone, and combretastatin A4) are still prevalently used as medical treatments around the world. These compounds exhibit a wide range of mechanisms of action. For example, flavopiridol is a cyclin-dependent kinase inhibitor, homoharringtonine inhibits protein synthesis and blocks cell cycle progression, β-lapachone is a DNA topoisomerase I inhibitor, and combretastatin A4 inhibits tumor blood vessel growth. Other plant secondary metabolites, such as alkaloids, diterpenes, triterpenes, and polyphenolic type compounds, also exhibit great anti-cancer potential.[110,122,123]

Microorganisms that thrive in diverse environments are also sources of novel anti-cancer compounds, such as rapamycin and geldanamycin. Rapamycin possesses immunosuppressing and anti-neoplastic activity. Geldanamycin, a rapamycin analog, has the ability to suppress the protein kinase activity of mTOR. Its chemical derivatives also show a potential to prevent cancer cell line proliferation.[124] The tumor-inhibitory features of the bacterial enzyme L-asparaginase are wildly known. Since L-asparaginase inhibits protein biosynthesis in lymphoblasts, it is used to treat acute lymphoblastic leukemia.[125]

Marine organisms, including plants, algae, bacteria, actinomycetes, fungi, sponges, and soft corals, are also sources of many chemical products. The most important compounds with the anti-cancer activity that have been isolated from marine organisms include peptides, polyphenols, polysaccharides, and alkaloids.[126]

## Natural cytostatic substances in RMS therapy

A standard treatment scheme based on chemo- and radiotherapy with tumor resection is still the most common for patients with RMS. However, high tumor malignancy combined with the young age of the patients limits successful application of the current treatment methods. The genetic and molecular pathways activated in RMS oncogenesis may constitute an efficient target for novel and effective tumor therapy development.[89] Flavonoids, which are phytochemicals produced in fruits, nuts and vegetables, exhibit anti-oxidant activities and protect against cancer development. It has been shown that flavonoids inhibit cancer cell growth and migration. In RMS, the fusion oncogene PAX3/FOXO1 transcription factor and G9a1 (a histone methyltransferase) are believed to be highly pro-oncogenic factors. Expression of both genes is regulated by the NR4A1 nuclear receptor.[127] A study conducted by Shrestha et al. revealed that the flavonoids kaempferol and quercetin bind to the ligand binding domain of NR4A1 and act as its antagonists in RMS cells.[128] These flavonoids also inhibit the expression of G9a, PAX3/FOXO1 and other pro-oncogenic NR4A1-regulated genes/pathways. Complementary results both in vitro and in vivo have demonstrated that kaempferol and quercetin are NR4A1 ligands acting as antagonists in RMS cells and mimic the effects of NR4A1 knockdown by RNAi. The authors suggested that NR4A1-active flavonoids can be repurposed for clinical applications in the treatment of RMS or other cancers where NR4A1 is a potential drug target.

The well-known Mediterranean herb *Rosmarinus officinalis*, commonly known as rosemary, also has therapeutic properties. Rosemary extract has been well-studied in animal models and has been shown to have anti-mutagenic and nontoxic properties.[129] Extracts from the leaves of *R. officinalis* possess a variety of bioactivities, including antioxidant, anti-tumor, anti-inflammatory, and anti-human immunodeficiency virus (HIV) properties.[130] *Rosmarinus officinalis* leaves contain numerous bioactive compounds, such as flavonoids, phenolic diterpenes, triterpenes, and caffeic acid esters.[131] The RMS anti-cancer therapy can include doxorubicin or vinblastine separately, or a combination of these chemotherapeutics, with rosemary extracts. Research has shown that the use of rosemary combined with doxorubicin or vinblastine in anti-cancer therapy reduced their toxic effects.[131] However, Kakouri et al. revealed the cytotoxic effects of *R. officinalis* extract on RMS cell lines.[132] The rosemary extract used in the experiment contained a high phenolic content and showed strong antioxidant activity. According to the authors, *R. officinalis* extract is a potential alternative source of bioactive compounds which could be used in the future against RMS.

An extract from ginger (*Zingiber zerumbet*) also has promising therapeutic effects in the treatment of pediatric RMS cells. Zerumbone, a substance obtained from ginger, exhibits anti-tumor and anti-inflammatory properties. Evidence obtained so far indicates also that zerumbone has chemoprotective and chemotherapeutic effects on various cancers. Interestingly, it has been shown that the exposure of RMS cells to zerumbone results in cell growth inhibition, decreased proliferation and induction of apoptosis. The authors also showed that the treatment of pediatric RMS cell lines with zerumbone extract induces strong inhibitory and apoptotic effects through increased caspase 3/7 activity and increased reactive oxygen species (ROS) production, as well as through modulation of the nuclear factor kappa B (NF-κB) pathway.[133]

Curcumin, derived from the rhizomes of the turmeric *Curcuma longa*, also exhibits anti-tumor effects on pediatric RMS. Extracts from this plant induce apoptosis, inhibit cell proliferation and efficiently act on signaling pathways influencing tumor development. Studies carried out by Sorg et al. showed that curcumin decreases cell viability in RMS cell lines in a concentration-dependent manner, and enhances the effects of the cytotoxic drugs vincristine and dactinomycin, leading to reduced migration and increased cell apoptosis.[134]

The in vitro cytotoxic activity of various plant extracts on RMS human cell lines (RD) was assessed by Maqsood et al.[135] The authors collected plant material from 6 plant species. The results showed that all of the plant extracts had a cytotoxic tendency towards RD cells after 48 h of incubation. Additionally, every plant extract showed more efficient cell-killing activity compared to 10 μM cisplatine.

It has also been demonstrated that the natural isoquinoline alkaloid berberine exhibits anti-tumor activity in RMS cell lines. Berberine is present in various medicinal plants, such as the Amur cork tree (*Phellodendron amurense*), which has been used as a traditional Chinese herb. Besides berberine, these plants synthesize a series of protoberberine-type alkaloids, such as palmatine, coptisine and jatrorrhizine. All of these compounds are believed to have anti-bacterial, anti-diabetic, anti-inflammatory, anti-oxidative, cardiovascular protective, and neuroprotective effects. The anti-tumor effects of berberine and palmatine have been studied on 3 human embryonal RMS cell lines: ERMS1, KYM1 and RD. It was observed that the intracellular incorporation of berberine in every RMS cell line was relatively higher than that for palmatine. Berberine significantly inhibited the cell cycle of all RMS cells at the G1 phase, whereas palmatine only suppressed the growth of RD cells.[136]

The RMS is a highly malignant cancer most frequently found in children, accounting for 5% of all pediatric tumors. In the past decades, the survival rates for high-risk patients have not improved. At present, standard treatments, including chemotherapy, radiotherapy and surgical removal of the tumor, do not bring about the expected results and are often insufficient to avoid cancer development.[137] Therefore, the use of natural origin cytostatics may bring many benefits in RMS anti-cancer treatment. It is noteworthy that natural products can be obtained from 4 main sources: plants, animals, marine organisms, and microorganisms.[138] According to Mushtaq et al., natural substances are the foundation of novel therapeutic compounds with minimal side effects.[139] This is because of the presence of tremendous biodiversity among plants, animals, marine organisms, and microorganisms. The process of drug discovery from natural sources is slow and monotonous, and is associated with uncertain results. However, with the help of recent advancements, such as proteomics, genomics, transcriptomics and genetic modification, natural products can be screened for their bioactivity, which may contribute to future drug development.

## Perspectives

There have been a lot of studies on natural compounds for RMS treatment and, in recent years, interest in these agents has increased. Most studies have been conducted in vitro and there are only scant results from the use of animal models. In vitro tests have their limitations and do not reflect the processes taking place in a living organism. Thus, the introduction of model species into RMS anticancer studies using natural cytostatic compounds will allow for a further assessment of their effects on cancerous and healthy tissues.

Among the vertebrates, zebrafish (*Danio rerio*) are a very good and commonly used model in cancer research.[140–144] The zebrafish model is characterized by conserved

physiology and anatomy with mammals. A large number of progeny, rapid ex vivo development and transparent larvae allowing for real-time imaging of all developmental stages are the most valuable advantages of using this model organism.[145] The zebrafish model is also convenient for cancer research due to time- and cost-efficient genetic manipulations. Additionally, cancer tumors developed in zebrafish show a high histological and molecular similarity to human ones.[146]

It is worth emphasizing that numerous mutant and transgenic zebrafish have been generated for studies of human cancers. One of them is the casper mutant, a transparent organism (because of a lack of melanocyte and iridophore cell populations) that allows for the investigation of labeled cancer cell growth in embryos or adult individuals.[147] In contrast to the mouse model, the transplantation of human tumor cells into zebrafish larvae does not require immunosuppressive drugs because the *D. rerio* adaptive immune system only becomes fully functional at 3 weeks post-fertilization.[148] The xenotransplantation of human cancer cells into zebrafish is a widely used method to analyze tumor biology and has enormous potential for the further evaluation of cancer progression and drug discovery. The xenotransplantation zebrafish model provides a unique opportunity to monitor cancer proliferation, tumor angiogenesis, metastasis, cancer stem cell self-renewal, and in vivo drug responses in real time.[149] Thus, zebrafish may be a valuable and efficient tool to evaluate novel therapeutic strategies for cancer and can contribute to new insights into tumor biology and cancer drug development.

## ORCID iDs

Damian Lewandowski https://orcid.org/0000-0003-3158-5956
Anna Szewczyk https://orcid.org/0000-0003-0934-9180
Justyna Radzka https://orcid.org/0000-0001-5052-7642
Magda Dubińska-Magiera https://orcid.org/0000-0003-2725-3924
Weronika Kazimierczak https://orcid.org/0000-0003-3894-5088
Małgorzata Daczewska https://orcid.org/0000-0001-7208-1012
Marta Migocka-Patrzałek https://orcid.org/0000-0003-0077-7779

## References

1. Arndt CAS, Crist WM. Common musculoskeletal tumors of childhood and adolescence. *N Engl J Med*. 1999;341(5):342–352. doi:10.1056/NEJM199907293410507
2. Ries L, Smith M, Gurney J, et al., eds. *Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995*. Bethesda, USA: National Cancer Institute, SEER Program. NIH Pub. No. 99-4649. https://seer.cancer.gov/archive/publications/childhood/childhood-monograph.pdf. Accessed November 10, 2022.
3. Skubitz KM, D'Adamo DR. Sarcoma. *Mayo Clin Proc*. 2007;82(11):1409–1432. doi:10.4065/82.11.1409
4. Hoang NT, Acevedo LA, Mann MJ, Tolani B. A review of soft-tissue sarcomas: Translation of biological advances into treatment measures. *Cancer Manag Res*. 2018;10:1089–1114. doi:10.2147/CMAR.S159641
5. Ognjanovic S, Linabery AM, Charbonneau B, Ross JA. Trends in childhood rhabdomyosarcoma incidence and survival in the United States, 1975–2005. *Cancer*. 2009;115(18):4218–4226. doi:10.1002/cncr.24465
6. Dasgupta R, Fuchs J, Rodeberg D. Rhabdomyosarcoma. *Semin Pediatr Surg*. 2016;25(5):276–283. doi:10.1053/j.sempedsurg.2016.09.011
7. Ingley KM, Cohen-Gogo S, Gupta AA. Systemic therapy in pediatric-type soft-tissue sarcoma. *Curr Oncol*. 2020;27(11):6–16. doi:10.3747/co.27.5481
8. Hustu HO, Pinkei D, Pratt CB. Treatment of clinically localized Ewing's sarcoma with radiotherapy and combination chemotherapy. *Cancer*. 1972;30(6):1522–1527. doi:10.1002/1097-0142(197212)30:6<1522::AID-CNCR2820300617>3.0.CO;2-J
9. Skapek SX, Ferrari A, Gupta AA, et al. Rhabdomyosarcoma. *Nat Rev Dis Primers*. 2019;5(1):1. doi:10.1038/s41572-018-0051-2
10. Martin-Giacalone BA, Weinstein PA, Plon SE, Lupo PJ. Pediatric rhabdomyosarcoma: Epidemiology and genetic susceptibility. *J Clin Med*. 2021;10(9):2028. doi:10.3390/jcm10092028
11. Barr FG, Qualman SJ, Macris MH, et al. Genetic heterogeneity in the alveolar rhabdomyosarcoma subset without typical gene fusions. *Cancer Res*. 2002;62(16):4704–4710. PMID:12183429.
12. Shern JF, Selfe J, Izquierdo E, et al. Genomic classification and clinical outcome in rhabdomyosarcoma: A report from an international consortium. *J Clin Oncol*. 2021;39(26):2859–2871. doi:10.1200/JCO.20.03060
13. Panda SP, Chinnaswamy G, Vora T, et al. Diagnosis and management of rhabdomyosarcoma in children and adolescents: ICMR Consensus Document. *Indian J Pediatr*. 2017;84(5):393–402. doi:10.1007/s12098-017-2315-3
14. Dias P, Chen B, Dilday B, et al. Strong immunostaining for myogenin in rhabdomyosarcoma is significantly associated with tumors of the alveolar subclass. *Am J Pathol*. 2000;156(2):399–408. doi:10.1016/S0002-9440(10)64743-8
15. Ilanthodi S, Pallipady A, Jayaprakash K, Monteiro F. Secondary cardiac pleomorphic rhabdomyosarcoma: A case report and review of literature. *J Clin Diagn Res*. 2011;5(2):364–366. https://www.jcdr.net/articles/pdf/1260/1648_2024_9_4_11.pdf. Accessed November 10, 2022.
16. Yin J, Liu Z, Yang K. Pleomorphic rhabdomyosarcoma of the liver with a hepatic cyst in an adult: Case report and literature review. *Medicine (Baltimore)*. 2018;97(29):e11335. doi:10.1097/MD.0000000000011335
17. Shirafkan A, Boroumand N, Komak S, Duchini A, Cicalese L. Pancreatic pleomorphic rhabdomyosarcoma. *Int J Sur Case Rep*. 2015;13:33–36. doi:10.1016/j.ijscr.2015.05.029
18. Alaggio R, Zhang L, Sung YS, et al. A molecular study of pediatric spindle and sclerosing rhabdomyosarcoma: Identification of novel and recurrent VGLL2-related fusions in infantile cases. *Am J Surg Pathol*. 2016;40(2):224–235. doi:10.1097/PAS.0000000000000538
19. Smith MH, Atherton D, Reith JD, Islam NM, Bhattacharyya I, Cohen DM. Rhabdomyosarcoma, spindle cell/sclerosing variant: A clinical and histopathological examination of this rare variant with three new cases from the oral cavity. *Head Neck Pathol*. 2017;11(4):494–500. doi:10.1007/s12105-017-0818-x
20. Rudzinski ER, Anderson JR, Hawkins DS, Skapek SX, Parham DM, Teot LA. The World Health Organization Classification of Skeletal Muscle Tumors in Pediatric Rhabdomyosarcoma: A report from the Children's Oncology Group. *Arch Pathol Lab Med*. 2015;139(10):1281–1287. doi:10.5858/arpa.2014-0475-OA
21. Williams LA, Richardson M, Kehm RD, et al. The association between sex and most childhood cancers is not mediated by birthweight. *Cancer Epidemiol*. 2018;57:7–12. doi:10.1016/j.canep.2018.09.002
22. Dorak MT, Karpuzoglu E. Gender differences in cancer susceptibility: An inadequately addressed issue. *Front Genet*. 2012;3:268. doi:10.3389/fgene.2012.00268
23. van Erp AEM, Versleijen-Jonkers YMH, van der Graaf WTA, Fleuren EDG. Targeted therapy-based combination treatment in rhabdomyosarcoma. *Mol Cancer Ther*. 2018;17(7):1365–1380. doi:10.1158/1535-7163.MCT-17-1131
24. Schlessinger J. Receptor tyrosine kinases: Legacy of the first two decades. *Cold Spring Harb Perspect Biol*. 2014;6(3):a008912. doi:10.1101/cshperspect.a008912
25. Temin HM. Studies on carcinogenesis by avian sarcoma viruses. V. Requirement for new DNA synthesis and for cell division. *J Cell Physiol*. 1967;69(1):53–63. doi:10.1002/jcp.1040690108
26. El-Badry OM, Minniti C, Kohn EC, Houghton PJ, Daughaday WH, Helman LJ. Insulin-like growth factor II acts as an autocrine growth and motility factor in human rhabdomyosarcoma tumors. *Cell Growth Differ*. 1990;1(7):325–331. PMID:2177632.
27. Wang Q, Wu Y, Aerts T, Slegers H, Clauwaert J. Expression of IGF-I and IGF-II receptors in rat C6 glioma cells as a function of the growth phase. *Cell Physiol Biochem*. 1998;8(6):304–313. doi:10.1159/000016292

