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# Insights in geriatric medicine 2021

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# Insights in geriatric medicine: 2021

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# Editorial: Insights in geriatric medicine: 2021

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Editorial on the Research Topic
Insights in geriatric medicine: 2021

The COVID-19 pandemic caught the world by surprise. The initial response was largely one of uncertainty, instability, even panic (1). As countries began to gain insight into the behavior of a highly contagious virus with serious health consequences on the individual and on health care services, responses became more organized and focused. On this background of the COVID-19 pandemic, Frontiers in Medicine took the lead in providing clinicians and scientists an opportunity to share their innovative research involving older people in a special Research Topic entitled *Insights in Geriatric Medicine: 2021*. The impact of the pandemic on this Research Topic was clear, with the submission of a number of studies relating specifically to the older population and COVID-19. However, it was encouraging to see the submission of an array of scientific research focusing on many aspects of the health of older people. The resulting Research Topic is interesting and innovative.

Focusing on COVID-19, Kyriazis et al. describe the consequences of prolonged lockdown and social distancing on older people in Cyprus. The authors found an increased risk of death from causes other than COVID-19 in older people. The authors call for a clear policy based on a comprehensive multifaceted approach, including medical, social, physical and psychological elements.

An interesting and timely meta-analysis that estimated the prevalence of insomnia symptoms in older Chinese adults during the COVID-19 pandemic evaluated nine studies with a total of 27,207 older Chinese adults. Zhang et al. found that almost one-quarter of those studied reported moderate insomnia symptoms, while 11.1% rated symptoms as severe. Not surprisingly, prevalence rates of insomnia symptoms were significantly higher for those living in the COVID-19 epicenter. The authors correctly highlight the need for supportive mental health services, with a specific focus on the assessment of insomnia symptoms.

Long COVID is increasingly recognized as a true entity that should be characterized and studied further. The study reported by Damanti et al. explored the prevalence of Long COVID-19 symptoms following discharge from admission for acute hospital care. The authors found that compared to robust patients, those who were frail were more likely to be malnourished and had a higher risk of sarcopenia and poor physical performance following discharge from hospital.

Moving away from COVID-19, the collection of manuscripts in this Research Topic also focus on other important areas relating to Geriatric care. One such condition is acute acalculous cholecystitis. A delay in the diagnosis of this condition may result in serious life-threatening complications, such as gangrene or perforation of the gallbladder. Lin et al. retrospectively investigated 374 older bedridden patients with clinical manifestations of acute acalculous cholecystitis. Patients with acute acalculous cholecystitis had a significantly higher incidence of complications, and a longer duration of symptoms and of antibiotic therapy. The authors emphasize the importance of early imaging using recognized diagnostic criteria in older patients with clinical manifestations of acute acalculous cholecystitis.

Acute hospital admission is challenging for older people, who are more prone to delirium and functional decline. The correct approach is clearly one of prevention, since pharmacotherapeutic interventions for delirium are associated with adverse events and have limited efficacy (2, 3). Aomura et al. posed an interesting hypothesis, questioning whether admission to a window-side bed was associated with the development of delirium in older patients admitted to a general ward. Within the limitations of this study, no significant association was found between the position of the hospital bed near a window and the development of delirium.

Recognizing the many factors contributing to increased post-hospitalization mortality, Lattanzio et al. conducted a well-designed multicenter prospective. Dependency in basic activities of daily living and anticholinergic cognitive burden were highly associated with poorer survival at 1 year post discharge, and to a lesser degree those with cognitive impairment had a significant risk of mortality. This study provides an important insight into those risk factors that should be identified and monitored to improve post-hospitalization prognosis.

An important etiological factor leading to acute hospitalization is sepsis. Lang et al. postulate that changes in DNA methylation, which have been shown to be linked to the aging process and to age-related diseases, may play a role in the mechanism and prognosis of sepsis in the older patient. Indeed, they found a significant correlation between 161 CpG methylation sites and poorer outcomes in the older sepsis group. This interesting finding may contribute to improving our understanding as to why older patients are more susceptible to sepsis with poorer outcomes.

The need to undergo major surgery challenges the health care team caring for the older patient. Factors influencing the course of surgery and outcome should be clearly identified. One such factor is polypharmacy, which has been clearly recognized as a geriatric giant with untoward effects on the health and wellbeing of older patients (4). Lertkovit et al. conducted a single-center prospective study to determine the prevalence of polypharmacy in 250 older patients undergoing elective major surgery. While there was no significant association between polypharmacy and postoperative cognitive dysfunction, there was some association between intraoperative therapeutic compounds and postoperative cognitive dysfunction.

The biology of human aging is complex and fascinating. Conte et al. provide an interesting insight onto one of the most interesting aspects of aging, namely the presence of chronic low-grade inflammation or inflammaging. Low-grade inflammation is associated with an increased risk for atherosclerosis and cardiovascular disease, and this inflammation is promoted by visceral adiposity. The authors relate to the epicardial adipose tissue, which constitutes the visceral fat depot of the heart, and which has been shown to be associated with a number of heart diseases.

The study by de Mello et al. helps to contribute further evidence in improving the healthcare of older adults. The authors aimed to determine the appropriate drug scheduling regimen for the administration of L-thyroxine in treating hypothyroidism in those older than 60 years of age. They found that thyroid-stimulating hormone (TSH) levels were similar during the follow-up period, and that there was no significant difference between morning or bedtime doses. This finding has clinical ramifications for drug scheduling in older adults.

Prostate cancer is the second leading cause of cancer death in American men, and the incidence increases with age. The two most important treatment modalities for localized prostate cancer are radical prostatectomy and intensity-modulated radiation therapy. Wu et al. conducted an important study to evaluate the differences in long-term medical resource consumption between these two forms of treatment. They found that the long-term medical resource consumption was higher in older men with high-risk localized prostate cancer undergoing intensity-modulated radiation therapy than in those undergoing radical prostatectomy.

The World Health Organization Clinical Consortium on healthy aging focused on frailty and intrinsic capacity (5). A clear emphasis was placed on functional ability, representing a combination of the intrinsic capacity of individuals, the environments they inhabit, and the interaction between them. Zhao et al. found that a higher impairment in intrinsic capacity at baseline was more likely to manifest with functional disability after a year than those with multimorbidity (three or more chronic diseases).

I believe that this Research Topic provides the researcher and clinician with interesting and varied *Insights in Geriatric Medicine* and serves as an important addition to the growing field of knowledge in Geriatric Medicine.