28. Blandford MC, Barr FG, Lynch JC, Randall RL, Qualman SJ, Keller C. Rhabdomyosarcomas utilize developmental, myogenic growth factors for disease advantage: A report from the children's oncology group. *Pediatr Blood Cancer*. 2006;46(3):329–338. doi:10.1002/pbc.20466
29. Makawita S, Ho M, Durbin AD, Thorner PS, Malkin D, Somers GR. Expression of insulin-like growth factor pathway proteins in rhabdomyosarcoma: IGF-2 expression is associated with translocation-negative tumors. *Pediatr Dev Pathol*. 2009;12(2):127–135. doi:10.2350/08-05-0477.1
30. Rikhof B, de Jong S, Suurmeijer AJ, Meijer C, van der Graaf WT. The insulin-like growth factor system and sarcomas. *J Pathol*. 2009;217(4):469–482. doi:10.1002/path.2499
31. Martins AS, Olmos D, Missiaglia E, Shipley J. Targeting the insulin-like growth factor pathway in rhabdomyosarcomas: Rationale and future perspectives. *Sarcoma*. 2011;2011:209736. doi:10.1155/2011/209736
32. Fuss E. Lignans in plant cell and organ cultures: An overview. *Phytochem Rev*. 2003;2(3):307–320. doi:10.1023/B:PHYT.0000045500.56476.f5
33. Vasilcanu D, Girnita A, Girnita L, Vasilcanu R, Axelson M, Larsson O. The cyclolignan PPP induces activation loop-specific inhibition of tyrosine phosphorylation of the insulin-like growth factor-1 receptor: Link to the phosphatidyl inositol-3 kinase/Akt apoptotic pathway. *Oncogene*. 2004;23(47):7854–7862. doi:10.1038/sj.onc.1208065
34. Tarnowski M, Tkacz M, Zgutka K, Bujak J, Kopytko P, Pawlik A. Picropodophyllin (PPP) is a potent rhabdomyosarcoma growth inhibitor both in vitro and in vivo. *BMC Cancer*. 2017;17(1):532. doi:10.1186/s12885-017-3495-y
35. Bagatell R, Norris R, Ingle AM, et al. Phase 1 trial of temsirolimus in combination with irinotecan and temozolomide in children, adolescents and young adults with relapsed or refractory solid tumors: A children's oncology group study. *Pediatr Blood Cancer*. 2014;61(5):833–839. doi:10.1002/pbc.24874
36. van de Velde ME, Kaspers GL, Abbink FCH, Wilhelm AJ, Ket JCF, van den Berg MH. Vincristine-induced peripheral neuropathy in children with cancer: A systematic review. *Crit Rev Oncol Hematol*. 2017;114:114–130. doi:10.1016/j.critrevonc.2017.04.004
37. Morgenstern DA, Rees H, Sebire NJ, Shipley J, Anderson J. Rhabdomyosarcoma subtyping by immunohistochemical assessment of myogenin: Tissue array study and review of the literature. *Pathol Oncol Res*. 2008;14(3):233–238. doi:10.1007/s12253-008-9012-5
38. Ramadan F, Fahs A, Ghayad SE, Saab R. Signaling pathways in rhabdomyosarcoma invasion and metastasis. *Cancer Metastasis Rev*. 2020;39(1):287–301. doi:10.1007/s10555-020-09860-3
39. Anderson JL, Park A, Akiyama R, Tap WD, Denny CT, Federman N. Evaluation of in vitro activity of the class I PI3K inhibitor buparlisib (BKM120) in pediatric bone and soft tissue sarcomas. *PLoS One*. 2015;10(9):e0133610. doi:10.1371/journal.pone.0133610
40. Guenther MK, Graab U, Fulda S. Synthetic lethal interaction between PI3K/Akt/mTOR and Ras/MEK/ERK pathway inhibition in rhabdomyosarcoma. *Cancer Lett*. 2013;337(2):200–209. doi:10.1016/j.canlet.2013.05.010
41. Renshaw J, Taylor KR, Bishop R, et al. Dual blockade of the PI3K/AKT/mTOR (AZD8055) and RAS/MEK/ERK (AZD6244) pathways synergistically inhibits rhabdomyosarcoma cell growth in vitro and in vivo. *Clin Cancer Res*. 2013;19(21):5940–5951. doi:10.1158/1078-0432.CCR-13-0850
42. Almazán-Moga A, Zarzosa P, Molist C, et al. Ligand-dependent Hedgehog pathway activation in rhabdomyosarcoma: The oncogenic role of the ligands. *Br J Cancer*. 2017;117(9):1314–1325. doi:10.1038/bjc.2017.305
43. Ingham PW, McMahon AP. Hedgehog signaling in animal development: Paradigms and principles. *Genes Dev*. 2001;15(23):3059–3087. doi:10.1101/gad.938601
44. Teglund S, Toftgård R. Hedgehog beyond medulloblastoma and basal cell carcinoma. *Biochim Biophys Acta Rev Cancer*. 2010;1805(2):181–208. doi:10.1016/j.bbcan.2010.01.003
45. Zibat A, Missiaglia E, Rosenberger A, et al. Activation of the Hedgehog pathway confers a poor prognosis in embryonal and fusion gene-negative alveolar rhabdomyosarcoma. *Oncogene*. 2010;29(48):6323–6330. doi:10.1038/onc.2010.368
46. Badagabettu S, Shetty P, D'Souza M. A unique variation of azygos system of veins. *J Cardiovasc Echography*. 2016;26(2):61–64. doi:10.4103/2211-4122.183761
47. Crist W, Gehan EA, Ragab AH, et al. The Third Intergroup Rhabdomyosarcoma Study. *J Clin Oncol*. 1995;13(3):610–630. doi:10.1200/JCO.1995.13.3.610
48. Srivastava RK, Kaylani SZ, Edrees N, et al. GLI inhibitor GANT-61 diminishes embryonal and alveolar rhabdomyosarcoma growth by inhibiting Shh/AKT-mTOR axis. *Oncotarget*. 2014;5(23):12151–12165. doi:10.18632/oncotarget.2569
49. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. *Nat Rev Drug Discov*. 2009;8(7):547–566. doi:10.1038/nrd2907
50. Francis AM, Alexander A, Liu Y, et al. CDK4/6 inhibitors sensitize Rb-positive sarcoma cells to Wee1 kinase inhibition through reversible cell-cycle arrest. *Mol Cancer Ther*. 2017;16(9):1751–1764. doi:10.1158/1535-7163.MCT-17-0040
51. Montoya-Cerrillo DM, Diaz-Perez JA, Velez-Torres JM, Montgomery EA, Rosenberg AE. Novel fusion genes in spindle cell rhabdomyosarcoma: The spectrum broadens. *Genes Chromosomes Cancer*. 2021;60(10):687–694. doi:10.1002/gcc.22978
52. Hugle M, Belz K, Fulda S. Identification of synthetic lethality of PLK1 inhibition and microtubule-destabilizing drugs. *Cell Death Differ*. 2015;22(12):1946–1956. doi:10.1038/cdd.2015.59
53. Rogojanu R, Thalhammer T, Thiem U, et al. Quantitative image analysis of epithelial and stromal area in histological sections of colorectal cancer: An emerging diagnostic tool. *Biomed Res Int*. 2015;2015:569071. doi:10.1155/2015/569071
54. Scheinman MM, Morady F. Invasive cardiac electrophysiologic testing: The current state of the art. *Circulation*. 1983;67(6):1169–1173. doi:10.1161/01.cir.67.6.1169
55. Kahen E, Yu D, Harrison DJ, et al. Identification of clinically achievable combination therapies in childhood rhabdomyosarcoma. *Cancer Chemother Pharmacol*. 2016;78(2):313–323. doi:10.1007/s00280-016-3077-8
56. Stewart E, Federico SM, Chen X, et al. Orthotopic patient-derived xenografts of paediatric solid tumours. *Nature*. 2017;549(7670):96–100. doi:10.1038/nature23647
57. Brandsma I, Fleuren EDG, Williamson CT, Lord CJ. Directing the use of DDR kinase inhibitors in cancer treatment. *Exp Opin Investig Dugs*. 2017;26(12):1341–1355. doi:10.1080/13543784.2017.1389895
58. Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. *Science*. 2017;355(6330):1152–1158. doi:10.1126/science.aam7344
59. Fam HK, Walton C, Mitra SA, et al. TDP1 and PARP1 deficiency are cytotoxic to rhabdomyosarcoma cells. *Mol Cancer Res*. 2013;11(10):1179–1192. doi:10.1158/1541-7786.MCR-12-0575
60. Smith MA, Reynolds CP, Kang MH, et al. Synergistic activity of PARP inhibition by talazoparib (BMN 673) with temozolomide in pediatric cancer models in the pediatric preclinical testing program. *Clin Cancer Res*. 2015;21(4):819–832. doi:10.1158/1078-0432.CCR-14-2572
61. Basit F, Humphreys R, Fulda S. RIP1 protein-dependent assembly of a cytosolic cell death complex is required for inhibitor of apoptosis (IAP) inhibitor-mediated sensitization to lexatumumab-induced apoptosis. *J Biol Chem*. 2012;287(46):38767–38777. doi:10.1074/jbc.M112.398966
62. Ueno M, Ikeda M, Morizane C, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: A non-randomised, multicentre, open-label, phase 1 study. *Lancet Gastroenterol Hepatol*. 2019;4(8):611–621. doi:10.1016/S2468-1253(19)30086-X
63. Dantonello TM, Int-Veen C, Schuck A, et al. Survival following disease recurrence of primary localized alveolar rhabdomyosarcoma. *Pediatr Blood Cancer*. 2013;60(8):1267–1273. doi:10.1002/pbc.24488
64. Malempati S, Hawkins DS. Rhabdomyosarcoma: Review of the Children's Oncology Group (COG) Soft-Tissue Sarcoma Committee experience and rationale for current COG studies. *Pediatr Blood Cancer*. 2012;59(1):5–10. doi:10.1002/pbc.24118
65. Maurer HM, Crist W, Lawrence W, et al. The Intergroup Rhabdomyosarcoma Study I: A final report. *Cancer*. 1988;61(2):209–220. doi:10.1002/1097-0142(19880115)61:2<209::AID-CNCR2820610202>3.0.CO;2-L
66. Maurer HM, Gehan EA, Beltangady M, et al. The Intergroup Rhabdomyosarcoma Study II: Objectives and study design. *Cancer*. 1993;71(5):1904–1922. doi:10.1002/1097-0142(19930301)71:5<1904::AID-CNCR2820710530>3.0.CO;2-X

67. Bayoumy M, Wynn T, Jamil A, Kahwash S, Klopfenstein K, Ruymann F. Prenatal presentation supports the in utero development of congenital leukemia: A case report. *J Pediatr Hematol Oncol*. 2003;25(2): 148–152. doi:10.1097/00043426-200302000-00013
68. Najem S, Langemann D, Appl B, et al. Smac mimetic LCL161 supports neuroblastoma chemotherapy in a drug class-dependent manner and synergistically interacts with ALK inhibitor TAE684 in cells with ALK mutation F1174L. *Oncotarget*. 2016;7(45):72634–72653. doi:10.18632 /oncotarget.12055
69. Martino E, Casamassima G, Castiglione S, et al. Vinca alkaloids and analogues as anti-cancer agents: Looking back, peering ahead. *Bioorg Med Chem Lett*. 2018;28(17):2816–2826. doi:10.1016/j.bmcl.2018. 06.044
70. Avendaño C, Menéndez JC. Anticancer drugs acting via radical species, photosensitizers and photodynamic therapy of cancer. In: *Medicinal Chemistry of Anticancer Drugs*. Amsterdam, the Netherlands: Elsevier; 2008:93–138. doi:10.1016/B978-0-444-52824-7.00004-4
71. Sobell HM. Actinomycin and DNA transcription. *Proc Natl Acad Sci U S A*. 1985;82(16):5328–5331. doi:10.1073/pnas.82.16.5328
72. Koscielniak E, Harms D, Henze G, et al. Results of treatment for soft tissue sarcoma in childhood and adolescence: A final report of the German Cooperative Soft Tissue Sarcoma Study CWS-86. *J Clin Oncol*. 1999;17(12):3706–3719. doi:10.1200/JCO.1999.17.12.3706
73. Furlanut M, Franceschi L. Pharmacology of ifosfamide. *Oncology*. 2003;65(Suppl 2):2–6. doi:10.1159/000073350
74. Kenney LB, Laufer MR, Grant FD, Grier H, Diller L. High risk of infertility and long term gonadal damage in males treated with high dose cyclophosphamide for sarcoma during childhood. *Cancer*. 2001;91(3):613–621. doi:10.1002/1097-0142(20010201)91:3<613::AID-CNCR1042>3.0.CO;2-R
75. Eaton BR, McDonald MW, Kim S, et al. Radiation therapy target volume reduction in pediatric rhabdomyosarcoma: Implications for patterns of disease recurrence and overall survival. *Cancer*. 2013;119(8): 1578–1585. doi:10.1002/cncr.27934
76. Bisogno G, De Salvo GL, Bergeron C, et al. Vinorelbine and continuous low-dose cyclophosphamide as maintenance chemotherapy in patients with high-risk rhabdomyosarcoma (RMS 2005): A multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2019; 20(11):1566–1575. doi:10.1016/S1470-2045(19)30617-5
77. Ladra MM, Szymonifka JD, Mahajan A, et al. Preliminary results of a phase II trial of proton radiotherapy for pediatric rhabdomyosarcoma. *J Clin Oncol*. 2014;32(33):3762–3770. doi:10.1200/JCO.2014.56.1548
78. McDonald MW, Esiashvili N, George BA, et al. Intensity-modulated radiotherapy with use of cone-down boost for pediatric head-and-neck rhabdomyosarcoma. *Int J Radiat Oncol Biol Phys*. 2008;72(3): 884–891. doi:10.1016/j.ijrobp.2008.01.058
79. Saltzman AF, Cost NG. Current treatment of pediatric bladder and prostate rhabdomyosarcoma. *Curr Urol Rep*. 2018;19(1):11. doi:10.1007/ s11934-018-0761-8
80. Terezakis SA, Wharam MD. Radiotherapy for rhabdomyosarcoma: Indications and outcome. *Clin Oncol*. 2013;25(1):27–35. doi:10.1016/j. clon.2012.07.009
81. Weigel BJ, Breitfeld PP, Hawkins D, Crist WM, Baker KS. Role of high-dose chemotherapy with hematopoietic stem cell rescue in the treatment of metastatic or recurrent rhabdomyosarcoma. *J Pediatr Hematol Oncol*. 2001;23(5):272–276. doi:10.1097/00043426-200106000-00007
82. Arndt CAS, Stoner JA, Hawkins DS, et al. Vincristine, actinomycin, and cyclophosphamide compared with vincristine, actinomycin, and cyclophosphamide alternating with vincristine, topotecan, and cyclophosphamide for intermediate-risk rhabdomyosarcoma: Children's Oncology Group Study D9803. *J Clin Oncol*. 2009;27(31):5182–5188. doi:10.1200/JCO.2009.22.3768
83. Mercado G, Barr F. Fusions involving PAX and FOX genes in the molecular pathogenesis of alveolar rhabdomyosarcoma: Recent advances. *Curr Mol Med*. 2007;7(1):47–61. doi:10.2174/156652407779940440
84. Williamson D, Missiaglia E, Chisholm J, Shipley J. Inconvenience of convenience cohort: Letter. *Cancer Epidemiol Biomarkers Prev*. 2012;21(8): 1388. doi:10.1158/1055-9965.EPI-12-0724
85. Vo TT, Ryan J, Carrasco R, et al. Relative mitochondrial priming of myeloblasts and normal HSCs determines chemotherapeutic success in AML. *Cell*. 2012;151(2):344–355. doi:10.1016/j.cell.2012.08.038
86. Gryder BE, Yohe ME, Chou HC, et al. PAX3–FOXO1 establishes myogenic super enhancers and confers BET bromodomain vulnerability. *Cancer Discov*. 2017;7(8):884–899. doi:10.1158/2159-8290.CD-16-1297
87. Salesse S, Verfaillie CM. BCR/ABL: From molecular mechanisms of leukemia induction to treatment of chronic myelogenous leukemia. *Oncogene*. 2002;21(56):8547–8559. doi:10.1038/sj.onc.1206082
88. Sasaki T, Rodig SJ, Chirieac LR, Jänne PA. The biology and treatment of EML4-ALK non-small cell lung cancer. *Eur J Cancer*. 2010; 46(10):1773–1780. doi:10.1016/j.ejca.2010.04.002
89. Chen C, Dorado Garcia H, Scheer M, Henssen AG. Current and future treatment strategies for rhabdomyosarcoma. *Front Oncol*. 2019;9: 1458. doi:10.3389/fonc.2019.01458
90. Wan X, Yeung C, Heske C, Mendoza A, Helman LJ. IGF-1R inhibition activates a YES/SFK bypass resistance pathway: Rational basis for co-targeting IGF-1R and Yes/SFK kinase in rhabdomyosarcoma. *Neoplasia*. 2015;17(4):358–366. doi:10.1016/j.neo.2015.03.001
91. van Gaal JC, Roeffen MHS, Flucke UE, et al. Simultaneous targeting of insulin-like growth factor-1 receptor and anaplastic lymphoma kinase in embryonal and alveolar rhabdomyosarcoma: A rational choice. *Eur J Cancer*. 2013;49(16):3462–3470. doi:10.1016/j.ejca.2013.06.022
92. van den Broeke LT, Pendleton CD, Mackall C, Helman LJ, Berzofsky JA. Identification and epitope enhancement of a PAX-FKHR fusion protein breakpoint epitope in alveolar rhabdomyosarcoma cells created by a tumorigenic chromosomal translocation inducing CTL capable of lysing human tumors. *Cancer Res*. 2006;66(3):1818–1823. doi:10.1158/0008-5472.CAN-05-2549
93. Camero S, Ceccarelli S, De Felice F, et al. PARP inhibitors affect growth, survival and radiation susceptibility of human alveolar and embryonal rhabdomyosarcoma cell lines. *J Cancer Res Clin Oncol*. 2019; 145(1):137–152. doi:10.1007/s00432-018-2774-6
94. Fulda S. Promises and challenges of Smac mimetics as cancer therapeutics. *Clin Cancer Res*. 2015;21(22):5030–5036. doi:10.1158/1078-0432.CCR-15-0365
95. Dobson CC, Naing T, Beug ST, et al. Oncolytic virus synergizes with Smac mimetic compounds to induce rhabdomyosarcoma cell death in a syngeneic murine model. *Oncotarget*. 2017;8(2):3495–3508. doi:10.18632/oncotarget.13849
96. Heinicke U, Haydn T, Kehr S, Vogler M, Fulda S. BCL-2 selective inhibitor ABT-199 primes rhabdomyosarcoma cells to histone deacetylase inhibitor-induced apoptosis. *Oncogene*. 2018;37(39):5325–5339. doi:10.1038/s41388-018-0212-5
97. Damia G, D'Incalci M. Mechanisms of resistance to alkylating agents. *Cytotechnology*. 1998;27(1/3):165–173. doi:10.1023/A:1008060720608
98. Malhotra V, Perry MC. Classical chemotherapy: Mechanisms, toxicities and the therapeutc window. *Cancer Biol Ther*. 2003;2(Suppl 1): 1–3. doi:10.4161/cbt.199
99. Lind MJ. Principles of cytotoxic chemotherapy. *Medicine (Baltimore)*. 2008;36(1):19–23. doi:10.1016/j.mpmed.2007.10.003
100. Zółtowska K, Sobczak M. Perspectives of use of polymer carriers of epidoxorubicin and cyclophosphamide in cancer therapy [in Polish]. *Polim Med*. 2014;44(1):51–62. PMID:24918656.
101. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: A brief review. *Adv Pharm Bull*. 2017;7(3):339–348. doi:10.15171/apb.2017.041
102. Kaseb H, Kuhn J, Babiker HM. Rhabdomyosarcoma. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2022:Bookshelf ID: NBK507721. http://www.ncbi.nlm.nih.gov/books/NBK507721/. Accessed February 13, 2023.
103. Luo H, Vong CT, Chen H, et al. Naturally occurring anti-cancer compounds: Shining from Chinese herbal medicine. *Chin Med*. 2019; 14(1):48. doi:10.1186/s13020-019-0270-9
104. Enam SF, Kilic CY, Huang J, et al. Cytostatic hypothermia and its impact on glioblastoma and survival. *Sci Adv*. 2022;8(47):eabq4882. doi:10.1126/sciadv.abq4882
105. Hashem S, Ali TA, Akhtar S, et al. Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. *Biomed Pharmacother*. 2022;150:113054. doi:10.1016/j.biopha.2022. 113054
106. Naeem M, Iqbal MO, Khan H, et al. A review of twenty years of research on the regulation of signaling pathways by natural products in breast cancer. *Molecules*. 2022;27(11):3412. doi:10.3390/molecules27113412
107. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod*. 2016;79(3):629–661. doi:10.1021/acs. jnatprod.5b01055
108. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol*. 2005;100(1–2):72–79. doi:10.1016/j.jep.2005.05.011