## Author contributions

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# COVID-19 Isolation and Risk of Death in Cyprus Elderly People

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Social isolation is associated with a higher risk of morbidity and death in older people. The quarantine and social distancing measures due to Covid-19 imposed in most countries and particularly in Cyprus, aim to isolate individuals from direct contact with others. This has resulted in vulnerable older people being isolated at their places of residence for several months, while the recommendations for continuing lockdowns do not appear to be ending. The risk of death from causes other than those related to Covid-19 increases in such individuals and it is due to the effects of social isolation. We estimate that in the next years, there will be a significant increase in the death numbers of such older people in Cyprus. The health authorities must develop a program of support for these older individuals to include medical, social, physical, and psychological elements. Examples of such support are given here.

**Keywords: COVID-19, social isolation, older people, death risk, Cyprus elderly, prevention of mortality**

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## INTRODUCTION

As the spread of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) continues to affect Cyprus, the elderly population has remained, and it is likely to remain, in enforced isolation for a long time. This is in line with similar policies in other countries. For example, the recommendation in the United Kingdom has been that people aged 70 years and older should be isolated at home for several months (1). However, it is well-accepted that social isolation in such age groups is a significant risk factor for morbidity and mortality and is therefore a serious public health concern (2, 3). This isolation exacerbates a variety of problems affecting older people which includes cardiovascular, cognitive, autoimmune, psychological, and hormonal disturbances (4).

We already knew before the current pandemic that there is consistent evidence linking social isolation and loneliness to worse cardiovascular and mental health outcomes (5). Moreover, we knew in advance that living alone and social disengagement is associated with a 24–32% higher hazard for hospital admission for elderly people suffering from respiratory disease (6).

In a recent qualitative study in Cyprus (7), it was confirmed that social isolation during the Covid-19 era is directly correlated with loneliness, a negative emotion associated with a perceived gap between existing relationships and desired ones. Strongest risk factors for morbidity and death were loss of a loved one, inactive lifestyle even before Covid-19, and clinical depression.

We are already beginning to experience increases in the numbers of non-Covid-19 deaths, compared to previous years (8). Estimates indicate a 17.7% increase in the number of deaths in the USA during 2020, compared to those in 2019, with heart disease and cancer-related deaths being the top two causes (9). It is possible that social isolation, associated with missed hospital treatments

and other reasons connected to the strict lockdown measures, contributed to such an increase in deaths (10).

## MORBIDITY AND MORTALITY

Valtorta et al. (11), examined 5,397 people aged 50 years and above, for an average period of 5.4 years. They found that there was an association between loneliness and increased risk of cardiovascular disease. Their conclusion was that: *"Loneliness is associated with an increased risk of developing coronary heart disease and stroke, independently of traditional cardiovascular disease risk factors. Our findings suggest that primary prevention strategies targeting loneliness could help to prevent cardiovascular disease."* Another study of 479,054 people over 7 years (12) confirmed the above findings concluding that "*Isolated and lonely persons are at increased risk of acute myocardial infarction (AMI) and stroke, and, among those with a history of AMI or stroke, increased risk of death."*

In a study of 6,500 participants aged 52 years and above, selected from The English Longitudinal Study of Aging in the UK (13), it was reported that social isolation (a state of complete or near-complete lack of contact between an individual and society) was associated with elevated risk of mortality (Hazzard Ratio of 1.26). Other studies also found significant association between loneliness (a temporary and involuntary lack of contact with others) and increased mortality (14). The increased mortality is not only linked to cardiovascular conditions. For example, it has been shown that other problems such as impaired immunity, altered hypothalamic pituitary–adrenocortical activity, and a pro-inflammatory gene expression profile are linked to increased mortality in this patients (15).

Another issue that needs to be considered is that of increased psychological morbidity. Pshychotropic drug use is on the increase during the Covid-19 pandemic (16). It is known that isolated older people are more likely to overuse medication such as antidepressants, anxiolytics, sedatives, or hypnotics (17). Chronic overuse of hypnotics and anxiolytics can lead to confusional states, is linked to dementia (18), and is associated with falls that can lead to hip fractures, head injuries, and even death (19).

Evidence is now emerging that the Covid-19 pandemic has widespread negative effects on cognitive abilities and on the mental well-being of older people (4, 20, 21). In addition, loneliness is significantly associated with a reduction in brain volume, particularly in areas associated with memory such as the left medial temporal lobe (22). Other problems include poor sleep patterns and a decline in executive function (15), all of which increase the risk of mortality in older people.

When thinking of socially isolated people, we tend to exclude people who live in institutional settings. However, people, and particularly people with dementia, living in institutional settings (residential and nursing homes) have suffered the strongest additional negative impact both due to the ban of external visitors, but also internal social distancing measures (guidelines to keep patients in their rooms instead of letting them socialize freely in common living areas, placing patients in single occupancy rooms when possible etc. (23)). Psychiatric symptoms, leading to overuse of psychiatric medications and negative side-effects such as tardive dyskinesia and akathisia, have also increased, accelerating the deterioration of these patients and leading to complications and death (24).

It is also notable that elderly people hospitalized with Covid-19 have negative short and long -term effects that impair their functionality because of the associated fatigue, muscle weakness and sarcopenia that can exacerbate frailty, dependence and disability, making this vulnerable group even more vulnerable leading to a vicious cycle of isolation and dependence (25).

## THE CYPRUS EXPERIENCE

Considering that cardiovascular disease is the leading cause of death in Cyprus, it is particularly important to realize that social isolation and loneliness may cause significant morbidity and mortality in these individuals. During 2018 there were 5,768 (all causes) deaths in Cyprus. Of these, 3,829 were people aged 75 years and older (26). The leading causes of death in Cyprus for the year 2017 were ischemic heart disease, stroke, Alzheimer's disease, lung cancer, diabetes, and chronic obstructive pulmonary disease (27). All of these conditions have enormous significance for older people as they are negatively affected by social isolation. Estimates of isolation-related mortality vary, with Hazzard Ratios (HR) of 1.26–3.7 (28). Even if we consider the lowest HR estimates we should expect substantial increases in the numbers of excess deaths of older Cypriot people, from causes other than Covid-19.

In addition to morbidity and mortality due to social isolation, there is an issue of extra deaths due to other effects of the lockdowns such as delayed diagnoses and lack of suitable follow-up for conditions other than Covid-19 (29). For instance, it is known that cancer risk in these situations is a real concern, particularly due to delayed diagnosis (30). We could apply the findings of this study to cancers in Cyprus. A total of 1,325 deaths occurred in Cyprus due to cancers in 2016 (31). Based on an approximate estimate of increased deaths by 10% (30), we could expect ∼130 extra deaths in Cyprus per year due to delayed cancer diagnosis alone. As reduced contact with healthcare staff contributes to higher risk, efforts to address this issue are likely to lead to improved health outcomes. Therefore, there is an urgent need to implement preventative strategies for physical and mental health that may reduce death risk in older populations (21).