109. Bernardini S, Tiezzi A, Laghezza Masci V, Ovidi E. Natural products for human health: An historical overview of the drug discovery approaches. *Nat Prod Res*. 2018;32(16):1926–1950. doi:10.1080/14786419.2017.1356838
110. Nobili S, Lippi D, Witort E, et al. Natural compounds for cancer treatment and prevention. *Pharmacol Res*. 2009;59(6):365–378. doi:10.1016/j.phrs.2009.01.017
111. Sauter ER. Cancer prevention and treatment using combination therapy with natural compounds. *Exp Rev Clin Pharmacol*. 2020;13(3):265–285. doi:10.1080/17512433.2020.1738218
112. Wilson L, Jordan MA. New microtubule/tubulin-targeted anticancer drugs and novel chemotherapeutic strategies. *J Chemother*. 2004;16(Suppl 4):83–85. doi:10.1179/joc.2004.16.Supplement-1.83
113. Altinoz MA, Ozpinar A, Alturfan EE, Elmaci I. Vinorelbine's anti-tumor actions may depend on the mitotic apoptosis, autophagy and inflammation: Hypotheses with implications for chemo-immunotherapy of advanced cancers and pediatric gliomas. *J Chemother*. 2018;30(4):203–212. doi:10.1080/1120009X.2018.1487149
114. Magge RS, DeAngelis LM. The double-edged sword: Neurotoxicity of chemotherapy. *Blood Rev*. 2015;29(2):93–100. doi:10.1016/j.blre.2014.09.012
115. Kudlowitz D, Muggia F. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel): Extending its indications. *Exp Opin Drug Safety*. 2014;13(6):681–685. doi:10.1517/14740338.2014.910193
116. Willson ML, Burke L, Ferguson T, Ghersi D, Nowak AK, Wilcken N. Taxanes for adjuvant treatment of early breast cancer. *Cochrane Database Syst Rev*. 2019;9(9):CD004421. doi:10.1002/14651858.CD004421.pub3
117. Li Y, Yu H, Han F, Wang M, Luo Y, Guo X. Biochanin A induces S phase arrest and apoptosis in lung cancer cells. *Biomed Res Int*. 2018;2018:3545376. doi:10.1155/2018/3545376
118. Cheng YM, Shen CJ, Chang CC, Chou CY, Tsai CC, Hsu YC. Inducement of apoptosis by cucurbitacin E, a tetracyclic triterpenes, through death receptor 5 in human cervical cancer cell lines. *Cell Death Discov*. 2017;3(1):17014. doi:10.1038/cddiscovery.2017.14
119. Delgado JL, Hsieh CM, Chan NL, Hiasa H. Topoisomerases as anticancer targets. *Biochem J*. 2018;475(2):373–398. doi:10.1042/BCJ20160583
120. Venditto VJ, Simanek EE. Cancer therapies utilizing the camptothecins: A review of the in vivo literature. *Mol Pharm*. 2010;7(2):307–349. doi:10.1021/mp900243b
121. Marinello J, Delcuratolo M, Capranico G. Anthracyclines as topoisomerase II poisons: From early studies to new perspectives. *Int J Mol Sci*. 2018;19(11):3480. doi:10.3390/ijms19113480
122. Seca A, Pinto D. Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. *Int J Mol Sci*. 2018;19(1):263. doi:10.3390/ijms19010263
123. Ashraf MA. Phytochemicals as potential anticancer drugs: Time to ponder nature's bounty. *Biomed Res Int*. 2020;2020:8602879. doi:10.1155/2020/8602879
124. Kim SJ, Kim HS, Seo YR. Understanding of ROS-inducing strategy in anticancer therapy. *Oxid Med Cell Longev*. 2019;2019:5381692. doi:10.1155/2019/5381692
125. Batool T, Makky EA, Jalal M, Yusoff MM. A comprehensive review on l-asparaginase and its applications. *Appl Biochem Biotechnol*. 2016;178(5):900–923. doi:10.1007/s12010-015-1917-3
126. Khalifa SAM, Elias N, Farag MA, et al. Marine natural products: A source of novel anticancer drugs. *Marine Drugs*. 2019;17(9):491. doi:10.3390/md17090491
127. Marshall AD, Grosveld GC. Alveolar rhabdomyosarcoma: The molecular drivers of PAX3/7-FOXO1-induced tumorigenesis. *Skelet Muscle*. 2012;2(1):25. doi:10.1186/2044-5040-2-25
128. Shrestha R, Mohankumar K, Martin G, et al. Flavonoids kaempferol and quercetin are nuclear receptor 4A1 (NR4A1, Nur77) ligands and inhibit rhabdomyosarcoma cell and tumor growth. *J Exp Clin Cancer Res*. 2021;40(1):392. doi:10.1186/s13046-021-02199-9
129. Ciolino HP, Clarke R, Yeh GC, Plouzek CA. Inhibition of P-glycoprotein activity and reversal of multidrug resistance in vitro by rosemary extract. *Eur J Cancer*. 1999;35(10):1541–1545. doi:10.1016/S0959-8049(99)00180-X
130. Peng CH, Su JD, Chyau CC, et al. Supercritical fluid extracts of rosemary leaves exhibit potent anti-inflammation and anti-tumor effects. *Biosci Biotechnol Biochem*. 2007;71(9):2223–2232. doi:10.1271/bbb.70199
131. Ibañez E, Kubátová A, Señoráns FJ, Cavero S, Reglero G, Hawthorne SB. Subcritical water extraction of antioxidant compounds from rosemary plants. *J Agric Food Chem*. 2003;51(2):375–382. doi:10.1021/jf025878j
132. Kakouri E, Nikola O, Kanakis C, et al. Cytotoxic effect of *Rosmarinus officinalis* extract on glioblastoma and rhabdomyosarcoma cell lines. *Molecules*. 2022;27(19):6348. doi:10.3390/molecules27196348
133. Urla C, Stagno MJ, Fuchs J, Warmann SW, Schmid E. Anticancer bioactivity of zerumbone on pediatric rhabdomyosarcoma cells [published online as ahead of print on August 5, 2022]. *J Cancer Res Clin Oncol*. 2022. doi:10.1007/s00432-022-04237-1
134. Sorg C, Schmid E, Bortel N, Fuchs J, Ellerkamp V. Antitumor effects of curcumin in pediatric rhabdomyosarcoma in combination with chemotherapy and phototherapy in vitro. *Int J Oncol*. 2020;58(2):266–274. doi:10.3892/ijo.2020.5155
135. Maqsood M, Qureshi R, Ikram M, et al. Preliminary screening of methanolic plant extracts against human rhabdomyosarcoma cell line from salt range, Pakistan. *Pak J Bot*. 2022;47(1):353–357. https://www.academia.edu/21032120. Accessed October 11, 2022.
136. Meng FC, Wu ZF, Yin ZQ, Lin LG, Wang R, Zhang QW. Coptidis rhizoma and its main bioactive components: Recent advances in chemical investigation, quality evaluation and pharmacological activity. *Chin Med*. 2018;13:13. doi:10.1186/s13020-018-0171-3
137. Ferrari A, Gasparini P, Casanova M. A home run for rhabdomyosarcoma after 30 years: What now? *Tumori*. 2020;106(1):5–11. doi:10.1177/0300891619888021
138. Jabeen S, Hanif M, Mumtaz Khan M, Khan Quadri M. Natural products sources and their active compounds on disease prevention: A review. *Int J Chem Biol Sci*. 2016;6:76–83. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=9859431f3143cb7457ee2d7cf87bed205c423fe6. Accessed October 11, 2022.
139. Mushtaq S, Abbasi BH, Uzair B, Abbasi R. Natural products as reservoirs of novel therapeutic agents. *EXCLIJ*. 2018;17:420–451. doi:10.17179/EXCLI2018-1174
140. Dubińska-Magiera M, Niedbalska-Tarnowska J, Migocka-Patrzałek M, Posyniak E, Daczewska M. Characterization of Hspb8 in zebrafish. *Cells*. 2020;9(6):1562. doi:10.3390/cells9061562
141. Dubińska-Magiera M, Migocka-Patrzałek M, Lewandowski D, Daczewska M, Jagla K. Zebrafish as a model for the study of lipid-lowering drug-induced myopathies. *Int J Mol Sci*. 2021;22(11):5654. doi:10.3390/ijms22115654
142. Migocka-Patrzałek M, Lewicka A, Elias M, Daczewska M. The effect of muscle glycogen phosphorylase (Pygm) knockdown on zebrafish morphology. *Int J Biochem Cell Biol*. 2020;118:105658. doi:10.1016/j.biocel.2019.105658
143. Migocka-Patrzałek M, Elias M. Muscle glycogen phosphorylase and its functional partners in health and disease. *Cells*. 2021;10(4):883. doi:10.3390/cells10040883
144. Niedbalska-Tarnowska J, Ochenkowska K, Migocka-Patrzałek M, Dubińska-Magiera M. Assessment of the preventive effect of L-carnitine on post-statin muscle damage in a zebrafish model. *Cells*. 2022;11(8):1297. doi:10.3390/cells11081297
145. Plantié E, Migocka-Patrzałek M, Daczewska M, Jagla K. Model organisms in the fight against muscular dystrophy: Lessons from drosophila and zebrafish. *Molecules*. 2015;20(4):6237–6253. doi:10.3390/molecules20046237
146. Feitsma H, Cuppen E. Zebrafish as a cancer model. *Mol Cancer Res*. 2008;6(5):685–694. doi:10.1158/1541-7786.MCR-07-2167
147. Bian C, Chen W, Ruan Z, et al. Genome and transcriptome sequencing of casper and roy zebrafish mutants provides novel genetic clues for iridophore loss. *Int J Mol Sci*. 2020;21(7):2385. doi:10.3390/ijms21072385
148. Yan C, Yang Q, Do D, Brunson DC, Langenau DM. Adult immune compromised zebrafish for xenograft cell transplantation studies. *EBioMedicine*. 2019;47:24–26. doi:10.1016/j.ebiom.2019.08.016
149. Zhang B, Xuan C, Ji Y, Zhang W, Wang D. Zebrafish xenotransplantation as a tool for in vivo cancer study. *Fam Cancer*. 2015;14(3):487–493. doi:10.1007/s10689-015-9802-3

# Qualitative and quantitative assessment of headaches in people with temporomandibular joint disorders: A pilot study

Magdalena Gębska[1,A–D], Magdalena Frąszczak[2,A–C], Bartosz Dalewski[3,D,E], Łukasz Kołodziej[4,D,F]

[1] Department of Rehabilitation Musculoskeletal System, Pomeranian Medical University, Szczecin, Poland
[2] Student Science Club at the Department of Rehabilitation of Musculoskeletal System, Pomeranian Medical University, Szczecin, Poland
[3] Department of Dental Prosthetics, Pomeranian Medical University, Szczecin, Poland
[4] Department of Orthopedic Traumatology and Musculoskeletal Oncology, Pomeranian Medical University, Szczecin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1193–1199

**Address for correspondence**
Magdalena Gębska
E-mail: mgebska@pum.edu.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

Received on February 21, 2023
Reviewed on April 29, 2023
Accepted on September 4, 2023

Published online on September 25, 2023

## Abstract

**Background.** Headaches (HAs) and temporomandibular joint dysfunction (TMD) are common comorbidities, and the presence of one of them in a patient increases the incidence of the other. The relationship between these 2 conditions may involve common pathophysiological processes. Considering the topicality of the problem, it is justified to conduct research in this field. In this study, we assessed HA type and severity in people with TMD.

**Objectives.** The aim of the study was to conduct qualitative and quantitative assessments of HAs in people with temporomandibular joint (TMJ) disorders.

**Materials and methods.** The study group consisted of 51 subjects of both sexes with a TMD diagnosed using the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) test. A self-report questionnaire was utilized to self-assess the presence of TMD symptoms, while the standardized Short-Form of the McGill Pain Questionnaire was used to qualitatively and quantitatively assess HAs.

**Results.** People with TMD were significantly more likely to report HA occurrences ($p < 0.001$). Pain intensity was statistically significantly higher among individuals with TMD compared to those without TMD symptoms ($p < 0.001$). Most often, the HA was associated with a pressing pain ($r = 0.82$) and least often, it was described as cutting ($r = 0.30$). Neck and shoulder girdle pain ($p = 0.059$; 82.9%) and clenching and/or grinding of teeth ($p = 0.021$; 92.7%) were significantly more common among patients who declared HAs than among those without HAs. The results obtained so far may indicate a significant relationship between HA and TMD.

**Conclusions.** We have described the relationship between the occurrence of HAs and TMD. Headaches are more frequent and more severe in people with TMD.

**Key words:** pain, temporomandibular joint, headache, dysfunction, craniofacial pain

**Cite as**
Gębska M, Frąszczak M, Dalewski B, Kołodziej Ł. Qualitative and quantitative assessment of headaches in people with temporomandibular joint disorders: A pilot study. *Adv Clin Exp Med*. 2023;32(10):1193–1199. doi:10.17219/acem/171821

**DOI**
10.17219/acem/171821

## Background

According to the World Health Organization (WHO) report, temporomandibular joint disorders (TMDs) are the 3rd most common dental disease after tooth decay and periodontal disease.[1] Schiffman et al. states that TMD can affect between 5% and 12% of the world population.[2] Some studies have shown a higher prevalence of up to 25% and 33–40% in the general population.[3–5]

A study by Chisnoiu et al. indicates that 60–70% of the general population have at least 1 sign of TMD, but only 1 in 4 people with TMD symptoms are referred to specialists.[6] In the USA, between 40% and 75% of adults have at least 1 sign of TMD.[7] In Korea, the percentage of people with TMD is 3.1% of the adult population, among whom 10.5% had more than 1 symptom related to the presence of this dysfunction.[8]

Patients with TMD symptoms span a wide age range; however, the peak occurrence is between the ages of 20 and 40.[9] Temporomandibular joint disorders affect women 1.5–2.5 times more often than men.[9]

The etiopathology of TMD is multifactorial and under constant investigation by researchers and clinicians. Genetic conditions, factors located inside the oral cavity, and environmental determinants, with a particular emphasis on psycho-emotional factors, are mentioned as etiological factors.[10–12] The most common symptoms of TMD include, among others, temporomandibular joint (TMJ) mobility disorder, pain in the masticatory muscles and/or TMJ, headaches (HAs), and/or cervical spine pain.[13,14]

According to the literature, there is a high rate of comorbidities in patients with multiple chronic pain conditions, including fibromyalgia and HAs.[15,16]

The International Classification of Headache Disorders (ICHD-3) published by The International Headache Society (HIS) states that within the facial region of the skull, TMD HAs are the second most common symptom after toothache.[17]

Headaches have been shown to occur more frequently in patients with TMD symptoms (27.4% compared to 15.2%) and can be divided into 2 main types: primary HAs (migraine and tension-type HA (TTH)) and secondary HAs (e.g., pain attributed to TMD, cervicogenic HA).[18,19] Unlike primary HAs, HAs attributed to TMD have a known cause of pain, which is TMD. The underlying condition of TMD needs to be treated in order to reduce HAs attributed to TMD. There is also a strong correlation between cervical HA and TMD, as patients with TMD often report cervical spine dysfunction, and patients with cervical HA often report TMD symptoms.[20,21]

Other studies have shown that TMD patients report HAs more frequently (68–85%) than the general population (50%).[22–24] Aggarwal et al. stated that HAs are more common in patients with TMD symptoms (27.4% compared to 15.2%).[25] Other studies have reported that approx. 70% of TMD patients complain of HAs.[26] In the study by Ballegaard et al., the incidence of TMD and HA was 56.1%.[27] Franco et al. examined 226 patients, and according to their study, TMD and HAs coexist more often compared to a control group without TMD (85.5% compared to 45.6%).[28] Reiter et al. observed that the HA attributed to TMD in painful TMD may be related to a central sensitization process.[29] In contrast, other studies have found that people with TMD are more likely to suffer from a migraine and episodic TTH.[30]

Also, Magnusson and Carlsson observed that TMD patients, especially women, suffered from migraines more often than the control group.[31] The correlation between TMD and HA was also studied by Gonçalves et al., who confirmed a higher frequency of TMD in the migraine and chronic migraine groups than in the control group.[32] This relationship may be associated with increased masticatory muscle tone, which generates nociceptive impulses in the central nervous system, increasing the likelihood of a migraine.[32] According to previous scientific studies, migraines are associated with the activation and sensitization of the trigeminal vascular system.[32] This leads to the release of several pro-inflammatory neuropeptides and neurotransmitters, and causes a cascade of inflammatory tissue responses, including vasodilation, plasma extravasation secondary to capillary leakage, edema, and mast cell degranulation. It is now believed that neurogenic inflammation contributes to the development of a migraine attack.[33,34] Other researchers have also described a link between HAs and TMD.[35–38]

Currently, numerous scientific studies are being carried out in search of innovative methods of HA treatment.[39–42] Also, in the context of TMD, there is more and more new scientific evidence confirming the effectiveness of pharmacological treatment as well as splint therapy and physiotherapy.[43,44]

Therefore, it can be assumed with great caution that the coexistence of TMD and HAs may suggest a common pathogenesis, causation, or common confounding factor, although this issue is not fully understood and may seem controversial.[45] Therefore, further research on this topic seems justified. In this study, the authors set themselves the goal of assessing the nature of the HAs, their frequency and severity in people with diagnosed disorders of the TMJs.

## Objectives

The aim of the study was to conduct qualitative and quantitative assessments of HAs in people with TMJ disorders.

## Materials and methods

As described above, HA is a common symptom in people with TMD. The diagnosis of TMD can be difficult, with some controversy regarding the relative importance

of clinical and radiological evidence.[46] Therefore, it is recommended to use the diagnostic criteria developed by the International Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) Consortium Network and the Orofacial Pain Special Interest Group.[46–49]

Our pilot study was conducted between April and September 2022 at the Department of Rehabilitation Musculoskeletal System of the Pomeranian Medical University in Szczecin, Poland. The study group consisted of 51 individuals of both sexes (women: n = 45, 85.5%; men: n = 6, 14.5%), aged 18–35 years, with TMD diagnosed using the RDC/TMD (n = 51, 82.3%).

The inclusion criteria were consent to participate in the study and the absence of neurological, autoimmune, hormonal, and degenerative joint diseases. The exclusion criteria were orthodontic, prosthetic and implant treatment, history of head trauma, condition after head and neck surgery, and pregnancy.

A study involved completing an anonymous questionnaire consisting of 2 parts:

1. An original 17-question survey. The first question – personal data – concerned the gender and age of the respondents. The next questions were related to the occurrence of TMJ symptoms (HA, pain in the neck and shoulder girdle, pain in the masticatory muscles, ear pain, pain in the TMJ, clenching/grinding of teeth, excessive or limited mobility of the jaw, acoustic symptoms, and feeling of increased tension in the masticatory muscles);
2. McGill Pain Questionnaire – Short-Form[50–52] – questions related to the quantitative and qualitative pain assessment. The questionnaire contains 15 adjectives, divided into 2 categories: sensory (adjectives 1–11) and affective (adjectives 12–15), each rated on a 4-point scale of pain severity (pain quality: 0 – not at all; 3 – very much). Furthermore, it includes a visual analog scale (ranging from no pain to the worst possible pain) and an index of the severity of the currently experienced pain (0 – no pain, 10 – unbearable pain). The higher the score, the more severe the patient's pain.

## Statistical analyses

The analysis was performed using the IBM SPSS v. 24 software (IBM Corp., Armonk, USA). Tests included the $\chi^2$ tests of independence for comparison of response proportions and one-sample Wilcoxon tests for comparison of pain severity to a null value. If the expected value criterion was not met in the contingency tables, Fisher's exact test and the Fisher–Freeman–Halton test were applied. Due to the observation of distributions deviating from the normal distribution based on the Shapiro–Wilk test as well as skewness and kurtosis indices, analyses were performed using non-parametric statistical methods.[53,54] Variable statistics are presented in Supplementary Table 1. A threshold of $\alpha = 0.05$ was used as the level of significance.

# Results

The $\chi^2$ test was used to compare the proportion of patients with HAs to those who reported no pain. Statistically significant differences have been observed ($\chi^2 = 18.84$, degrees of freedom (df) = 1, $p < 0.001$). Patients with TMD had significantly more HAs, which is confirmed by 80% of respondents (Fig. 1).

The type of HA was verified using a one-sample Wilcoxon test due to the fact that patients who declared no HAs at the same time declared a HA frequency of 0. Thus, it was decided to compare the 41 people with HAs

**Table 1.** The average severity of the headache and type of headache

| Dependent variable | | Control group (Mdn = 0) | | W | Z | p-value | r |
|---|---|---|---|---|---|---|---|
| | | Mdn | IQR | | | | |
| Exacerbation of headaches | | 3.00 | 1.00 | 820.00 | 5.60 | <0.001 | 0.87 |
| Type of pain | pricking | 0.00 | 1.00 | 171.00 | 3.94 | <0.001 | 0.62 |
| | rushing | 0.00 | 0.00 | 36.00 | 2.59 | 0.010 | 0.41 |
| | pressing | 2.00 | 1.00 | 595.00 | 5.18 | <0.001 | 0.82 |
| | breaking | 0.00 | 1.75 | 91.00 | 3.24 | 0.001 | 0.51 |
| | cutting | 0.00 | 0.00 | 10.00 | 1.89 | 0.059 | 0.30 |
| | persistent | 1.00 | 1.00 | 231.00 | 4.16 | <0.001 | 0.66 |
| | blunt | 0.00 | 1.00 | 91.00 | 3.24 | 0.001 | 0.51 |
| | dimmed | 0.00 | 1.00 | 153.00 | 3.76 | <0.001 | 0.59 |
| | continuous | 0.00 | 1.00 | 78.00 | 3.11 | 0.002 | 0.49 |
| | intermittent | 0.00 | 0.00 | 45.00 | 2.89 | 0.004 | 0.46 |
| | momentary | 0.00 | 1.00 | 120.00 | 3.77 | <0.001 | 0.60 |

W – Wilcoxon test value; Z – standardized test value; r – effect size index of the difference between the obtained value and the null value; IQR – interquartile range; Mdn – median.

**Table 2.** The relationship between the occurrence of pain and symptoms of temporomandibular joint dysfunction

| Current symptom | | Control group | | Headache | | p-value | φ/V |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | |
| Neck and shoulder girdle pain | I don't know | 0 | 0.0 | 1 | 2.4 | 0.059 | 0.34 |
| | no | 5 | 50.0 | 6 | 14.6 | | |
| | yes | 5 | 50.0 | 34 | 82.9 | | |
| Masticatory muscle pain | I don't know | 0 | 0.0 | 2 | 4.9 | 0.096 | 0.32 |
| | no | 5 | 50.0 | 7 | 17.1 | | |
| | yes | 5 | 50.0 | 32 | 78.0 | | |
| Ear pain | I don't know | 0 | 0.0 | 4 | 9.8 | 0.332 | 0.25 |
| | no | 6 | 60.0 | 30 | 73.2 | | |
| | yes | 4 | 40.0 | 7 | 17.1 | | |
| TMJ pain | I don't know | 0 | 0.0 | 2 | 4.9 | 1.000 | 0.10 |
| | no | 3 | 30.0 | 12 | 29.3 | | |
| | yes | 7 | 70.0 | 27 | 65.9 | | |
| TMJ joint blocking | I don't know | 0 | 0.0 | 3 | 7.3 | 0.034 | 0.39 |
| | no | 3 | 30.0 | 10 | 24.4 | | |
| | yes | 7 | 70.0 | 28 | 68.3 | | |
| TMJ hypermobility | I don't know | 1 | 10.0 | 7 | 17.1 | 0.590 | 0.14 |
| | no | 6 | 60.0 | 27 | 65.9 | | |
| | yes | 3 | 30.0 | 7 | 17.1 | | |
| Clenching and/or grinding of teeth | I don't know | 1 | 10.0 | 1 | 2.4 | 0.021 | 0.38 |
| | no | 3 | 30.0 | 2 | 4.9 | | |
| | yes | 6 | 60.0 | 38 | 92.7 | | |
| Limited mandibular mobility | I don't know | 0 | 0.0 | 3 | 7.3 | 1.000 | 0.13 |
| | no | 5 | 50.0 | 21 | 51.2 | | |
| | yes | 5 | 50.0 | 17 | 41.5 | | |
| Acoustic symptoms | no | 2 | 20.0 | 8 | 19.5 | 1.000 | 0.00 |
| | yes | 8 | 80.0 | 33 | 80.5 | | |
| Masticatory muscle tension | no | 3 | 30.0 | 8 | 19.5 | 0.669 | 0.10 |
| | yes | 7 | 70.0 | 33 | 80.5 | | |

TMJ – temporomandibular joint.

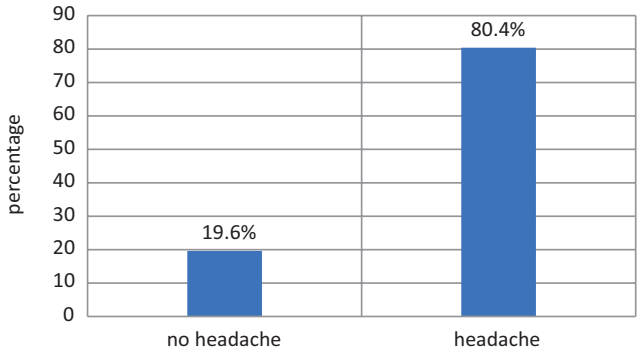



**Fig. 1.** The occurrence of headaches in the patients (n = 51)

to the null value (declared by people with no HAs). The results are presented in Table 1.

Pain severity is statistically significantly higher among individuals with TMD compared to the value of 0, which indicates no pain. Furthermore, almost every type of pain was statistically significantly more frequent compared to the null value, with the exception of cutting pain (p = 0.059), where the average score was close to the value indicating no pain. The obtained difference results (compared to the null value) were moderate for rushing, continuous and intermittent pain (r ≥ 0.30), and strong for other types of pain (r ≥ 0.50, Table 1).

Table 2 shows the results of Fisher's exact test for the 2×2 tables and the Fisher–Freeman–Halton exact test for larger contingency tables on the comparison of TMD and HA symptom prevalence.

When analyzing the results, it was found that neck and shoulder girdle pain, masticatory muscle pain, ear pain, and TMJ hypermobility, as well as limited mandibular mobility, a feeling of tension in the masticatory muscles, and acoustic complaints, were not differentiated by the perception of a HA. However, TMJ joint blocking was significantly

less common among individuals with HAs (68.3%) compared to the group without HAs. In addition, clenching and/or grinding of teeth occurred significantly more frequently among patients with HAs (92.7%, Table 2) compared to the controls.

# Discussion

The study performed the quantitative and qualitative assessment of HAs in people with TMD, and did not examine the basis of their occurrence. Patients with TMD declared the presence of HAs significantly more often, which was confirmed by 80% of the respondents. The obtained difference results (compared to the null value) were moderate for continuous (r = 0.49), intermittent (r = 0.46), rushing (r = 0.41), and cutting (r = 0. 30) pain, and were strong for pressing (r = 0.82), persistent (r = 0.66), pickling (r = 0.62), momentary (r = 0.60), dimmed (r = 0.59), blunt (r = 0.51), and breaking pain (r = 0.51).

According to the literature, patients diagnosed with TTH more often described the type of HA they experience as pressing.[55,56] Therefore, at the initial stage of the conducted research, it could have been assumed with great caution that the oppressive type of pain reported by patients with TMD would indicate the presence of TTH, which should be taken into account in future research.

Previous epidemiological studies have shown an association between TMD pain and TTH.[55] Clinically, pain associated with TMD and TTH share a combination of distinct head and face signs and symptoms, i.e., tenderness of the masticatory muscles for TMD and pericranial muscle tenderness for TTH in the active phase of both conditions.[57] Other clinical intersections between TMD and TTH include the age of subjects in terms of peak incidence, pain severity, pharmacotherapy, and even non-pharmacological treatment.[58–60] A recent study also showed a high prevalence of active myofascial trigger points in TTH patients.[61] This finding may support the hypothesis that peripheral muscle mechanisms are involved in the pathophysiology of TTH.

Current research indicates an important role of central sensitization in the pathogenesis of chronic TTH.[62] Continuous episodes of pain involving the pericranial muscles (such as the temporalis muscle) can hypersensitize the central nervous system, activating higher brain centers, which can lead to chronic TTH transformation.[63] Despite some clinical similarities and overlaps, both TMD HAs and primary TTH HAs are distinct entities.

Other studies have reported that TMD symptoms are more common in individuals with primary HAs compared to those without HAs.[64] This relationship is bidirectional, and several studies have shown that most TMD patients report HAs.[65] One prospective study claimed that the presence of TMD predicted future HAs.[66] In addition, the onset of TMD was followed by an increased incidence of HAs.[67] Several randomized controlled trials have shown a beneficial effect of treating the masticatory muscles for HAs.[68,69]

The conducted research shows that pain in the masticatory muscles, ear pain, TMJ pain and hypermobility, as well as limited TMJ mobility, the feeling of masticatory muscle tension, and acoustic complaints are not differentiated by the sensation of a HA presence. Pain in the neck and shoulder girdle (82.9%, p = 0.059) as well as clenching and/or grinding of teeth (92.7%, p = 0.021) are significantly more common among patients with both TMD and HAs, which has been confirmed by previous studies. On the other hand, blocking TMJ occurred less frequently in people with HAs than in those without pain. Studies by other authors showed a significant relationship between HAs, TMJ pain and acoustic symptoms. However, in the study by Melo et al., masticatory muscle pain and TMJ pain were found to be more common in patients with HAs. In addition, TMD seems to be more severe in patients with HAs.[70] Temporomandibular joint dysfunction is more common in people with HAs than in the pain-free group.[71]

## Limitations

Our preliminary research has some limitations. The main limitation is the small research group, the lack of a neurological examination of patients with HAs, and the lack of additional imaging diagnostics of the TMJs, which should be taken into account when continuing these studies in the future. Further research is needed on the correct diagnosis and causative treatment of patients suffering from HAs resulting from TMD. Patients with suspected primary or secondary HAs due to TMD should be referred to a dental practitioner to ensure effective causative treatment.

# Conclusions

According to the conducted pilot studies, HAs are a serious health problem in people with TMD. At the initial stage of the research, it was assumed that TMD patients were more likely to experience a tension HA. Subsequently, it was observed that people with HAs were more likely to have pain in the neck and shoulder girdle, as well as parafunctions such as clenching and/or teeth grinding. In contrast, TMJ blocking occurred significantly less frequently. Therefore, while continuing the research, it is necessary to differentiate TMJ diseases into those of joint and muscle origin. According to research, the treatment of patients with TMD and HAs may require close interdisciplinary cooperation between specialties (dentistry and neurology). Vigilance should be exercised in the differentiation of these 2 disease entities during their treatment.

## Supplementary data

The supplementary materials are available at https://doi.org/10.5281/zenodo.8272638. The package contains the following files:

Supplementary Table 1. Descriptive statistics of indicators of tested variables.

### ORCID iDs

Magdalena Gębska https://orcid.org/0000-0002-1477-8302
Magdalena Frąszczak https://orcid.org/0009-0003-2958-4644
Bartosz Dalewski https://orcid.org/0000-0002-0652-664X
Łukasz Kołodziej https://orcid.org/0000-0002-8607-7097