## MEASURES TO MITIGATE RISKS

It is necessary to devise strategies for supporting older people at risk from social isolation and health deterioration. A series of interventions by the State in association with volunteers could provide such support for vulnerable older people. Initiatives that would be beneficial include the following:

- A reformed Government policy is urgently needed to allow relatives and carers of isolated older individuals to visit them (whether at home, in a care home or hospital) in order

to attend to their basic psychological and physical health needs and help to prevent their deterioration. As Covid-19 PCR tests are widely available and have sensitivity of 71–98% (32), there is no reason why visitors with no Covid-19 symptoms and a negative PCR cannot meet vulnerable individuals. In cases where extra reassurance is desired, a second negative PCR test is known to confer the highest accuracy (32). In addition, spreading population strategies in favor of vaccination against Covid-19 offers glimpses of hope to reassess isolation protocols.

- Primary care services can maintain regular contact with vulnerable individuals at high risk in the community, via telephone calls from healthcare professionals in order to ensure early identification of, and intervention for, medical and other needs. The calls should be initiated by the health professional and not by the patient (33). Likewise, provision of specialist psychological support can have beneficial outcomes (34). Furthermore, volunteers, friends, and relatives can enable meaningful and supportive telephone conversations on a regular basis (35).
- Promote physical activities, preferably a mixture of resistance, strength, and balance exercises as even light intensity exercise has positive health outcomes (36). For some individuals, technology such as use of the internet, social media sites, and media broadcasts can support these exercises programs (37). Some of these programs could be presented without charge by television stations within their sphere of corporate social responsibility.
- Consider "hidden" negative aspects of issues affecting older people, such as for example, the social stigma associated with Covid-19, negative perpetuating factors promoting the pandemic, and general fear (38).
- Smart ICT solutions, such as ReMember-Me (39). These may help prevent and detect cognitive decline, promote cognitive function and social inclusion among older adults. Smart solutions offer an innovative paradigm to improve cognition, emotional well-being, activity, sleep patterns and online socialization, promoting interactions in the context of cognitive fitness and individualized suggestions for a healthy brain.
- Digital technologies can be harnessed further, and opportunities include online social and entertainment activities, networking, religious services, and board games (40), as well as cognitive training exercises and video-games (41). However, there is an issue of inequality with regards access to such technologies, and not all able, older individuals can participate (42).

## CONCLUSION

Social isolation due to Covid-19 restrictive measures, has been shown to have a negative impact on health outcomes, with increasing morbidity and mortality among older people. It is imperative to develop nationwide strategies to prevent adverse outcomes, other than those related to Covid-19, in socially isolated people. In our attempt to prevent Covid-19 related deaths, we should not cause additional morbidity and deaths resulting from the isolation measures we have instigated. A wide range of interventions can be implemented that can promote social engagement and interactions, improve physical functions and psychological well-being, maintain optimal health status, and prevent deterioration. Although the examples of such interventions are best suited to the older people in Cyprus, the basic principles can be applicable to older people living in any country.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

MK conceptualized and wrote the manuscript. GM, HP, and MP edited, revised, and added to the manuscript. BP edited and revised the manuscript and enhanced the concept. All approved the final version of the manuscript.

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# Hospital Admission to a Window-Side Bed Does Not Prevent Delirium: A Retrospective Cohort Study of Older Medical Inpatients in General Wards

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**Background:** Delirium in older inpatients is a serious problem. The presence of a window in the intensive care unit has been reported to improve delirium. However, no study has investigated whether window-side bed placement is also effective for delirium prevention in a general ward.

**Objectives:** This study aims to clarify the association between admission to a window-side bed and delirium development in older patients in a general ward.

**Design:** This research is designed as a retrospective cohort study of older patients admitted to the internal medicine departments of Shinshu University Hospital, Japan.

**Participants:** The inclusion criteria were the following: (1) admitted to hospital internal medicine departments between April 2009 and December 2018, (2) older than 75 years, (3) admitted to a multi-patient room in a general ward, and (4) unplanned admission. The number of eligible patients was 1,556.

**Exposure:** This study is a comparison of 495 patients assigned to a window-side bed (window group) with 1,061 patients assigned to a non-window-side bed (non-window group). When patients were transferred to the other type bed after admission, observation was censored.

**Main Measures:** The main outcome of interest was "delirium with event" (e.g., the use of medication or physical restraint for delirium) within 14 days after admission as surveyed by medical chart review in a blinded manner.

**Key Results:** The patients had a median age of 80 years and 38.1% were female. The main outcome was recorded in 36 patients in the window group (10.7 per thousand person-days) and 84 in the non-window group (11.7 per thousand person-days). Log-rank testing showed no significant difference between the groups ($p = 0.78$). Multivariate analysis with Cox regression modeling also revealed no significant association for the window group with main outcome development (adjusted hazard ratio 0.90, 95% confidence interval of 0.61–1.34).

**Conclusions:** Admission to a window-side bed did not prevent delirium development in older patients admitted to a general ward.

**Keywords: delirium, general ward, internal medicine, older patients, window-side bed**

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# INTRODUCTION

Characterized by an acute disturbance in mental abilities resulting in confusion and abnormal behavior (1), delirium is a serious problem in older inpatients. Although delirium develops the most in inpatients admitted to the intensive care unit (ICU) or after surgery, older inpatients with medical diseases were also reported to exhibit delirium at rates of 6–26% (2). The condition harms patients by increasing mortality risk during hospitalization, prolonging hospital stay, and diminishing independence and cognitive function after discharge (3–8). Furthermore, delirium imposes considerable financial costs on health care systems (9).

To prevent or treat delirium, non-pharmacologic multicomponent approaches are highly encouraged, including orientation to time and place, cognitive stimulation, early mobilization, sleep enhancement, identification of underlying delirium causes, detecting early signs of delirium, and educating nursing staff and family members (10–12). However, those approaches can be labor intensive (12) and are difficult to perform for large numbers of inpatients admitted into general wards. Pharmacological approaches with neuroleptics and sedative medicines are also employed to control the symptoms of delirium. However, no convincing evidence supports the use or effect of any drug against delirium (13), with some studies even reporting potential harm (14, 15). Further prophylactic and therapeutic strategies are needed for general wards.

Delirium in patients on a general ward may be prevented and improved by assignment to a window-side bed, with circadian regulation by exposure to sunlight, phototherapy, and melatonin agonists being reportedly effective (16–18). As patients in window-side beds receive considerably more direct exposure to sunlight, their circadian rhythm may be better regulated to help prevent delirium. Furthermore, visibility to the outside through a window could suppress delirium by maintenance of cognition (19, 20). Indeed, several studies have described that the presence of a window in the inpatient room is effective for managing delirium in the ICU (21, 22), and several expert opinions recommend placing delirious patients near a window (23, 24). Considering that window-side bed assignment bears no additional costs or labor requirements and causes no side effects, this management strategy may be a simple and effective approach against delirium.