### References

1. Checherita L, Stamatin O, Liana A, Luca E. Parafunctions in dysfunctional syndrome of the stomatognathic system: Literature review. *Rom J Med Dent Educ*. 2020;9(1):53–61. https://journal.adre.ro/wp-content/uploads/2020/05/PARAFUNCTIONS-IN-DYSFUNCTIONAL-SYNDROME-OF-THE-STOMATOGNATHIC-SYSTEM-LITERATURE-REVIEW.pdf. Accessed January 3, 2023.
2. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
3. Murphy MK, MacBarb RF, Wong ME, Athanasiou KA. Temporomandibular disorders: A review of etiology, clinical management, and tissue engineering strategies. *Int J Oral Maxillofac Implants*. 2013;28(6):e393–e414. doi:10.11607/jomi.te20
4. Aceves-Avila FJ, Chávez-López M, Chavira-González JR, Ramos-Remus C. Temporomandibular joint dysfunction in various rheumatic diseases. *Reumatismo*. 2013;65(3):126–130. doi:10.4081/reumatismo.2013.126
5. Calixtre LB, Gruninger BLDS, Chaves TC, Oliveira ABD. Is there an association between anxiety/depression and temporomandibular disorders in college students? *J Appl Oral Sci*. 2014;22(1):15–21. doi:10.1590/1678-775720130054
6. Chisnoiu AM, Picos AM, Popa S, et al. Factors involved in the etiology of temporomandibular disorders: A literature review. *Med Pharm Rep*. 2015;88(4):473–478. doi:10.15386/cjmed-485
7. Scrivani SJ, Keith DA, Kaban LB. Temporomandibular disorders. *N Engl J Med*. 2008;359(25):2693–2705. doi:10.1056/NEJMra0802472
8. Sim SH, Ha M. Association between psychological factors and temporomandibular disorders in Korean adults: The fouth Korean national health and nutritional examination survey (2009). *J Korean Soc Dent Hyg*. 2013;13(5):739–747. doi:10.13065/jksdh.2013.13.05.739
9. Bueno CH, Pereira DD, Pattussi MP, Grossi PK, Grossi ML. Gender differences in temporomandibular disorders in adult population-al studies: A systematic review and meta-analysis. *J Oral Rehabil*. 2018;45(9):720–729. doi:10.1111/joor.12661
10. Valesan LF, Da-Cas CD, Réus JC, et al. Prevalence of temporomandibular joint disorders: A systematic review and meta-analysis. *Clin Oral Invest*. 2021;25(2):441–453. doi:10.1007/s00784-020-03710-w
11. Mitrowska-Guźmińska M, Gębska M, Jonko K, et al. Effect of personality type on the occurrence of temporomandibular disorders: A cross-sectional study. *Int J Environ Res Public Health*. 2022;20(1):352. doi:10.3390/ijerph20010352
12. Gębska M, Dalewski B, Pałka Ł, Kołodziej Ł, Sobolewska E. Chronotype profile, stress, depression level, and temporomandibular symptoms in students with type D personality. *J Clin Med*. 2022;11(7):1886. doi:10.3390/jcm11071886
13. Sagripanti M, Viti C. Primary headaches in patients with temporomandibular disorders: Diagnosis and treatment of central sensitization pain. *Cranio*. 2018;36(6):381–389. doi:10.1080/08869634.2017.1359353
14. Liu F, Steinkeler A. Epidemiology, diagnosis, and treatment of temporomandibular disorders. *Dent Clin North Am*. 2013;57(3):465–479. doi:10.1016/j.cden.2013.04.006
15. Dao TT, LeResche L. Gender differences in pain. *J Orofac Pain*. 2000;14(3):169–184; discussion 184–195. PMID:11203754.
16. Andreu Y, Galdon MJ, Durá E, et al. An examination of the psychometric structure of the Multidimensional Pain Inventory in temporomandibular disorder patients: A confirmatory factor analysis. *Head Face Med*. 2006;2(1):48. doi:10.1186/1746-160X-2-48
17. Ambroziak-Kudła K, Kostrzewa-Janicka J. Headaches and TMD patients: Literature review. *Prosthodontics*. 2023;73(1):81–88. doi:10.5114/ps/162658
18. Bender SD. Temporomandibular disorders, facial pain, and headaches: May 2012. *Headache*. 2012;52:22–25. doi:10.1111/j.1526-4610.2012.02134.x
19. Abouelhuda AM, Kim HS, Kim SY, Kim YK. Association between headache and temporomandibular disorder. *J Korean Assoc Oral Maxillofac Surg*. 2017;43(6):363. doi:10.5125/jkaoms.2017.43.6.363
20. Mingels S, Dankaerts W, Granitzer M. Preclinical signs of a temporomandibular disorder in female patients with episodic cervicogenic headache versus asymptomatic controls: A cross-sectional study. *PM R*. 2019;11(12):1287–1295. doi:10.1002/pmrj.12156
21. Piekartz HV, Rösner C, Batz A, Hall T, Ballenberger N. Bruxism, temporomandibular dysfunction and cervical impairments in females: Results from an observational study. *Muscoskelet Sci Pract*. 2020;45:102073. doi:10.1016/j.msksp.2019.102073
22. Stovner LJ, Andree C. Prevalence of headache in Europe: A review for the Eurolight project. *J Headache Pain*. 2010;11(4):289–299. doi:10.1007/s10194-010-0217-0
23. Van Der Meer HA, Speksnijder CM, Engelbert RHH, Lobbezoo F, Nijhuis-van Der Sanden MWG, Visscher CM. The association between headaches and temporomandibular disorders is confounded by bruxism and somatic symptoms. *Clin J Pain*. 2017;33(9):835–843. doi:10.1097/AJP.0000000000000470
24. Lendraitiene E, Smilgiene L, Petruseviciene D, Savickas R. Changes and associations between cervical range of motion, pain, temporomandibular joint range of motion and quality of life in individuals with migraine applying physiotherapy: A pilot study. *Medicina*. 2021;57(6):630. doi:10.3390/medicina57060630
25. Aggarwal VR, McBeth J, Zakrzewska JM, Lunt M, Macfarlane GJ. The epidemiology of chronic syndromes that are frequently unexplained: Do they have common associated factors? *Int J Epidemiol*. 2006;35(2):468–476. doi:10.1093/ije/dyi265
26. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia*. 2013;33(9):629–808. doi:10.1177/0333102413485658
27. Ballegaard V, Thede-Schmidt-Hansen P, Svensson P, Jensen R. Are headache and temporomandibular disorders related? A blinded study. *Cephalalgia*. 2008;28(8):832–841. doi:10.1111/j.1468-2982.2008.01597.x
28. Franco AL, Gonçalves DAG, Castanharo SM, Speciali JG, Bigal ME, Camparis CM. Migraine is the most prevalent primary headache in individuals with temporomandibular disorders. *J Orofac Pain*. 2010; 24(3):287–292. PMID:20664830.
29. Reiter S, Emodi-Perlman A, Kasiel H, et al. Headache attributed to temporomandibular disorders: Axis I and II findings according to the diagnostic criteria for temporomandibular disorders. *J Oral Facial Pain Headache*. 2021;35(2):119–128. doi:10.11607/ofph.2863
30. Gonçalves DAG, Camparis CM, Speciali JG, Franco AL, Castanharo SM, Bigal ME. Temporomandibular disorders are differentially associated with headache diagnoses: A controlled study. *Clin J Pain*. 2011;27(7):611–615. doi:10.1097/AJP.0b013e31820e12f5
31. Magnusson T, Carlsson GE. Recurrent headaches in relation to temporomandibular joint pain-dysfunction. *Acta Odontol Scand*. 1978;36(5–6):333–338. doi:10.3109/00016357809029084
32. Gonçalves MC, Florencio LL, Chaves TC, Speciali JG, Bigal ME, Bevilaqua-Grossi D. Do women with migraine have higher prevalence of temporomandibular disorders? *Braz J Phys Ther*. 2013;17(1):64–68. doi:10.1590/S1413-35552012005000054
33. Spekker E, Tanaka M, Szabó Á, Vécsei L. Neurogenic inflammation: The participant in migraine and recent advancements in translational research. *Biomedicines*. 2021;10(1):76. doi:10.3390/biomedicines10010076
34. Tanaka M, Török N, Tóth F, Szabó Á, Vécsei L. Co-players in chronic pain: Neuroinflammation and the tryptophan–kynurenine metabolic pathway. *Biomedicines*. 2021;9(8):897. doi:10.3390/biomedicines9080897

35. Réus JC, Polmann H, Souza BDM, et al. Association between primary headaches and temporomandibular disorders. *J Am Dent Assoc*. 2022;153(2):120–131.e6. doi:10.1016/j.adaj.2021.07.021
36. Greenbaum T, Emodi-Perlman A. Headache and orofacial pain: A traffic-light prognosis-based management approach for the musculoskeletal practice. *Front Neurol*. 2023;14:1146427. doi:10.3389/fneur.2023.1146427
37. Wright EF, Clark EG, Paunovich ED, Hart RG. Headache improvement through TMD stabilization appliance and self-management therapies. *Cranio*. 2006;24(2):104–111. doi:10.1179/crn.2006.017
38. Glaros A, Urban D, Locke J. Headache and temporomandibular disorders: Evidence for diagnostic and behavioural overlap. *Cephalalgia*. 2007;27(6):542–549. doi:10.1111/j.1468-2982.2007.01325.x
39. Tajti J, Szok D, Csáti A, Szabó Á, Tanaka M, Vécsei L. Exploring novel therapeutic targets in the common pathogenic factors in migraine and neuropathic pain. *Int J Mol Sci*. 2023;24(4):4114. doi:10.3390/ijms24044114
40. Southey BR, Rodriguez-Zas SL. Alternative splicing of neuropeptide prohormone and receptor genes associated with pain sensitivity was detected with zero-inflated models. *Biomedicines*. 2022;10(4):877. doi:10.3390/biomedicines10040877
41. Kleczkowska P, Nowicka K, Bujalska-Zadrozny M, Hermans E. Neurokinin-1 receptor-based bivalent drugs in pain management: The journey to nowhere? *Pharmacol Ther*. 2019;196:44–58. doi:10.1016/j.pharmthera.2018.11.007
42. Tanaka M, Török N, Vécsei L. Are 5-HT$_1$ receptor agonists effective anti-migraine drugs? *Exp Opin Pharmacother*. 2021;22(10):1221–1225. doi:10.1080/14656566.2021.1910235
43. Dalewski B, Kamińska A, Kiczmer P, et al. Pressure algometry evaluation of two occlusal splint designs in bruxism management: Randomized, controlled clinical trial. *J Clin Med*. 2021;10(11):2342. doi:10.3390/jcm10112342
44. Crăciun MD, Geman O, Leuciuc FV, Holubiac IȘ, Gheorghiță D, Filip F. Effectiveness of physiotherapy in the treatment of temporomandibular joint dysfunction and the relationship with cervical spine. *Biomedicines*. 2022;10(11):2962. doi:10.3390/biomedicines10112962
45. Horswell BB, Sheikh J. Evaluation of pain syndromes, headache, and temporomandibular joint disorders in children. *Oral Maxillofac Surg Clin North Am*. 2018;30(1):11–24. doi:10.1016/j.coms.2017.08.007
46. Osiewicz M, Lobbezoo F, Loster B, Wilkosz M, Naeije M, Ohrbach R. Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD): The Polish version of a dual-axis system for the diagnosis of TMD.* RDC/TMD Form. *J Stomatol*. 2013;66(5):576–649. https://protetyka.cm-uj.krakow.pl/cm/uploads/2019/06/JofS_2013_RDC.pdf. Accessed January 5, 2023.
47. List T, Greene CS. Moving forward with the RDC/TMD. *J Oral Rehabil*. 2010;37(10):731–733. doi:10.1111/j.1365-2842.2010.02135.x
48. Petersson A. What you can and cannot see in TMJ imaging: An overview related to the RDC/TMD diagnostic system. *J Oral Rehabil*. 2010;37(10):771–778. doi:10.1111/j.1365-2842.2010.02108.x
49. Ohrbach R, Dworkin SF. The evolution of TMD diagnosis: Past, present, future. *J Dent Res*. 2016;95(10):1093–1101. doi:10.1177/0022034516653922
50. Bakhtadze MA, Lusnikova IV, Kuzminov KO, Bolotov DA. The Second Short Form of the McGill Pain Questionnaire as the useful additional tool for differential diagnostics of migraine and cervicogenic headache [in Russian]. *Zh Nevrol Psikhiatr Im S S Korsakova*. 2021;121(3):70. doi:10.17116/jnevro202112103170
51. Adelmanesh F, Jalali A, Attarian H, et al. Reliability, validity, and sensitivity measures of expanded and revised version of the Short-Form McGill Pain Questionnaire (SF-MPQ-2) in Iranian patients with neuropathic and non-neuropathic pain. *Pain Med*. 2012;13(12):1631–1638. doi:10.1111/j.1526-4637.2012.01517.x
52. Strand LI, Ljunggren AE, Bogen B, Ask T, Johnsen TB. The Short-Form McGill Pain Questionnaire as an outcome measure: Test-retest reliability and responsiveness to change. *Eur J Pain*. 2008;12(7):917–925. doi:10.1016/j.ejpain.2007.12.013
53. George D, Mallery P. *IBM SPSS Statistics 27 Step by Step: A Simple Guide and Reference*. 17th ed. New York, USA: Routledge; 2022. ISBN:978-1-03-207090-2.
54. Watkins MW. *A Step-by-Step Guide to Exploratory Factor Analysis with SPSS*. New York, USA: Routledge; 2021. ISBN:978-0-367-71031-6.
55. Ashina S, Mitsikostas DD, Lee MJ, et al. Tension-type headache. *Nat Rev Dis Primers*. 2021;7(1):24. doi:10.1038/s41572-021-00257-2
56. Scripter C. Headache: Tension-type headache. *FP Essent*. 2018;473:17–20. PMID:30346680.
57. Ciancaglini R, Radaelli G. The relationship between headache and symptoms of temporomandibular disorder in the general population. *J Dent*. 2001;29(2):93–98. doi:10.1016/S0300-5712(00)00042-7
58. Graff-Radford SB, Newman AC. The role of temporomandibular disorders and cervical dysfunction in tension-type headache. *Curr Pain Headache Rep*. 2002;6(5):387–391. doi:10.1007/s11916-002-0081-y
59. Costa Y, Porporatti A, Calderon P, Conti P, Bonjardim L. Can palpation-induced muscle pain pattern contribute to the differential diagnosis among temporomandibular disorders, primary headaches phenotypes and possible bruxism? *Med Oral Patol Oral Cir Bucal*. 2016;21(1):e59–e65. doi:10.4317/medoral.20826
60. Neblett R, Cohen H, Choi Y, et al. The Central Sensitization Inventory (CSI): Establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. *J Pain*. 2013;14(5):438–445. doi:10.1016/j.jpain.2012.11.012
61. Fernández-de-las-Peñas C, Cuadrado ML. Physical therapy for headaches. *Cephalalgia*. 2016;36(12):1134–1142. doi:10.1177/0333102415596445
62. Do TP, Heldarskard GF, Kolding LT, Hvedstrup J, Schytz HW. Myofascial trigger points in migraine and tension-type headache. *J Headache Pain*. 2018;19(1):84. doi:10.1186/s10194-018-0913-8
63. Yu S, Han X. Update of chronic tension-type headache. *Curr Pain Headache Rep*. 2015;19(1):469. doi:10.1007/s11916-014-0469-5
64. Bendtsen L, Ashina S, Moore A, Steiner TJ. Muscles and their role in episodic tension-type headache: Implications for treatment. *Eur J Pain*. 2016;20(2):166–175. doi:10.1002/ejp.748
65. Gonçalves DAG, Bigal ME, Jales LCF, Camparis CM, Speciali JG. Headache and symptoms of temporomandibular disorder: An epidemiological study. *Headache*. 2010;50(2):231–241. doi:10.1111/j.1526-4610.2009.01511.x
66. Anderson GC, John MT, Ohrbach R, et al. Influence of headache frequency on clinical signs and symptoms of TMD in subjects with temple headache and TMD pain. *Pain*. 2011;152(4):765–771. doi:10.1016/j.pain.2010.11.007
67. Marklund S, Wiesinger B, Wänman A. Reciprocal influence on the incidence of symptoms in trigeminally and spinally innervated areas. *Eur J Pain*. 2010;14(4):366–371. doi:10.1016/j.ejpain.2009.06.004
68. Lim PF, Smith S, Bhalang K, Slade GD, Maixner W. Development of temporomandibular disorders is associated with greater bodily pain experience. *Clin J Pain*. 2010;26(2):116–120. doi:10.1097/AJP.0b013e3181c507ef
69. Schokker RP, Hansson TL, Ansink BJ. The result of treatment of the masticatory system of chronic headache patients. *J Craniomandib Disord*. 1990;4(2):126–130. PMID:2133472.
70. Hara K, Shinozaki T, Okada-Ogawa A, et al. Headache attributed to temporomandibular disorders and masticatory myofascial pain. *J Oral Sci*. 2016;58(2):195–204. doi:10.2334/josnusd.15-0491
71. Melo CEB, Oliveira JLG, Jesus ACF, et al. Temporomandibular disorders dysfunction in headache patients. *Med Oral Patol Oral Cir Bucal*. 2012;17(6):e1042–e1046. doi:10.4317/medoral.18007

# Profiling of microRNA as a tool to introduce rAAV vectors in gene therapy of breast cancer: A preliminary report

Agnieszka Zajkowska[1,A–D], Milena Czajka[1,D,E], Krystian Gulik[2,C], Krzysztof Gawrychowski[1,B], Maciej Małecki[1,3,A,E,F]

[1] Department of Applied Pharmacy, Faculty of Pharmacy, Medical University of Warsaw, Poland
[2] Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Poland
[3] Laboratory of Gene Therapy, Faculty of Pharmacy, Medical University of Warsaw, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2023;32(10):1201–1210

**Address for correspondence**
Agnieszka Zajkowska
E-mail: agnieszka.zajkowska@wum.edu.pl

**Funding sources**
This work was supported by a grant from the National Centre of Research and Development (Strategmed 1/233264/4/NCBR/2014, MentorEYE).

**Conflict of interest**
None declared

Received on September 1, 2022
Reviewed on April 4, 2023
Accepted on August 31, 2023

Published online on September 29, 2023

## Abstract

**Background.** Despite the wide range of diagnostic and therapeutic methods, breast cancer is responsible for many deaths each year. One of the original and novel cancer therapeutic approaches is gene therapy based on recombinant adeno-associated viral vectors. Among the molecular factors with the potential to become useful diagnostic biomarkers, microRNA (miRNA) molecules are being considered for personalized therapies.

**Objectives.** The aim of the study was to examine the utility of miRNA profiling in the design of personalized recombinant adeno-associated virus (rAAV)-based gene therapy for breast cancer patients.

**Materials and methods.** The analysis of 754 miRNAs in 7 breast cancer samples and control samples was performed using real-time polymerase chain reaction (PCR) based on TaqMan® Low-density Array (TLDA) cards. Online repositories were used to explore the relationship between miRNAs and genes encoding rAAV receptors (*KIAA0319L*, *HSPG2*, *FGFR1*, *c-MET*, *PDGFRA*, *ITGB5*, and *RPSA*). Then, we performed a comparative analysis of the results to examine the possibility of using miRNA profiling in the design of rAAV-based therapeutic protocols.

**Results.** Fifty-two percent of tested miRNAs were noted in at least 1 analyzed breast cancer and control tissue. Thirteen miRNAs were selected due to being outliers in the tested samples. In total, 155 miRNAs targeted genes encoding rAAV receptors in the tested samples (29 miRNAs for *KIAA0319L*, 60 miRNAs for *c-MET*, 31 miRNAs for *HSPG2*, 43 miRNAs for *FGFR1*, 36 miRNAs for *PDGFRA*, 18 miRNAs for *RPSA*, and 25 miRNAs for *ITGB5*). The expression of the selected miRNAs was not homogeneous across the 7 samples.

**Conclusions.** Profiling of microRNA could be a significant factor in the design of rAAV-based personalized gene therapy for breast cancer patients.

**Key words:** breast cancer, microRNA, gene therapy, rAAV

**Cite as**
Zajkowska A, Czajka M, Gulik K, Gawrychowski K, Małecki M. Profiling of microRNA as a tool to introduce rAAV vectors in gene therapy of breast cancer: A preliminary report. *Adv Clin Exp Med*. 2023;32(10):1201–1210. doi:10.17219/acem/171745

**DOI**
10.17219/acem/171745

# Background

As of 2020, breast cancer has become the most diagnosed cancer worldwide. Despite the development of anticancer treatments, the number of cases is still estimated to be approx. 2.26 million, causing more than 680,000 deaths in 2020.[1,2] Undoubtedly, an important factor in the treatment of the disease is effective oncological therapy, which depends on the selection of the therapeutic protocol.[3–5] Molecular diagnostics could be an extremely valuable tool in the treatment of breast cancer, which not only enables the detection of tumors but can also contribute to the selection of an appropriate oncological treatment protocol.[6–8] A novel approach in the selection of a therapeutic protocol for breast cancer could be the profiling of microRNAs (miRNAs).[8–12] MicroRNAs constitute small, single-stranded RNA molecules of 14–22 nucleotides, whose biological role is to inhibit gene expression at the level of translation.[13,14] In addition, miRNAs are characterized by their stability and tissue or cell specificity.[15] Because of these characteristics, they represent an interesting diagnostic solution with a great potential for the detection of organ injury (e.g., miRNA-208 in the diagnosis of cardiac injury[16,17]), determining the nature of the ongoing disease process (e.g., miRNA-21 as a biomarker for breast cancer[18–20]) or detecting congenital malformations of the fetus.[21–23] Moreover, miRNAs are involved in numerous physiological and pathophysiological processes, including tumorigenesis, which gives them the potential as a tool to personalize treatment, including oncological treatments, e.g., for breast cancer.[15,24,25]

The anticancer treatments that have been proposed for the treatment of breast cancer do not provide satisfactory results.[5,26,27] One of the novel therapeutic approaches could be gene therapy using recombinant adeno-associated virus (rAAV) vectors. The rAAV vectors are small, non-pathogenic, characterized by cell tropism, and display high transduction efficiency to selected cell types. Therefore, rAAV vectors are favorable candidates for the treatment of diseases, including cancer.[28–30] The use of rAAV vectors in oncology treatment could be associated with an increased survival rate of patients.[31–33] However, the transduction efficiency using rAAV vectors can be limited due to antigen-presenting cells, which stimulate the patient's immune system against transgene products that are expressed after the introduction of the rAAV vectors. This effect has the potential to reduce the effectiveness of gene therapy.[34–37] Furthermore, the effectiveness of gene therapy based on the use of viral vectors depends on the delivery of the therapeutic gene into the cell. This effect is related to the efficient binding of the viral vector to target cell surface receptors.[34–39] It is well known that miRNAs are involved in the regulation of gene expression.[13,14] Using the miRNA profile, gene therapy with the use of rAAV vectors could be personalized, resulting in increased transduction efficiency of cells in, e.g., breast cancer. The miRNA profiling could be used to develop safe and effective anticancer therapies for breast cancer.[28–30]

# Objectives

The study aimed to examine the utility of the miRNA profiling in the design of personalized rAAV-based gene therapy for breast cancer patients. In breast cancer, the presence of miRNAs targeting silencing of mRNAs encoding receptors for rAAV that condition rAAV vector transduction may result in ineffectiveness of rAAV vector-based anti-cancer therapy. Knowing the patient-specific miRNA signature could help select the best rAAV-based viral vector to guarantee the effectiveness of gene therapy.