To date, no study has focused on the effect of windows against delirium in general wards, and the impact of window-side placement has not been addressed. This study examined the association of admission to a window-side bed with delirium development in older inpatients in a general hospital ward.

# METHODS

## Study Design

This was a retrospective cohort study reviewing the medical charts of patients admitted to Shinshu University Hospital, Japan.

## Setting and Study Population

The inclusion criteria of this investigation were as follows: (1) admitted to any internal medicine department at Shinshu University Hospital, Japan, between April 2009 and December 2018, (2) older than 75 years, (3) admitted to a multi-patient room in a general ward, and (4) unplanned admission. Only patients in the internal medicine departments were included to eliminate the effect of operations on delirium. Patients with scheduled admission, including those for a medical check-up, were excluded to identify patients at a higher risk of delirium. The exclusion criteria were as follows: (1) transfer from another hospital, (2) regularly taking a medicine for delirium before admission, and (3) already delirious on admission. Patients satisfying exclusion criterion (1) were excluded due to a lack of data, while those meeting criteria (2) or (3) were dropped since they might have already been delirious at admission. Medication for delirium was defined as any antipsychotics, mianserin, trazodone, or yokukansan which have all been generally prescribed for delirium (13, 25–27). Delirious at admission was defined as already exhibiting "delirium with event" which was the main outcome of this study (described below), within 3 h after admission.

## Baseline Characteristics

The data of eligible patients were collected from hospital medical records and included bed position at admission, transfer to another bed during hospitalization, basic clinical information, sequential organ failure assessment (SOFA) score (28, 29), performance status (PS) (30), Charlson comorbidity index (CCI) (31), daily medicine use before admission, pre-existing dementia, and main disease for admission. To calculate SOFA score, blood test data obtained within 24 h after admission was referred and the partial pressure of oxygen in arterial blood from peripheral oxygen saturation was predicted using the Hill equation (32). The PS scores were obtained as assessed by nurses on patient admission. The CCI, pre-existing dementia, and the main disease for admission were ascertained using the records of the registered disease name on admission. The main disease for admission was classified as a central nervous system disorder, cardiovascular disease, infection, malignancy, or others.

## Exposure of Interest

Eligible patients were divided into the group admitted to a window-side bed (window group) and the group admitted to a non-window-side bed (non-window group). At Shinshu University Hospital, multi-patient rooms in general wards can accommodate up to four or six patients. In rooms for four patients, the two beds closest to the window were defined as window-side beds and the two remaining beds were considered non-window-side beds (**Figure 1**). In rooms for six patients, the two beds closest to the window were judged as window-side beds and the remaining four beds were defined as non-window-side beds. Each bed was separated by a dividing curtain. Bedside luminosity was measured to confirm the hypothesis that patients in window-side beds received more exposure to natural light. The luminosity of each of the two beds at window-side beds and non-window-side beds in rooms facing south and north

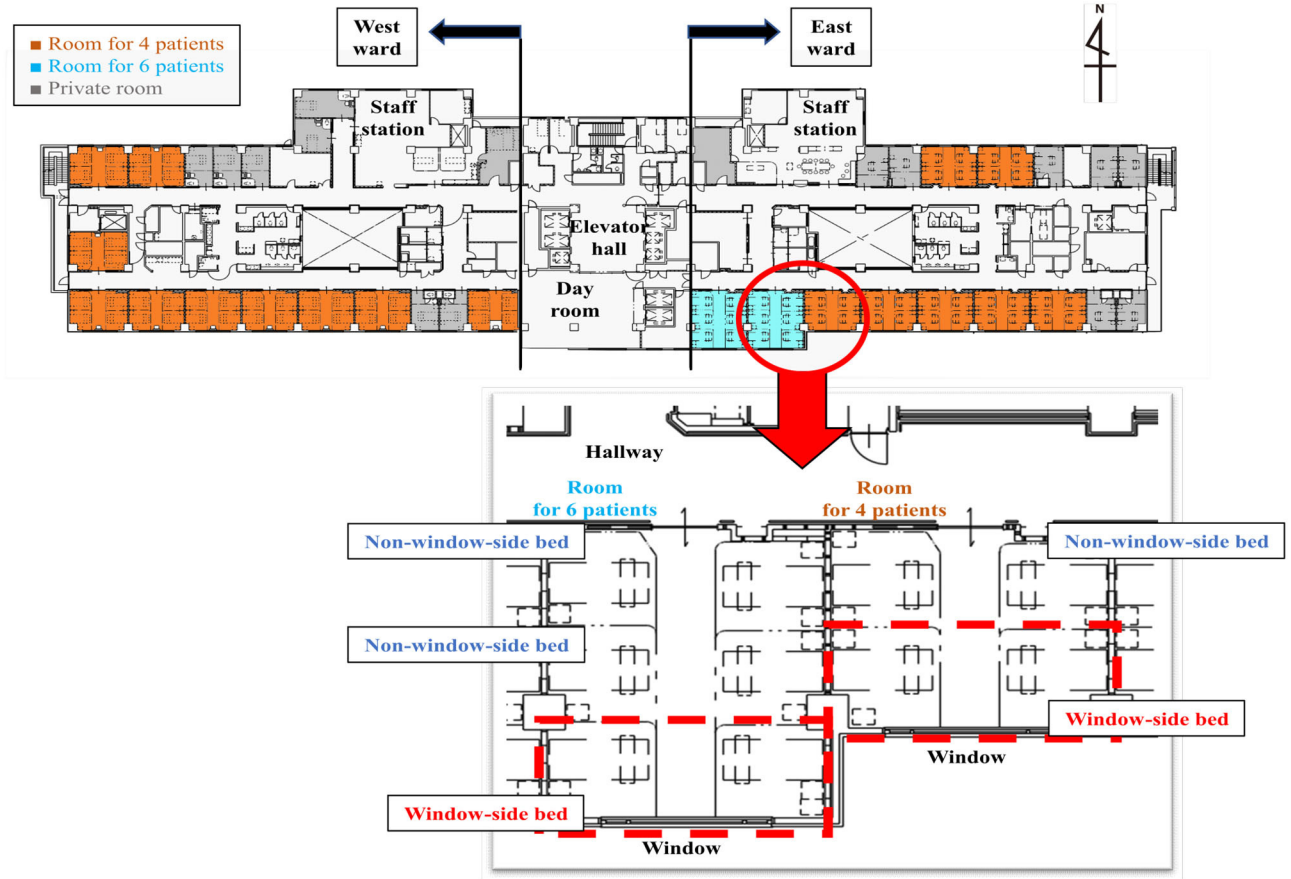



**FIGURE 1 |** Map of a general hospital ward. The map shows the layout of a general ward at Shinshu University Hospital. There are two types of multi-patient rooms: rooms for four patients and rooms for six patients. In both room types, the two beds nearest to the window are designated window-side beds (red dotted lines), while the others are designated non-window-side beds. Each bed is separated by a dividing curtain.

were determined with a luminometer (EM-9300SD, SATOSHOJI, Japan) every 3 h from 9:00 A.M. to 9:00 P.M. on March 30, 2019, on a clear day on the seventh floor of the hospital.

## Outcome Assessment

The primary outcome was "delirium with event" within 14 days after admission. The definition and abstraction method of this outcome are as follows. First, two physicians reviewed the medical charts of eligible hospitalized patients and identified delirium development using a chart-based method for identification (33). In this method, the physicians searched for key terms indicating acute mental change (e.g., delirium, mental state change, inattention, disorientation, hallucinations, agitation, inappropriate behavior, etc.). If the acute mental change could not be explained by reasons other than delirium (e.g., central nervous system disorder or dementia), the patient was defined as having "delirium." To enhance the reproducibility and specificity of the outcome, physicians further assessed whether the abstracted "delirium" was accompanied by any of the following events: (1) use of any drugs as sedatives for delirium, including antipsychotics, mianserin, trazodone, yokukansan, benzodiazepines, and first-generation antihistamines, (2) physical restraint, (3) transfer to another bed, (4) transient stay in the staff room for monitoring, and (5) self-removal of drip line or catheter (34, 35). If the delirium was accompanied by any such event, the case was classified as "delirium with event." The observation period for the primary outcome was limited to 14 days after admission to exclude the influence of a long hospital stay on delirium. Fourteen days was also chosen since Japanese medical staff are basically recommended to discharge patients within 14 days considering that some medical fees are covered by national healthcare for only 14 days of admission. Both physicians reviewed the medical charts independently and were blinded to whether the patient was in the window or non-window group. If their judgment differed on an outcome, mutual consensus was reached by discussion.

The secondary outcomes of "delirium with event" was also assessed within 30 days after admission, "delirium" within 14 or 30 days after admission, hospital stay longer than 14 days, transfer to the ICU, and death during hospitalization.

## Sample Size Decision

Previous literature suggested the primary outcome to occur at a frequency of approximately 10% (2). To detect an absolute

difference of 5% in the ratio of the primary outcome between the two groups (i.e., 7.5% in the window group and 12.5% in the non-window group) with 80% power at a 5% significance level, a total of 1,280 patients (divided at a 2:3 ratio) were required. Based on this calculation, the final recruitment target was set at 1,500 patients.

## Statistical Analysis

Descriptive statistics were employed to summarize the demographic factors of the patients stratified by two groups. Continuous variables were presented as the median and interquartile range (IQR) and compared using the Wilcoxon–Mann–Whitney test. Categorical variables were presented as the number and percentage and assessed by means of the chi-square test.

At the assessment of "delirium with event" or "delirium," observation was censored when the following events were recorded for reasons other than delirium: (1) use of antipsychotics, mianserin, trazodone, or yokukansan, (2) physical restraint, (3) transfer to another bed (apart from window-side bed to window-side bed or non-window-side bed to non-window-side bed), (4) transient stay in the staff room for monitoring, and (5) self-removal of drip line or catheter. Kaplan–Meier curves of cumulative outcome incidence were calculated and compared between the groups using the log-rank test. The hazard ratio (HR) of the window group for the main outcome was estimated using multivariable Cox proportional hazard models to adjust for such potential confounders as age, sex, low body weight (i.e., body mass index less than 18.5), SOFA score, regular use of risk drugs for delirium before admission (e.g., benzodiazepines, non-benzodiazepines, anti-histamines, and narcotic analgesics), PS, CCI, admission for central nervous system disorders, and pre-existing dementia (36–38). Concerning the assessments of hospital stay for longer than 14 days, transfer to the ICU, and death during hospitalization, the observation period was limited not to 14 days, but to the entire time of hospitalization, and censoring was not taken into account. The adjusted odds ratios of the window group for those outcomes were estimated using logistic regression models. Additional subgroup analyses were conducted using various factors related to: (1) the environment of the inpatient and bed, including the type of room, direction of ward, direction of room and window, and season of admission, and (2) patient characteristics including age, sex, low body weight, SOFA score, regular use of risk drugs for delirium before admission, PS, CCI, admission for central nervous system disorders, and pre-existing dementia. The adjusted HR of the window group was assessed for the primary outcome in each subgroup. Each subgroup factor was excluded from its own regression model (e.g., age was excluded from the regression model in the subgroup analysis relating to age). Regarding age, SOFA score, PS, and CCI, the patients were divided into subgroups according to median values.

Multiple imputation was performed to account for missing data values for PS and SOFA scores in 269 patients. Each missing value was replaced with a set of substituted plausible values by creating 20 filled-in complete data sets by multiple imputation using a chained equation method (39). To test the robustness of the results with the multiple imputation method, complete case analysis and median imputation analysis were also performed as sensitivity analyses regarding the assessment of the main outcome.