# Materials and methods

## Breast cancer and control samples

Breast cancer tissue samples were collected from 7 patients diagnosed with breast cancer (age: 70.1 ±8.8 years). Normal breast tissue samples adjacent to the tumor were collected from 3 enrolled patients (age: 73 ±6.7 years). The experiment was performed after obtaining the approval of the Medical University of Warsaw (WUM) Bioethical Committee, Poland (approval No. KB/62/2017).

## Isolation of miRNAs

Samples were stored at –80°C in RNAlater™ Stabilization Solution (Invitrogen, Waltham, USA) until the isolation of RNA. Total RNA containing the miRNA fraction was isolated from samples using the mirVana™ miRNA Isolation Kit (Ambion; Thermo Fisher Scientific, Waltham, USA), according to the manufacturer's protocol. The RNA quality and quantity were assessed using spectrophotometric measurement (Q3000; Quawell Technology, San Jose, USA) and RNA integrity testing (Agilent 2100, RNA 6000 Nano Kit; Agilent Technologies, Santa Clara, USA), with the assessment of miRNA quantity (Agilent 2100; Small RNA Kit; Agilent Technologies).

## Assessment of miRNA profiles in breast cancer/control samples

The miRNA profiling was performed using microfluidic cards with lyophilized TaqMan Molecular Probes (TaqMan® Low-density Array (TLDA) cards), namely TaqMan® Human MicroRNA Array A (Applied Biosystem, Foster City, USA) and TaqMan® Human MicroRNA Array B v3.0 (Applied Biosystem), to detect 754 different miRNAs and non-coding RNA molecules, known as endogenous controls (RNU44 (assay ID: 001094), RNU48

(assay ID: 001006) and U6 snRNA (assay ID: 001973). First, cDNA of all tested miRNAs and non-coding RNA was synthesized using the TaqMan® MicroRNA Reverse Transcription (RT) Kit (Applied Biosystems), according to the manufacturer's protocol. An RT reaction was carried out under thermal conditions according to the manufacturer's protocol. Then, a real-time polymerase chain reaction (PCR) reaction was performed. The obtained reaction mixture was dispersed into each port included in the TLDA card. The PCR reaction was carried out using a ViiA™ 7 Real-Time PCR thermocycler (Applied Biosystems), according to the thermal profile as recommended by the manufacturer. The PCR reaction was performed in duplicate for each analyzed sample. The results were examined with the use of ExpressionSuite Software v. 1.0.3 (Thermo Fisher Scientific). A manual threshold was set at 0.2 for all samples. The miRNAs whose copy threshold (Ct) values were found to be <35 in at least one of the analyzed samples, including the control sample, were included in the in silico analysis.[40] The miRNA level was presented as an ΔCt value, which expresses the normalized Ct value relative to the endogenous control. In this study, RNU48 (assay ID: 001006) was used as the endogenous control, having the least variation within the tested samples. The results are presented using the $2^{-\Delta Ct}$ method.

## Bioinformatic analysis

Online repositories were used to perform the association analysis between breast cancer miRNAs and selected genes encoding receptors for rAAV-based vectors: miRTarBase v. 7.0 (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php); miRDB v. 5.0 (http://www.mirdb.org/), miRanda (https://bioweb.pasteur.fr/packages/pack@miRanda@3.3a), and TarBase v. 7.0 (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index). The following genes encoding receptors for AAV-based vectors were selected for the analysis: *KIAA0319L* (NM_024874), *HSPG2* (NM_005529), *FGFR1* (NM_013439), *c-MET* (NM_000245), *PDGFRA* (NM_006206), *ITGB5* (NM_002213), and *RPSA* (NM_002295).

## Statistical analyses

The results are shown as mean and 95% confidence intervals (95% CIs). The one-way analysis of variance (ANOVA) was used to compare samples in relation to ΔCt. Tukey's post-hoc test was performed to estimate statistically significant differences between the test groups. Grubbs test was used to analyze ΔCt outliers within the study samples. The results are shown with a significance level of $p < 0.05$ (see Supplementary Table 1 for details of statistical test assumptions). The statistical analysis was performed using Statistica v. 13.3 software (StatSoft Polska Sp. z o.o., Kraków, Poland).

# Results

## Profiling miRNAs in breast cancer patients

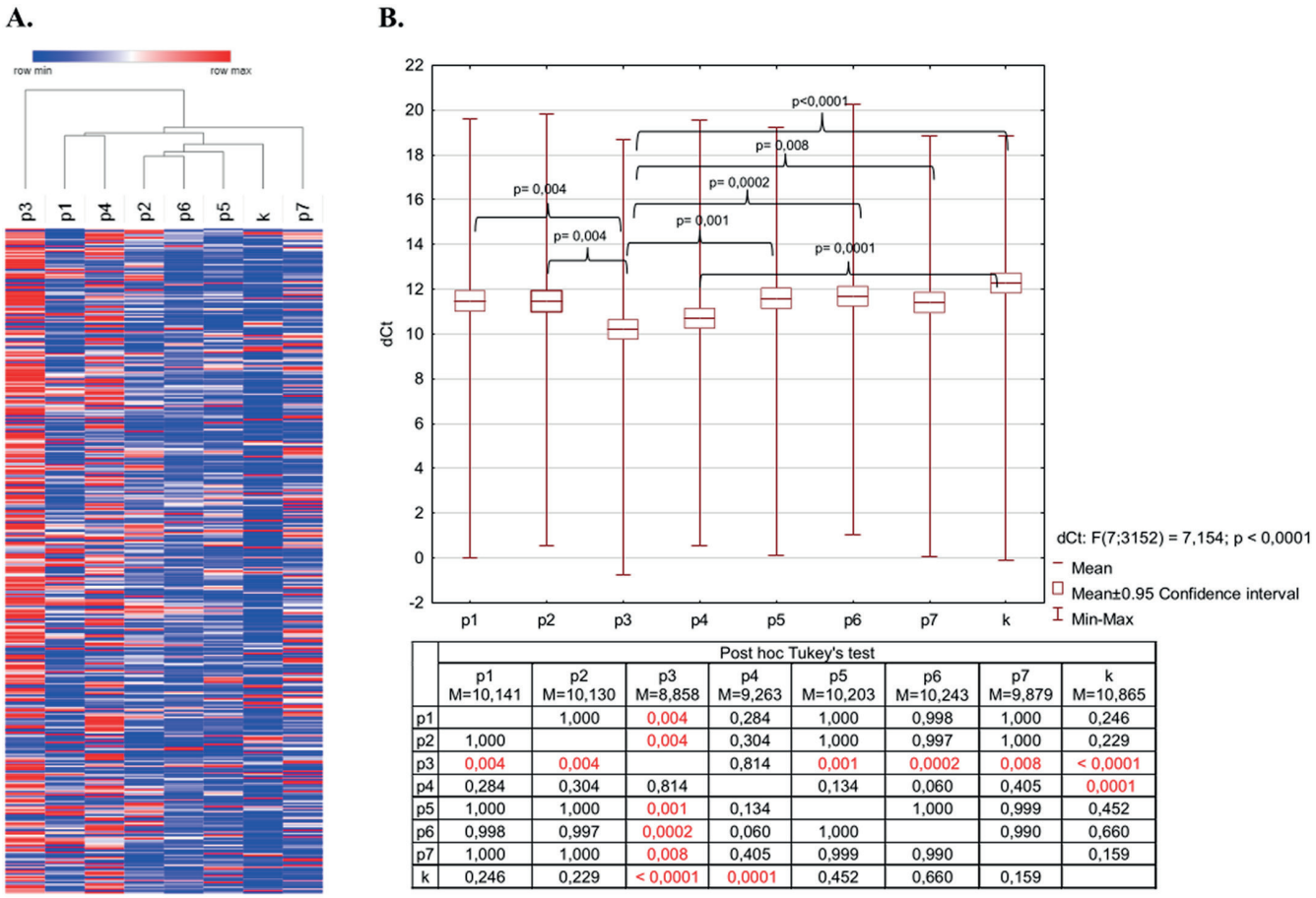
The miRNA profiling assay indicated that 52% of all miRNAs tested (representing 395 miRNAs) satisfied the sensitivity criteria of the method, i.e., showed Ct values <35 in at least one of the analyzed breast cancer and control tissues. A list of miRNAs present in breast cancer is available from the corresponding author. We found that the miRNA profile was found to be diversified across the 7 breast cancer samples (Fig. 1). The analysis of the ΔCt values in the samples showed significant differences amongst themselves and when compared to the controls. The visualization of miRNA expression in breast cancer using the heatmap (Fig. 1A) demonstrated heterogeneous levels of miRNAs, as shown for the p3 sample. It was characterized by the largest number of miRNA molecules with the highest levels of expression (highest $2^{-\Delta Ct}$ value) and was statistically different from most of the analyzed samples (Fig. 1B).

## Analysis of the most significant miRNAs in breast cancer

The statistical analysis (Grubbs test) was used to distinguish 13 miRNAs which were the most outliers among all

Table 1. miRNA with the potential to silence more than 1 gene encoding receptors for recombinant adeno-associated virus (rAAV)

| Number of simultaneously silenced rAAV receptor genes (n = 6) | miRNA |
|---|---|
| 5 | hsa-miR-22-3p, hsa-miR-34a-5p |
| 4 | hsa-let-7a-5p, hsa-let-7b-5p, hsa-miR-130a-3p, hsa-miR-218-5p, hsa-miR-335-5p, hsa-miR-449a, hsa-miR-455-3p |
| 3 | hsa-let-7e-5p, hsa-let-7f-5p, hsa-let-7g-5p, hsa-miR-141-3p, hsa-miR-15a-5p, hsa-miR-16-5p, hsa-miR-200a-3p, hsa-miR-27b-3p, hsa-miR-34c-5p, hsa-miR-532-3p, hsa-miR-543 |
| 2 | hsa-miR-101-3p, hsa-miR-125a-5p, hsa-miR-125b-5p, hsa-miR-130b-3p, hsa-miR-137, hsa-miR-146b-5p, hsa-miR-150-5p, hsa-miR-155-5p, hsa-miR-15b-5p, hsa-miR-17-5p, hsa-miR-181a-5p, hsa-miR-185-5p, hsa-miR-195-5p, hsa-miR-199a-3p, hsa-miR-20a-3p, hsa-miR-21-3p, hsa-miR-214-3p, hsa-miR-26b-5p, hsa-miR-27a-3p, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-320a, hsa-miR-330-3p, hsa-miR-362-3p, hsa-miR-424-5p, hsa-miR-425-5p, hsa-miR-449b-5p, hsa-miR-450b-5p, hsa-miR-454-3p, hsa-miR-484, hsa-miR-486-3p, hsa-miR-486-5p, hsa-miR-345-5p |




| | Post hoc Tukey's test | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | p1 M=10,141 | p2 M=10,130 | p3 M=8,858 | p4 M=9,263 | p5 M=10,203 | p6 M=10,243 | p7 M=9,879 | k M=10,865 |
| p1 | | 1,000 | 0,004 | 0,284 | 1,000 | 0,998 | 1,000 | 0,246 |
| p2 | 1,000 | | 0,004 | 0,304 | 1,000 | 0,997 | 1,000 | 0,229 |
| p3 | 0,004 | 0,004 | | 0,814 | 0,001 | 0,0002 | 0,008 | < 0,0001 |
| p4 | 0,284 | 0,304 | 0,814 | | 0,134 | 0,060 | 0,405 | 0,0001 |
| p5 | 1,000 | 1,000 | 0,001 | 0,134 | | 1,000 | 0,999 | 0,452 |
| p6 | 0,998 | 0,997 | 0,0002 | 0,060 | 1,000 | | 0,990 | 0,660 |
| p7 | 1,000 | 1,000 | 0,008 | 0,405 | 0,999 | 0,990 | | 0,159 |
| k | 0,246 | 0,229 | < 0,0001 | 0,0001 | 0,452 | 0,660 | 0,159 | |

Fig. 1. miRNA present in breast cancer and control samples. A. Heatmap shows the differential expression of miRNAs between breast cancer patients and controls ($2^{-\Delta Ct}$) (red color stands for the highest value of $2^{-\Delta Ct}$, and blue color indicates the lowest value of $2^{-\Delta Ct}$); B. The results are presented as a mean of ΔCt ±0.95 confidence interval. The statistical significance is shown (analysis of variance (ANOVA): F(7;3152) = 7.154; p < 0.0001; Tukey's post-hoc test)

p1–p7 – breast cancer samples; k – control.

the miRNAs studied in breast cancer: let-7c-5p (p = 0.0475), miRNA-1290 (p = 0.040), miRNA-187-3p (p = 0.029), miRNA-224-5p (p = 0.024), miRNA-30d-3p (p = 0.036), miRNA-425-3p (p = 0.047), miRNA-454-3p (p = 0.038), miRNA-454-5p (p = 0.043), miRNA-501-5p (p = 0.034), miRNA-766-3p (p = 0.032), miRNA-770-5p (p = 0.047), miRNA-885-5p (p = 0.026), and miRNA-99b-3p (p = 0.042). These were not characterized by homogeneous levels or even presence defined as a Ct value <35 in the tested samples. Specifically, miRNA-187-3p and miRNA-766-3p were considered absent in 28.6% of the samples, while miRNA-454-5p and miRNA-770-5p failed to meet the criterion of Ct value <35 in 71.4% and 57.1% of breast cancer samples, respectively (Fig. 2A,B).

## Analysis of the miRNA profile in breast cancer showed genes encoding rAAV vector receptors

Four online repositories were analyzed to explore the potential of miRNAs to silence receptor gene expression in breast cancer. The miRNAs were selected to potentially silence the expression of any of the tested mRNAs, namely genes encoding receptors for rAAV-mediated cell transduction (*KIAA0319L*, c-*MET*, *HSPG2*, *FGFR1*, *PDGFRA*, *RPSA*, and *ITGB5)*. The in silico analysis identified 155 miRNAs that could influence the mRNA expression of these cellular receptors for rAAV. Notably, heterogeneity was observed in miRNA levels between breast cancer samples. In addition, there were significant differences in the ΔCt values of miRNAs capable of regulating rAAV receptor expression between the control and 2 breast cancer samples (p3 and p4; Fig. 3A,B).

## miRNAs with the potential to inhibit the expression of particular rAAV receptors

Our studies have shown that all analyzed genes can be regulated by miRNAs identified in this study. The profile of the selected miRNAs was not homogeneous in the samples. However, there were no significant differences in the levels of miRNAs specific to any separately tested gene among breast cancer samples (Fig. 3). Interestingly, the selected miRNAs with the potential to silence genes encoding receptors for rAAV had not been present unequivocally in the tested samples (Table 1).