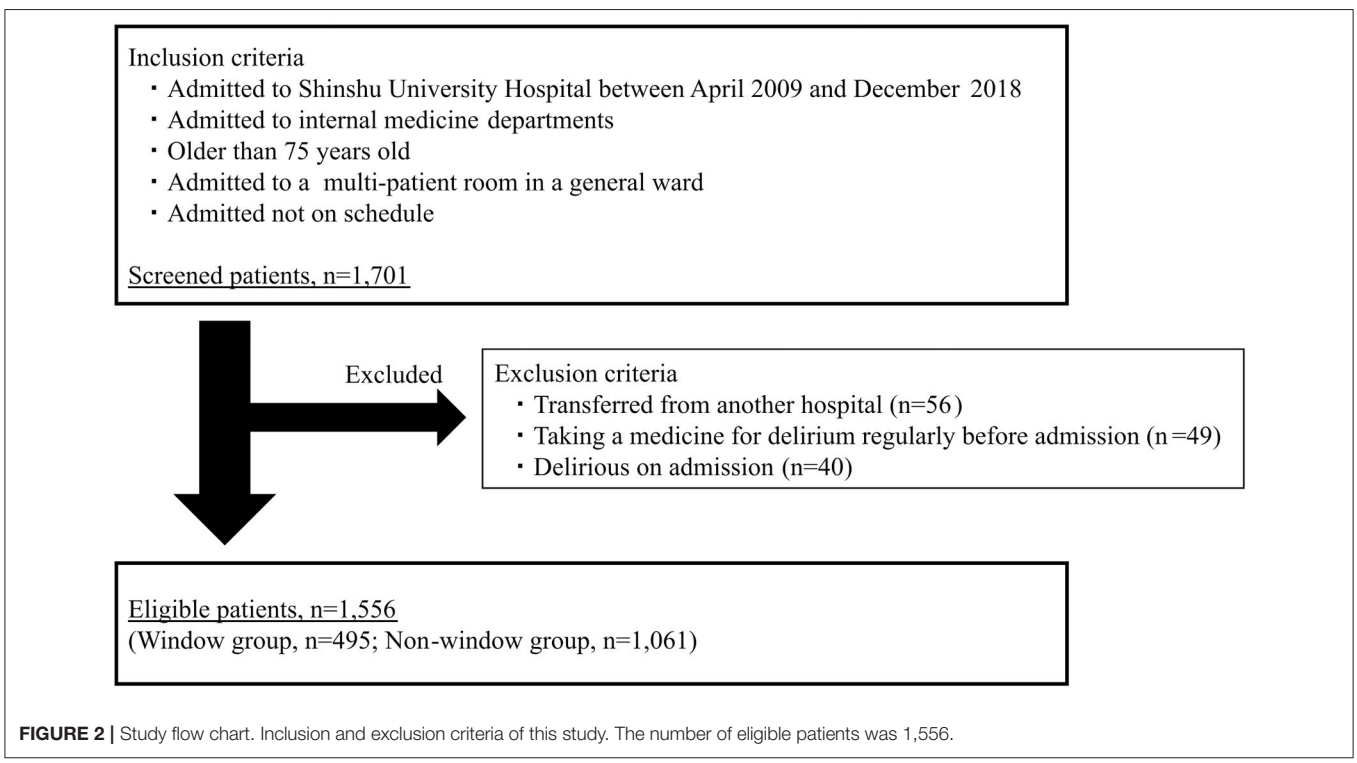



**FIGURE 2 |** Study flow chart. Inclusion and exclusion criteria of this study. The number of eligible patients was 1,556.

All statistical analyses were performed using IBM SPSS statistics version 27.0 (IBM, Armonk, NY). Values of $p < 0.05$ were considered statistically significant.

## Ethics Approval and Consent to Participate

This study followed the reporting guidelines of Strengthening the Reporting of Observational Studies in Epidemiology. It was performed in accordance with the tenets set forth in the Declaration of Helsinki and approved by the ethics committee of Shinshu University Hospital (authorization number: 4329). Informed written consent was waived in this study by the ethics committee of Shinshu University Hospital due to its retrospective nature using medical records that did not subject the patients to new interventions. The collected data were anonymously stored and used for analysis. As an alternative to written informed consent, an opt-out document was created and posted on the hospital website that contained information on the design of the research and publication of the results to provide subjects the opportunity to halt the provision of their medical data.

# RESULTS

## Bedside Luminosity

From 9:00 A.M. to 3:00 P.M, bedside luminosity was considerably higher at window-side beds (∼600–1,100 lux) than at non-window-side beds (∼300–400 lux), regardless of whether the room faced south or north (data not shown). Luminosity was undetectable at 9:00 P.M., after lights-out. These results strongly implied that the patients of window group received much more natural light cycle than those of the non-window group.

## Baseline Characteristics

The number of patients fulfilling the inclusion criteria was 1,701, among which eligible subjects totaled 1,556 after the exclusion of 145 patients (**Figure 2**). Regarding the characteristics of the eligible patients, median age was 80 years (IQR 77 to 84) and the proportion of female was 38.1%. All patients were Japanese. The characteristics of the patients in the window group ($n = 495$) and non-window group ($n = 1{,}061$) are presented in **Table 1**. There were no significant differences between the groups for basic characteristics or physical condition, such as SOFA score, PS, or CCI. The characteristics of patients with and without missing data differed significantly for age (median age: 79 and 80 years, respectively, $p = 0.03$) and admission with cardiovascular disease (16.7 and 11.6%, respectively, $p = 0.02$).

**TABLE 1** | Baseline cohort characteristics.

| | Window group ($n = 495$) | Non-window group ($n = 1{,}061$) | *p*-value |
|---|---|---|---|
| Age, median [IQR*], y | 80.0 [77.0, 84.0] | 80.0 [77.0, 84.0] | 0.32 |
| Female, *n* (%) | 183 (37.0) | 410 (38.6) | 0.54 |
| Body mass index, median [IQR*] | 21.0 [18.8, 23.6] | 21.3 [19.0, 23.7] | 0.34 |
| SOFA† score, median [IQR*] | 2.0 [1.0, 3.0] | 2.0 [1.0, 3.0] | 0.98 |
| Missing, *n* (%) | 16 (3.2) | 41 (3.9) | 0.66 |
| Regular drug use before admission, *n* (%) | | | |
| Benzodiazepines | 99 (20.2) | 208 (19.9) | 0.89 |
| Non-benzodiazepines | 36 (7.4) | 97 (9.3) | 0.24 |
| Anti-histamines | 46 (9.4) | 88 (8.5) | 0.56 |
| Narcotic analgesics | 21 (4.3) | 20 (1.9) | 0.01 |
| Any of the above drugs | 179 (36.2) | 349 (32.9) | 0.21 |
| Performance Status, median [IQR*] | 0.0 [0.0, 2.0] | 0.0 [0.0, 1.0] | 0.57 |
| Missing, *n* (%) | 59 (11.9) | 162 (15.3) | 0.09 |
| CCI‡, median [IQR*] | 2.0 [1.0, 3.0] | 2.0 [1.0, 3.0] | 0.78 |
| Type of main disease, *n* (%) | | | |
| Central nervous system disorder | 38 (7.7) | 57 (5.4) | 0.09 |
| Cardiovascular disease | 64 (12.9) | 130 (12.3) | 0.74 |
| Infection | 79 (16.0) | 125 (11.8) | 0.02 |
| Malignancy | 73 (14.7) | 166 (15.6) | 0.71 |
| Other | 241 (48.7) | 583 (54.9) | 0.02 |
| Pre-existing dementia | 13 (2.6) | 27 (2.5) | 1.00 |

**IQR, interquartile range; †SOFA, sequential organ failure assessment; ‡CCI, Charlson comorbidity index.*

## Association of Window Group With Primary Outcome

The incidence of "delirium with event" within 14 days after admission was 120 patients (7.7%; 11.4 per thousand person-days), and the breakdown of events was as follows: use of drugs for delirium in 56 cases (46.7%), physical restraint in 37 cases (30.8%), transfer to another bed in 12 cases (10%), transient stay in the staff room in 10 cases (9.8%), and self-removal of drip line or catheter in 4 cases (3.3%). The primary outcome was recorded in 36 cases in the window group (10.7 per thousand person-days) and in 84 cases in the non-window group (11.7 per thousand person-days). The unadjusted cumulative hazard curves for the primary outcome in the window and non-window groups are shown in **Figure 3**. Log-rank testing did not identify any remarkable difference between the groups ($p = 0.78$). Multivariate analysis with Cox regression models revealed no significant associations for the window group with the primary outcome [adjusted HR 0.90, 95% confidence interval (CI) 0.61–1.34, $p = 0.62$] (**Table 2**). The results of sensitivity analyses on missing data cases were similar for complete case analysis (adjusted HR 1.06, 95% CI 0.71–1.60, $p = 0.77$) and median imputation analysis (adjusted HR 0.89, 95% CI 0.60–1.32, $p = 0.56$).

## Association of Window Group With Secondary Outcomes

The unadjusted cumulative hazard curves for "delirium with event" and "delirium" within 30 days after admission are described in **Supplementary Figure 1**. Log-rank testing revealed no significant differences between the groups for "delirium with event" within 14 days after admission ($p = 0.72$) or "delirium" within 14 or 30 days after admission ($p = 0.99$ and 0.77, respectively). Multivariate analysis with a Cox regression model also identified no significant associations between the window group and "delirium with event" within 30 days after admission

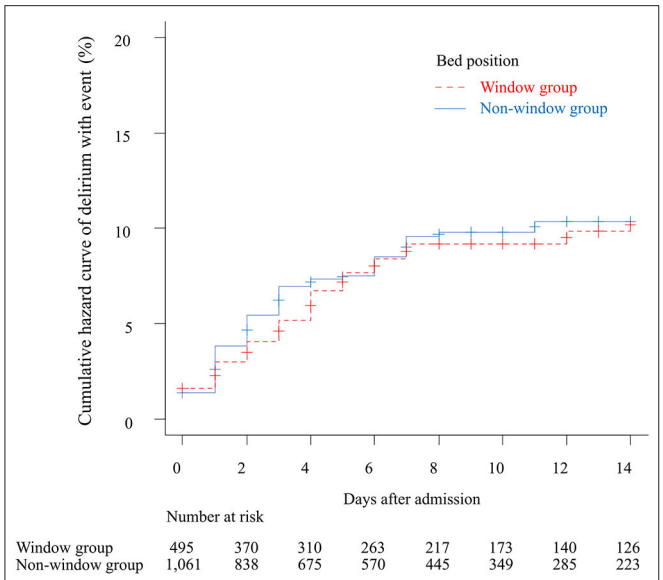



**FIGURE 3 |** Unadjusted cumulative curves for the primary outcome. The figure shows the cumulative curves of delirium with event within 14 days after admission. The dotted red line indicates the window group and the solid blue line indicates the non-window group.

**TABLE 2 |** Multivariable analysis on the association of window group and delirium with event.

| | Hazard ratio (95% CI*) | *p*-value |
|---|---|---|
| Crude model | 0.95 (0.64–1.40) | 0.78 |
| Adjusted model 1 | 0.96 (0.65–1.42) | 0.84 |
| Adjusted model 2 | 0.90 (0.61–1.34) | 0.62 |

*Table shows the hazard ratio of the window group for delirium with event within 14 days after admission by Cox regression models. In model 1, hazard ratio was adjusted for age and sex. In model 2, hazard ratio was adjusted for age, sex, low body weight, sequential organ failure assessment score, regular use of risk drugs for delirium before admission, performance status, Charlson comorbidity index, admission for central nervous system disorders, and pre-existing dementia. *CI, confidence interval.*

(adjusted HR 0.89, 95% CI 0.61–1.32, $p = 0.56$) or "delirium" within 14 or 30 days after admission (adjusted HR 0.97, 95% CI 0.71–1.34, $p = 0.95$ and adjusted HR 0.95, 95% CI 0.70–1.30, $p = 0.76$, respectively).

The adjusted odds ratios (ORs) of the window group for secondary outcomes were estimated using logistic regression models including hospital stay longer than 14 days (adjusted OR 1.19, 95% CI 0.95–1.49, $p = 0.11$), transfer to the ICU (adjusted OR 1.28, 95% CI 0.44–3.66, $p = 0.64$), and death during hospitalization (adjusted OR 1.18, 95% CI 0.85–1.63, $p = 0.30$). No significant relationships were observed between the window group and any outcome.

## Subgroup Analysis on the Association of Window Group With Primary Outcome

The results of subgroup analysis are shown in **Figure 4**. No significant relationship was detected between the window group and the primary outcome in any subgroup regarding the inpatient environment or bed or patient characteristics. Similarly, no significant interaction effect was detected between the window group and any subgroup.

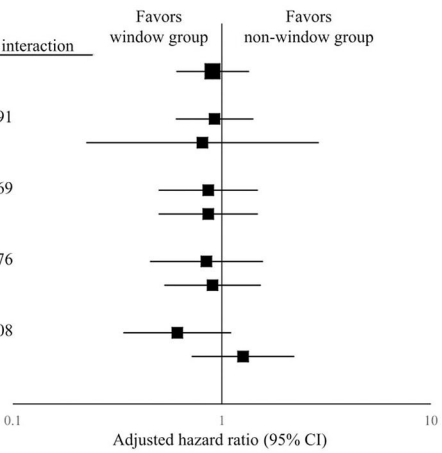
# DISCUSSION

To the knowledge of the researchers, this is the first study to examine the effects of window-side bed assignment on delirium development in general wards. As several reports from the ICU found that rooms with a window could suppress delirium (21, 22, 40, 41), the admission to window-side beds in general wards was hypothesized to have a similar effect. However, no significant association was found between admission to a window-side bed and delirium development, even after adjusting for possible confounders.

Several reasons may explain why the expected association was absent in this investigation. First, the rooms were not compared with and without windows, but rather compared window-side beds with non-window-side beds. Although earlier studies reported the effect of the presence of a window to suppress delirium by comparing rooms with and without a window (21, 22, 40, 41), those results could have been caused not by the window itself, but by window-associated accompanying factors, such as the newness and clarity of the room. The present study compared window-side beds with non-window-side beds in identical layout rooms of a single center, which was considered to assess the direct impact of windows on delirium through sunlight exposure and visibility of the outside world. Indeed, almost half of the ICU research reported that windows did not decrease the development of delirium (42), implying the possibility that the simple presence of a window did not associate with delirium prevention. Furthermore, any significant associations for window-side beds were not compared with delirium in subgroup analyses factoring the environments of patients or rooms. These results support the result that windows do not directly impact delirium. Second, the illnesses of the patients in this study were considerably milder than those of previous reports. According to the ICU research, window placement suppressed delirium by maintaining cognitive function and efficient sleep (43). However, patients in general wards like those in this study have generally milder illnesses and less frequently experience cognitive dysfunction or sleeping disorders (44). Thus, it was possible that the cohort study did not require such window effects as cognitive function and sleep maintenance, which in turn did not appreciably reduce delirium development. Third, it was conceivable that the study's statistical power was insufficient to detect differences between the test groups. The risk difference in the ratio of the primary outcome was 0.6% (7.3% in the window group and 7.9% in the non-window group) in this study, which was considerably less than the predicted 5%. Thus, the effect of a window-side bed on delirium suppression was much smaller than estimation, and hence the sample size might not have been sufficient to detect an effect. However, considering the very small risk difference between the groups, window-side bed placement may not be the main deciding factor to prevent delirium development in the clinical setting. The bed placement of patients at higher risk of