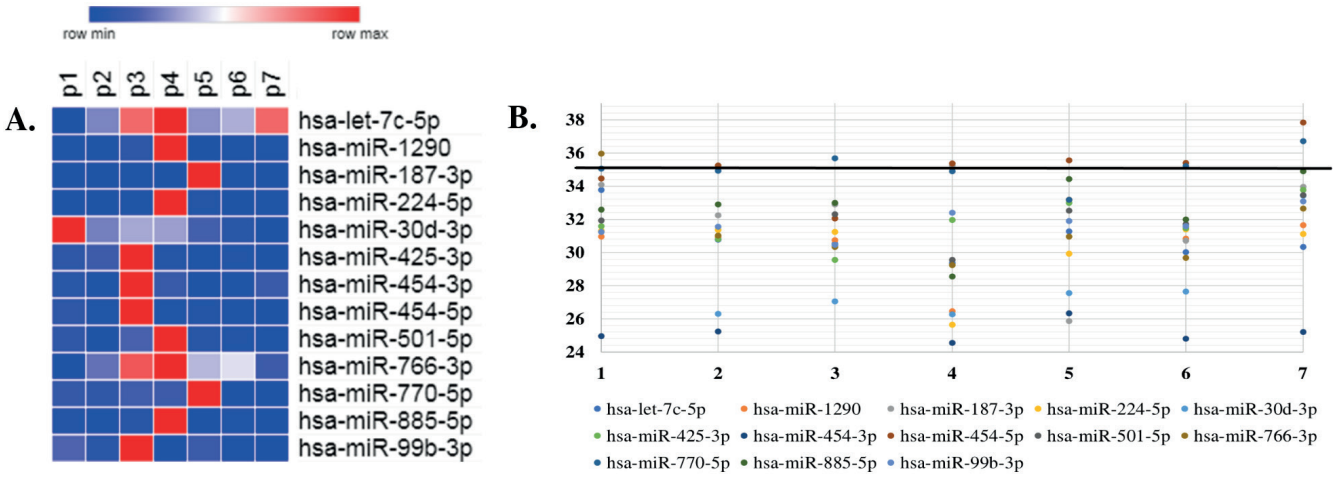
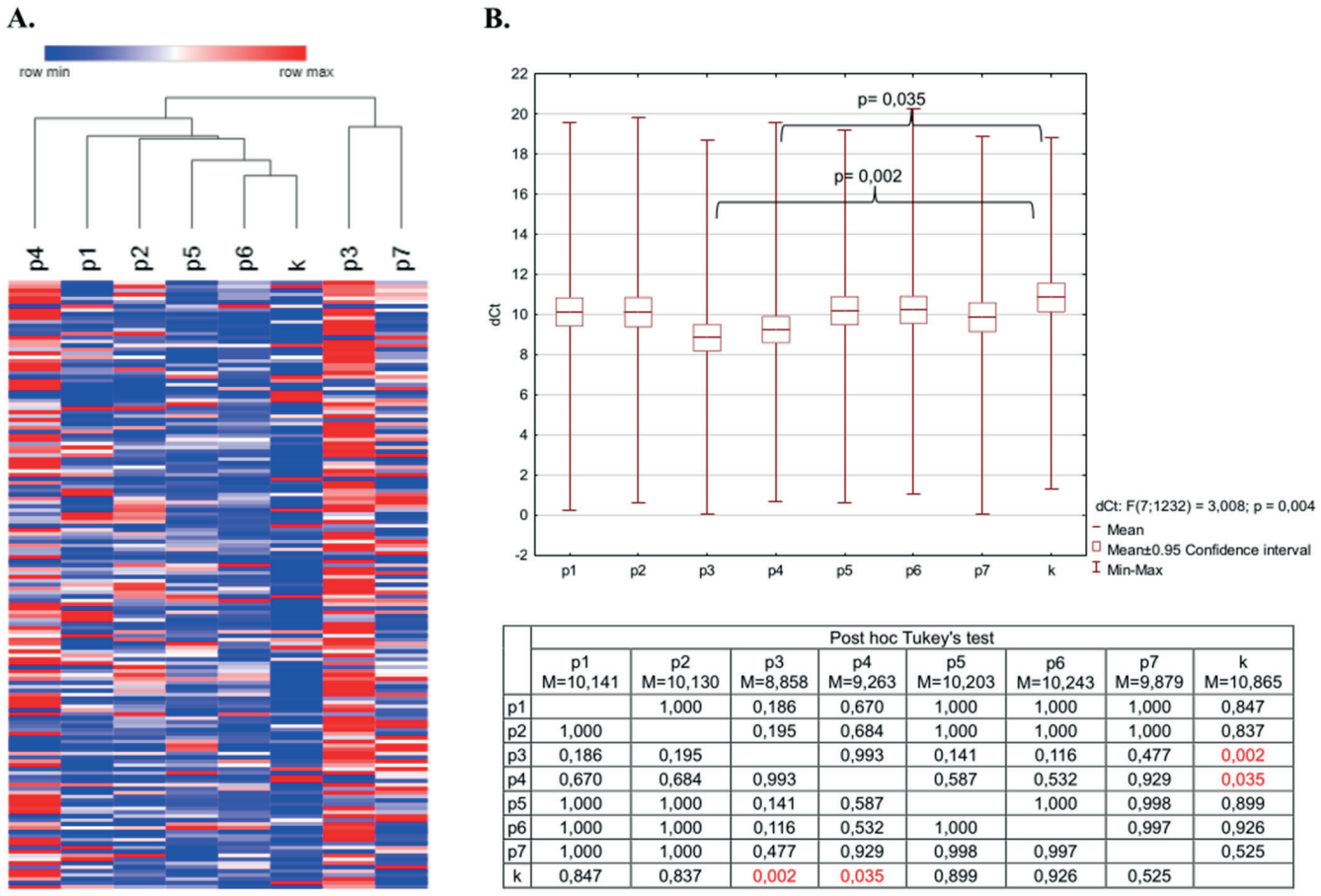



Fig. 2. The most outlier miRNAs in breast cancer sample (n = 7). A. Heatmap (red color stands for the highest value of $2^{-\Delta Ct}$, blue color indicates the lowest value of $2^{-\Delta Ct}$); B. The graph presents miRNA outliers for individual patients (p1–p7), the line indicates copy threshold (Ct) =35

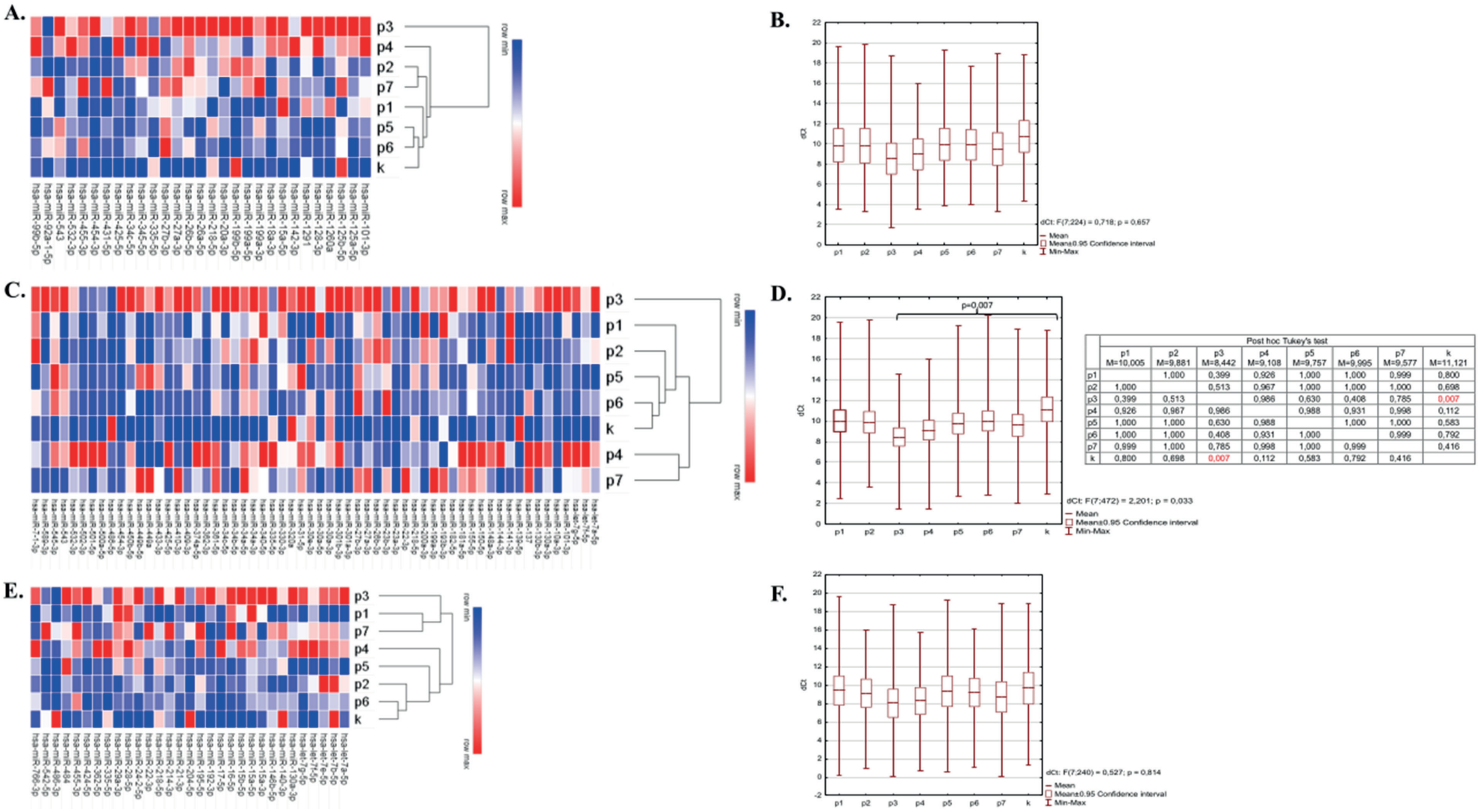



| | Post hoc Tukey's test | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | p1 M=10,141 | p2 M=10,130 | p3 M=8,858 | p4 M=9,263 | p5 M=10,203 | p6 M=10,243 | p7 M=9,879 | k M=10,865 |
| p1 | | 1,000 | 0,186 | 0,670 | 1,000 | 1,000 | 1,000 | 0,847 |
| p2 | 1,000 | | 0,195 | 0,684 | 1,000 | 1,000 | 1,000 | 0,837 |
| p3 | 0,186 | 0,195 | | 0,993 | 0,141 | 0,116 | 0,477 | 0,002 |
| p4 | 0,670 | 0,684 | 0,993 | | 0,587 | 0,532 | 0,929 | 0,035 |
| p5 | 1,000 | 1,000 | 0,141 | 0,587 | | 1,000 | 0,998 | 0,899 |
| p6 | 1,000 | 1,000 | 0,116 | 0,532 | 1,000 | | 0,997 | 0,926 |
| p7 | 1,000 | 1,000 | 0,477 | 0,929 | 0,998 | 0,997 | | 0,525 |
| k | 0,847 | 0,837 | 0,002 | 0,035 | 0,899 | 0,926 | 0,525 | |

Fig. 3. miRNAs potentially inhibit 7 genes encoding receptors for recombinant adeno-associated virus (rAAV). A. Heatmap shows the differential expression of miRNAs between breast cancer patients and controls ($2^{-\Delta Ct}$). Red color stands for the highest value of $2^{-\Delta Ct}$, and blue color indicates the lowest value of $2^{-\Delta Ct}$; B. The results are presented as mean of ΔCt ±0.95 confidence interval. The statistical significance is presented (analysis of variance (ANOVA): F(7;1232) = 3.008; p = 0.004; post-hoc Tukey's test)

p1–p7 – breast cancer samples; k – control.

We found that 36.8% of the selected miRNAs are complementary to more than one mRNA of the analyzed genes. It is particularly evident for miRNA-22-3p and miRNA-34a-5p, which have the potential to silence as many as 5 genes encoding rAAV receptors (*c-MET*, *HSPG2*, *FGFR1*, *RPSA*, *ITGB5*, and *c-MET*, *FGFR1*, *RPSA*, *ITGB5*, *PDGFRA*, respectively) (Table 1).




Post hoc Tukey's test

| | p1 M=10,005 | p2 M=9,881 | p3 M=8,442 | p4 M=9,108 | p5 M=9,757 | p6 M=9,995 | p7 M=9,577 | k M=11,121 |
|---|---|---|---|---|---|---|---|---|
| p1 | | 1,000 | 0,399 | 0,926 | 1,000 | 1,000 | 0,999 | 0,800 |
| p2 | 1,000 | | 0,513 | 0,967 | 1,000 | 1,000 | 1,000 | 0,698 |
| p3 | 0,399 | 0,513 | | 0,986 | 0,630 | 0,408 | 0,785 | 0,007 |
| p4 | 0,926 | 0,967 | 0,986 | | 0,988 | 0,931 | 0,998 | 0,112 |
| p5 | 1,000 | 1,000 | 0,630 | 0,988 | | 1,000 | 1,000 | 0,583 |
| p6 | 1,000 | 1,000 | 0,408 | 0,931 | 1,000 | | 0,999 | 0,792 |
| p7 | 0,999 | 1,000 | 0,785 | 0,998 | 1,000 | 0,999 | | 0,416 |
| k | 0,800 | 0,698 | 0,007 | 0,112 | 0,583 | 0,792 | 0,416 | |

**Fig. 4.** A,C,E. Heatmap showing the differential expression of miRNAs between breast cancer patients and controls ($2^{-\Delta Ct}$) for *KIAA0319L* (A), *c-MET* (C) and *HSPG2* (E) genes; B,D,F. The results are presented as mean of ΔCt ±0.95 confidence interval, and the outcomes of the analysis of variance for *KIAA0319L* (B), *c-MET* (D) and *HSPG2* (F) genes. The statistical significance is presented (analysis of variance (ANOVA): F(7;224) = 0.718; p = 0.657 (B), F(7;472) = 2.201; p = 0.033 (D), F(7;240) = 0.527; p = 0.814 (F); Tukey's post-hoc test (D)). Red color stands for the highest value of $2^{-\Delta Ct}$, and blue color indicates the lowest value of $2^{-\Delta Ct}$

p1–p7 – breast cancer samples; k – control.

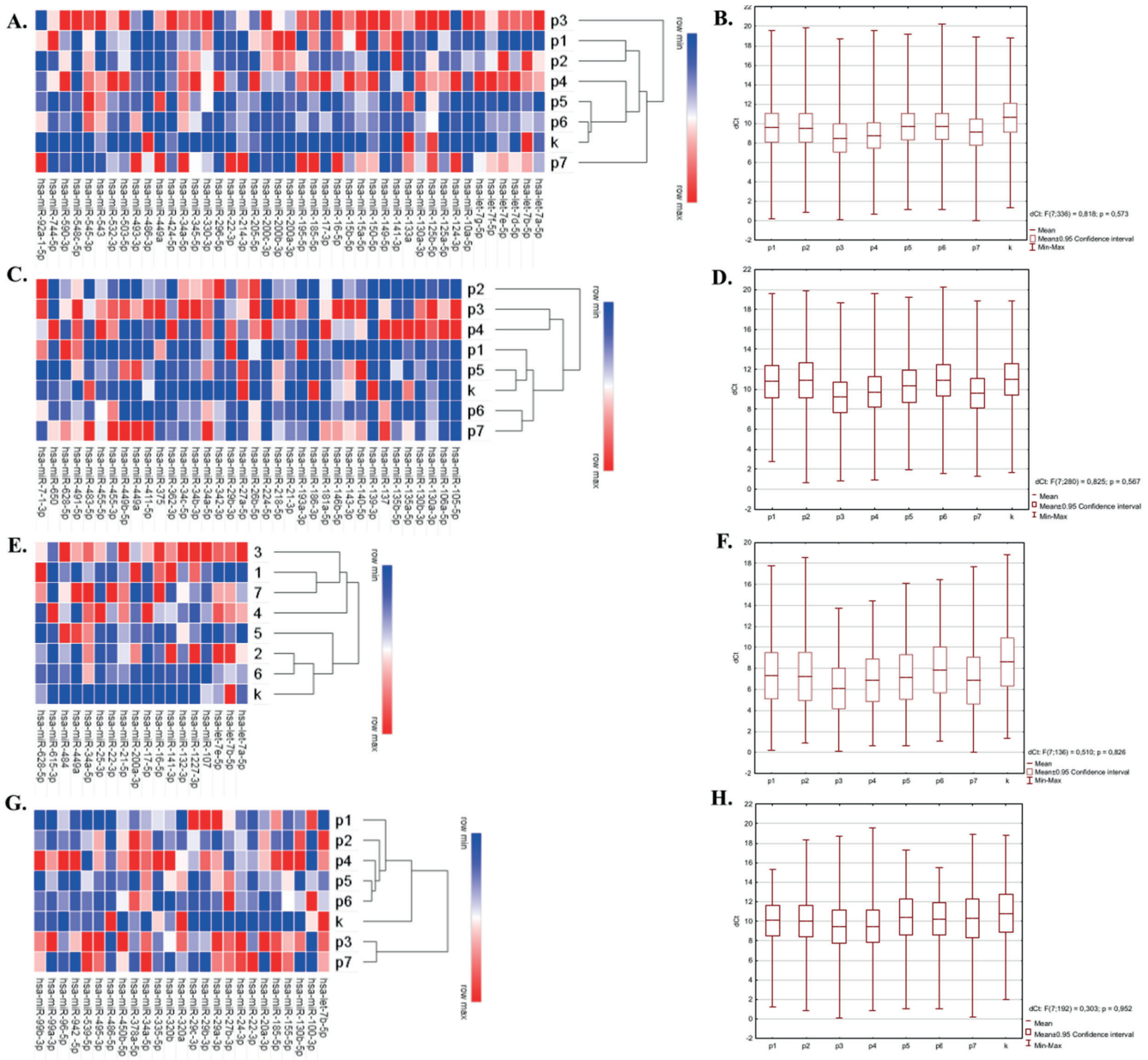



Fig. 5. A,C,E,G. Heatmap showing the differential expression of miRNAs between breast cancer patients and controls ($2^{-\Delta Ct}$) for *FGFR1* (A), *PDGFRA* (C), *RPSA* (E), and *ITGB5* (G) genes; B. The results are presented as mean, and the outcomes of the analysis of variance (ANOVA) for *FGFR1* (B), *PDGFRA* (D), *RPSA* (F), and *ITGB5* (H) genes are displayed. The statistical analysis is shown (ANOVA: F(7;336) = 0.818; p = 0.573 (B), F(7;280) = 0.825; p = 0.567 (D), F(7;136) = 0.510; p = 0.826 (F), F(7;192) = 0.303; p = 0.952 (H)). Red color stands for the highest value of $2^{-\Delta Ct}$, and blue color indicates the lowest value of $2^{-\Delta Ct}$

p1–p7 – breast cancer samples; k – control.

### *KIAA0319L*, *c-MET* and *HSPG2* genes

Among the analyzed miRNAs, 29 demonstrated a probability of silencing the *KIAA0319L* gene (Fig. 4A), 60 could silence the c-*MET* gene (Fig. 4C), and 31 could silence the *HSPG2* gene (Fig. 4E). The variation of miRNA levels was visualized using a heatmap, and cluster analysis was performed for the breast cancer samples (Fig. 4A,C,E, respectively). The miRNA profile of the p3 sample is distinguished in the range of $2^{-\Delta Ct}$ values (Fig. 4A,C,E). Furthermore, ANOVA showed no statistical significance between the ΔCt values among the breast cancer samples tested for the *KIAA0319L* gene (Fig. 4B) and *HSPG2* gene (Fig. 4F). However, it demonstrated significant differences between the ΔCt values of miRNAs in the p3 sample and control tissue (k) for the c-*MET* gene (Fig. 4D).

### *FGFR1*, *PDGFRA*, *RPSA*, and *ITGB5* genes

The current study found 43 miRNAs capable of silencing the *FGFR1* gene (Fig. 5A), 36 miRNAs that could silence the *PDGFRA* gene (Fig. 5C), 18 for the *RPSA* gene (Fig. 5E), and 25 for the *ITGB5* gene (Fig. 5G). These miRNAs could all potentially silence the expression of the genes encoding receptors necessary for the entry of rAAV. No significant differences were observed in the ΔCt of either the breast cancer or control samples (Fig. 5B,D,F,H).

## Discussion

Breast cancer is the most common cancer diagnosed globally in female patients,[1,2,41,42] thus various innovative treatment options and diagnostics continue to be developed.[5,6,27,43–45] Certainly, one of the novel treatment approaches could be gene therapy.[46,47] In this regard, the most frequently utilized gene carriers are viral vectors.[48,49] The rAAV vectors are of particular interest because of their unique features.[28–32] The synthesis of the corresponding protein is influenced by several factors, including miRNAs. Interestingly, previous studies demonstrated that the expression of up to 60% of human genes is regulated by miRNAs, which have the potential to inhibit the formation of correct protein. Therefore, miRNAs are extremely important in the design of gene therapy, including breast cancer therapy, in which personalization is the goal, and where the most ideal viral vector needs to be selected.[35,39] The purpose of this study was to highlight whether endogenous miRNAs can act as potential biomarkers in the personalization of rAAV vector-based gene therapy for breast cancer. We analyzed the miRNA profile in breast cancer samples, which were obtained from 7 patients using quantitative (q)PCR TLDA cards. The study demonstrated that 52% of miRNA molecules, representing 395 of the 754 molecules, were positive in at least 1 of the tested samples (Fig. 1A). The paper also examined the diversity of levels of all included miRNAs. The level expressed as ΔCt was found to vary over the range of tested samples (Fig. 1B). It is noteworthy that the p3 sample shows a high degree of variability and is significantly different from the other breast cancer and control samples (Fig. 1A). Moreover, the miRNA profile for the individual probes varies, which has the potential to be a consequence of the molecular subtype of breast cancer. Blenkiron et al. showed that the profile of miRNA is variable and characteristic, depending on the molecular subtype of breast cancer.[50] In addition, a cluster analysis of the breast cancer samples conducted by Blenkiron et al. presented a high degree of miRNA diversity. The authors suggested it to be a result of miRNA deregulation in cancer cells. Furthermore, Tsai et al. depicted the varying expression of miRNAs in breast cancer.[51] The researchers demonstrated a heterogeneous profile of miRNA molecules that depend on the age of patients, the tumor lesion and the hormone receptor profile. Similar studies indicating the presence of miRNA heterogeneity in breast cancer were conducted by Sempere et al.[52] In this study, the levels of miRNA heterogeneity were analyzed. In breast cancer samples tested herein, the levels of miRNA varied the most for the molecules presented in Fig. 2. A distinguishing property of miRNAs is that they can function both as molecules that promote or inhibit the neoplastic process within the same tumor.[53,54] Fluctuations in the level of miRNAs observed herein suggest the individualism of the studied sample, which was also shown in the study by Galka-Marciniak et al.[55] In this work, miRNAs in neoplastic tissue were analyzed in association with genes that are involved in the formation of protein surface receptors of cells targeted by rAAV vectors. These vectors are increasingly used in clinical trials of gene therapy, including anticancer therapy. More importantly, several drugs based on rAAV have been registered, including Zolgensma (U.S. Food and Drug Administration (FDA)), Luxturna (European Medicines Agency (EMA)) and Glybera (EMA). The in silico analysis showed that the expression of each of the selected genes could be regulated by dozens of miRNAs. Moreover, a single miRNA can influence the regulation of several genes encoding receptor proteins that target viral vectors. Examples include miR-22-3p and miR-34a-5p, both of which can silence 5 of the 7 genes tested in the rAAV vector (e.g., miR-22-3p inhibits the expression of c-*MET*, *HSPG2*, *FGFR1*, *RPSA*, and *ITGB5*) (Table 1). The significant variance across miRNA levels between the tested samples can influence the expression of receptor proteins, which are essential for the introduction of viral vectors into target cells. For instance, miRNA-224-5p is a molecular target of the *PDGFRA* gene, whose protein is a co-receptor for rAAV5. Conversely, miR-766-3p can potentially be involved in inhibiting the expression of the hspg2 protein, which is a receptor for the majority of rAAV vectors.[38,39] By identifying these miRNAs for individual patients, it is entirely appropriate to propose a gene therapy based on a particular targeted rAAV or to exclude it completely. It is also interesting to observe that out of the 754 miRNAs indicated in our breast cancer samples, 395 were identified with PCR, of which just 155 could potentially inhibit the expression of rAAV receptors (Fig. 3A). These findings highlight that designing an appropriate vector for gene therapy of breast cancer could prove to be a significant achievement. Among the most aberrant miRNAs in the tested breast cancer samples (Fig. 2A), 5 participated in the potential regulation of receptor protein expression for rAAV vectors. They were determinable for all samples except miR-766-3p, which was not determined in the p1 probe. Based on the results, the differences in ΔCt of miRNA levels for the p3 and p4 samples were observed (Fig. 3A). The ΔCt levels were lower in p3 and p4 samples, indicating higher miRNA levels compared to other samples. This higher level of miRNAs could directly influence the expression of genes they control. Based on the patient's susceptibility to a specific rAAV serotype, it was determined that the miRNA profile could be useful in determining the efficacy of gene therapy using viral vectors. Significant differences in miRNA expression can be observed in the investigated samples (Fig. 1). These differences could indicate that miRNA profiling is a useful step in the development and implementation of the selected gene therapy protocol. Concerning rAAV receptors, for example, in the p5 sample, 22.6% of the total miRNA was undetected (data not shown). In contrast, only 7.1% of miRNA molecules in the p4 sample were not detected (data not shown). Based on these results, it could

be concluded that the sample with the lowest number of undetectable miRNAs could be predisposed to gene therapy based on the rAAV vectors. Conversely, the sample with the highest number of miRNAs is less likely to be transduced by viral vectors. Among the miRNAs tested in the study, those not determined in association with silencing of the rAAV vector receptor genes made up a small percentage. The group of examined samples showed differences in the detection of 155 miRNAs, which were identified in this study as molecules that recognize receptor genes exploited by rAAVs.

## Limitations

The limitation of the study is the relatively small number of tested breast cancer samples and insufficient knowledge of medical history and sample collection. However, a major advantage of the study is the broad examination of miRNAs.

# Conclusions

In summary, the obtained results demonstrate that personalized gene therapy for breast cancer could be designed using rAAV vectors. According to the selected miRNA molecules in the performed studies, it would have been possible to estimate the presence of the receptor protein indirectly in a particular breast cancer patient. This novel approach could be of considerable clinical relevance and could indicate whether rAAVs are the ideal vectors for gene therapy in a particular patient. The main purpose of the work was to thoroughly investigate the molecular signature of breast cancer, which would be a prerequisite for personalized gene therapy.

## Supplementary data

The supplementary materials are available at https://doi.org/10.5281/zenodo.8213977. The package contains the following files:

Supplementary Table 1. Test assumptions in the statistical analysis.


### ORCID iDs

Agnieszka Zajkowska https://orcid.org/0000-0002-6664-9705
Milena Czajka https://orcid.org/0000-0001-5791-643X
Krystian Gulik https://orcid.org/0000-0002-4003-653X
Krzysztof Gawrychowski https://orcid.org/0000-0002-3666-3506
Maciej Małecki https://orcid.org/0000-0002-7078-4918


### References


1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA A Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Heng YJ, Lester SC, Tse GM, et al. The molecular basis of breast cancer pathological phenotypes. *J Pathol*. 2017;241(3):375–391. doi:10.1002/path.4847
4. Grogan Fleege NM, Cobain EF. Breast cancer management in 2021: A primer for the obstetrics and gynecology. *Best Pract Res Clin Obstet Gynaecol*. 2022;82:30–45. doi:10.1016/j.bpobgyn.2022.02.004
5. Trayes KP, Cokenakes SEH. Breast cancer treatment. *Am Fam Physician*. 2021;104(2):171–178. PMID:34383430.
6. Tamimi RM, Baer HJ, Marotti J, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. *Breast Cancer Res*. 2008;10(4):R67. doi:10.1186/bcr2128
7. Litton JK, Burstein HJ, Turner NC. Molecular testing in breast cancer. *Am Soc Clin Oncol Educ Book*. 2019;39:e1–e7. doi:10.1200/EDBK_237715
8. Afzal S, Hassan M, Ullah S, Abbas H, Tawakkal F, Khan MA. Breast cancer: Discovery of novel diagnostic biomarkers, drug resistance, and therapeutic implications. *Front Mol Biosci*. 2022;9:783450. doi:10.3389/fmolb.2022.783450
9. Hussen BM, Abdullah ST, Rasul MF, et al. MicroRNAs: Important players in breast cancer angiogenesis and therapeutic targets. *Front Mol Biosci*. 2021;8:764025. doi:10.3389/fmolb.2021.764025
10. Long X, Shi Y, Ye P, Guo J, Zhou Q, Tang Y. MicroRNA-99a suppresses breast cancer progression by targeting FGFR3. *Front Oncol*. 2020;9:1473. doi:10.3389/fonc.2019.01473
11. Jang J, Kim Y, Kang K, Kim K, Park Y, Kim C. Multiple microRNAs as biomarkers for early breast cancer diagnosis. *Mol Clin Oncol*. 2020;14(2):31. doi:10.3892/mco.2020.2193
12. Loh HY, Norman BP, Lai KS, Rahman NMANA, Alitheen NBM, Osman MA. The regulatory role of microRNAs in breast cancer. *Int J Mol Sci*. 2019;20(19):4940. doi:10.3390/ijms20194940
13. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. *Br J Haematol*. 2008;141(5):672–675. doi:10.1111/j.1365-2141.2008.07077.x
14. Mehta A, Baltimore D. MicroRNAs as regulatory elements in immune system logic. *Nat Rev Immunol*. 2016;16(5):279–294. doi:10.1038/nri.2016.40
15. Rupaimoole R, Slack FJ. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discov*. 2017;16(3):203–222. doi:10.1038/nrd.2016.246
16. Kalayinia S, Arjmand F, Maleki M, Malakootian M, Singh CP. MicroRNAs: Roles in cardiovascular development and disease. *Cardiovasc Pathol*. 2021;50:107296. doi:10.1016/j.carpath.2020.107296
17. Zhao X, Wang Y, Sun X. The functions of microRNA-208 in the heart. *Diabetes Res Clin Pract*. 2020;160:108004. doi:10.1016/j.diabres.2020.108004
18. Bautista-Sánchez D, Arriaga-Canon C, Pedroza-Torres A, et al. The promising role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics. *Mol Ther Nucleic Acids*. 2020;20:409–420. doi:10.1016/j.omtn.2020.03.003
19. Arisan ED, Rencuzogullari O, Cieza-Borrella C, et al. MiR-21 is required for the epithelial–mesenchymal transition in MDA-MB-231 breast cancer cells. *Int J Mol Sci*. 2021;22(4):1557. doi:10.3390/ijms22041557
20. Wang H, Tan Z, Hu H, et al. microRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1. *BMC Cancer*. 2019;19(1):738. doi:10.1186/s12885-019-5951-3
21. Seelan RS, Pisano MM, Greene RM. MicroRNAs as biomarkers for birth defects. *Microrna*. 2022;11(1):2–11. doi:10.2174/2211536611666220215123423
22. Légaré C, Clément AA, Desgagné V, et al. Human plasma pregnancy-associated miRNAs and their temporal variation within the first trimester of pregnancy. *Reprod Biol Endocrinol*. 2022;20(1):14. doi:10.1186/s12958-021-00883-1
23. Gu H, Chen L, Xue J, et al. Expression profile of maternal circulating microRNAs as non-invasive biomarkers for prenatal diagnosis of congenital heart defects. *Biomed Pharmacother*. 2019;109:823–830. doi:10.1016/j.biopha.2018.10.110
24. Khan P, Siddiqui JA, Lakshmanan I, et al. RNA-based therapies: A cog in the wheel of lung cancer defense. *Mol Cancer*. 2021;20(1):54. doi:10.1186/s12943-021-01338-2
25. Mollaei H, Safaralizadeh R, Rostami Z. MicroRNA replacement therapy in cancer. *J Cell Physiol*. 2019;234(8):12369–12384. doi:10.1002/jcp.28058

26. Barzaman K, Karami J, Zarei Z, et al. Breast cancer: Biology, biomarkers, and treatments. *Int Immunopharmacol*. 2020;84:106535. doi:10.1016/j.intimp.2020.106535
27. Nagini S. Breast cancer: Current molecular therapeutic targets and new players. *Anticancer Agents Med Chem*. 2017;17(2):152–163. doi:10.2174/1871520616666160502122724
28. Czajka M, Zajkowska A, Gawlak M, Bujalska-Zadrozny M, Malecki M. Mosaic recombinant adeno-associated virus vector rAAV/DJ/CAG for targeted gene delivery to melanoma cells metastasized to the lung. *Anticancer Res*. 2020;40(8):4425–4444. doi:10.21873/anticanres.14448
29. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*. 2019;18(5):358–378. doi:10.1038/s41573-019-0012-9
30. Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. *Nat Rev Genet*. 2020;21(4):255–272. doi:10.1038/s41576-019-0205-4
31. Santiago-Ortiz JL, Schaffer DV. Adeno-associated virus (AAV) vectors in cancer gene therapy. *J Control Release*. 2016;240:287–301. doi:10.1016/j.jconrel.2016.01.001
32. He ZH, Lei Z, Zhen Y, et al. Adeno-associated virus-mediated expression of recombinant CBD–HepII polypeptide of human fibronectin inhibits metastasis of breast cancer. *Breast Cancer Res Treat*. 2014;143(1):33–45. doi:10.1007/s10549-013-2783-8
33. Asad AS, Moreno Ayala MA, Gottardo MF, et al. Viral gene therapy for breast cancer: Progress and challenges. *Exp Opin Biol Ther*. 2017;17(8):945–959. doi:10.1080/14712598.2017.1338684
34. Geisler A, Fechner H. MicroRNA-regulated viral vectors for gene therapy. *World J Exp Med*. 2016;6(2):37. doi:10.5493/wjem.v6.i2.37
35. Lu Y, Song S. Distinct immune responses to transgene products from rAAV1 and rAAV8 vectors. *Proc Natl Acad Sci U S A*. 2009;106(40):17158–17162. doi:10.1073/pnas.0909520106
36. Shirley JL, De Jong YP, Terhorst C, Herzog RW. Immune responses to viral gene therapy vectors. *Mol Ther*. 2020;28(3):709–722. doi:10.1016/j.ymthe.2020.01.001
37. Muhuri M, Zhan W, Maeda Y, et al. Novel combinatorial microRNA-binding sites in AAV vectors synergistically diminish antigen presentation and transgene immunity for efficient and stable transduction. *Front Immunol*. 2021;12:674242. doi:10.3389/fimmu.2021.674242
38. Pillay S, Zou W, Cheng F, et al. Adeno-associated virus (AAV) serotypes have distinctive interactions with domains of the cellular AAV receptor. *J Virol*. 2017;91(18):e00391–17. doi:10.1128/JVI.00391-17
39. Pillay S, Meyer NL, Puschnik AS, et al. An essential receptor for adeno-associated virus infection. *Nature*. 2016;530(7588):108–112. doi:10.1038/nature16465
40. Roy B, Wang Q, Palkovits M, Faludi G, Dwivedi Y. Altered miRNA expression network in locus coeruleus of depressed suicide subjects. *Sci Rep*. 2017;7(1):4387. doi:10.1038/s41598-017-04300-9
41. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer: Epidemiology, risk factors, classification, prognostic markers, and current treatment strategies. An updated review. *Cancers*. 2021;13(17):4287. doi:10.3390/cancers13174287
42. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA A Cancer J Clin*. 2022;72(1):7–33. doi:10.3322/caac.21708
43. Bradley R, Braybrooke J, Gray R, et al. Aromatase inhibitors versus tamoxifen in premenopausal women with oestrogen receptor-positive early-stage breast cancer treated with ovarian suppression: A patient-level meta-analysis of 7030 women from four randomised trials. *Lancet Oncol*. 2022;23(3):382–392. doi:10.1016/S1470-2045(21)00758-0
44. Cortés J, Kim SB, Chung WP, et al. Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer. *N Engl J Med*. 2022;386(12):1143–1154. doi:10.1056/NEJMoa2115022
45. Portman N, Lim E. A new sophistication for breast cancer PDXs. *Nat Cancer*. 2022;3(2):138–140. doi:10.1038/s43018-021-00328-z
46. Belete TM. The current status of gene therapy for the treatment of cancer. *Biol Targets Ther*. 2021;15:67–77. doi:10.2147/BTT.S302095
47. Montaño-Samaniego M, Bravo-Estupiñan DM, Méndez-Guerrero O, Alarcón-Hernández E, Ibáñez-Hernández M. Strategies for targeting gene therapy in cancer cells with tumor-specific promoters. *Front Oncol*. 2020;10:605380. doi:10.3389/fonc.2020.605380
48. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Sig Transduct Target Ther*. 2021;6(1):53. doi:10.1038/s41392-021-00487-6
49. Schmit PF, Pacouret S, Zinn E, et al. Cross-packaging and capsid mosaic formation in multiplexed AAV libraries. *Mol Ther Methods Clin Dev*. 2020;17:107–121. doi:10.1016/j.omtm.2019.11.014
50. Blenkiron C, Goldstein LD, Thorne NP, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. *Genome Biol*. 2007;8(10):R214. doi:10.1186/gb-2007-8-10-r214
51. Tsai HP, Huang SF, Li CF, Chien HT, Chen SC. Differential microRNA expression in breast cancer with different onset age. *PLoS One*. 2018;13(1):e0191195. doi:10.1371/journal.pone.0191195
52. Sempere LF, Christensen M, Silahtaroglu A, et al. Altered microRNA expression confined to specific epithelial cell subpopulations in breast cancer. *Cancer Res*. 2007;67(24):11612–11620. doi:10.1158/0008-5472.CAN-07-5019
53. Szczepanek J, Skorupa M, Tretyn A. MicroRNA as a potential therapeutic molecule in cancer. *Cells*. 2022;11(6):1008. doi:10.3390/cells11061008
54. Tan W, Liu B, Qu S, Liang G, Luo W, Gong C. MicroRNAs and cancer: Key paradigms in molecular therapy. *Oncol Lett*. 2017;15(3):2735–2742. doi:10.3892/ol.2017.7638
55. Galka-Marciniak P, Urbanek-Trzeciak MO, Nawrocka PM, Kozlowski P. A pan-cancer atlas of somatic mutations in miRNA biogenesis genes. *Nucl Acids Res*. 2021;49(2):601–620. doi:10.1093/nar/gkaa1223