**A** Subgroup analysis relating to the environment of the inpatient and bed.

| | Patients, n (%) | Adjusted hazard ratio (95% CI) | p-value | p-value for interaction |
|---|---|---|---|---|
| Overall | 1,556 | 0.90 (0.61-1.34) | 0.62 | |
| Room type | | | | |
| Room for 4 patients | 1,295 (83.2) | 0.92 (0.60-1.40) | 0.71 | 0.91 |
| Room for 6 patients | 261 (16.8) | 0.80 (0.22-2.89) | 0.74 | |
| Direction of ward | | | | |
| East ward | 926 (59.5) | 0.86 (0.50-1.47) | 0.59 | 0.69 |
| West ward | 630 (40.5) | 0.86 (0.50-1.47) | 0.82 | |
| Direction of room and window | | | | |
| Facing south | 640 (41.1) | 0.84 (0.45-1.56) | 0.59 | 0.76 |
| Facing north or west | 916 (58. 9) | 0.90 (0.53-1.52) | 0.70 | |
| Season of admission | | | | |
| Spring or summer | 793 (51.0) | 0.61 (0.34-1.10) | 0.10 | 0.08 |
| Autumn or winter | 763 (49.0) | 1.26 (0.72-2.20) | 0.41 | |




**B** Subgroup analysis relating to patient's characteristics.

| | Patients, n (%) | Adjusted hazard ratio (95% CI) | p-value | p-value for interaction |
|---|---|---|---|---|
| Overall | 1,556 | 0.90 (0.61-1.34) | 0.62 | |
| Age (years) | | | | |
| < 81 | 819 (52.6) | 1.21 (0.68-2.16) | 0.53 | 0.12 |
| ≥ 81 | 737 (47.4) | 0.67 (0.38-1.17) | 0.16 | |
| Sex | | | | |
| Female | 593 (38.1) | 0.94 (0.49-1.80) | 0.86 | 0.74 |
| Male | 963 (61.9) | 0.97 (0.59-1.60) | 0.90 | |
| Body mass index | | | | |
| < 18.5 | 327 (21.0) | 1.54 (0.73-3.24) | 0.26 | 0.12 |
| ≥ 18.5 | 1,229 (79.0) | 0.74 (0.46-1.20) | 0.22 | |
| SOFA* score | | | | |
| < 3 | 920 (61.4) | 1.23 (0.71-2.13) | 0.46 | 0.68 |
| ≥ 3 | 579 (38.6) | 0.68 (0.38-1.24) | 0.21 | |
| Regular use of risk drugs for delirium | | | | |
| Yes | 528 (33.9) | 0.75 (0.39-1.43) | 0.38 | 0.63 |
| No | 1,028 (66.1) | 0.96 (0.58-1.59) | 0.87 | |
| Performance Status | | | | |
| = 0 | 910 (68.2) | 1.09 (0.58-2.02) | 0.79 | 0.95 |
| ≥ 1 | 425 (31.8) | 1.03 (0.60-1.78) | 0.92 | |
| Charlson Comorbidity Index | | | | |
| < 3 | 1,110 (71.3) | 0.75 (0.45-1.24) | 0.26 | 0.15 |
| ≥ 3 | 446 (28.7) | 1.42 (0.71-2.82) | 0.32 | |
| Type of main disease | | | | |
| Central nervous system disorder | 95 (6.1) | 0.75 (0.20-2.83) | 0.68 | 0.36 |
| Other | 1,461 (93.9) | 0.97 (0.64-1.48) | 0.89 | |
| Pre-existing dementia | | | | |
| Yes | 40 (2.6) | 0.80 (0.20-3.26) | 0.76 | 0.53 |
| No | 1,516 (97.4) | 0.95 (0.63-1.44) | 0.81 | |

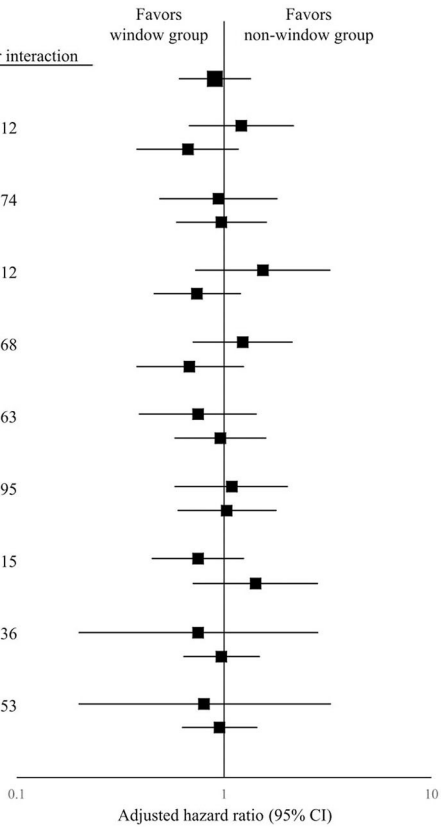



**FIGURE 4 |** Subgroup analysis on the association of window group with primary outcome. The table and forest plot show the adjusted hazard ratio of the window group for delirium with event within 14 days after admission for each subgroup. The results of subgroup analyses in terms of inpatient environment and bed are shown in **(A)** and those regarding patient characteristics are shown in **(B)**. In the forest plot, black squares show the adjusted hazard ratio and horizontal lines show 95% CIs. Hazard ratio was modified using the Cox regression model adjusted for age, sex, low body weight, sequential organ failure assessment score, regular use of risk drugs for delirium before admission, performance status, Charlson comorbidity index, admission for central nervous system disorders, and pre-existing dementia.

delirium may be better decided by such factors as patient request or proximity to a staff station rather than by window-side or not. Additional research with a larger sample size is needed for further assessment.

This study had several limitations that must be considered when interpreting the results. First, it was conducted retrospectively, and the patients were not allocated randomly to window or non-window groups. There was also the possibility that unmeasured confounding factors influenced the results. Indeed, some potential confounders could not be abstracted, including family structure of the patient, catheterization during hospitalization, whether the dividing curtain between beds was

opened or closed, and patient bed location request. Moreover, the number of patients in the window group was much smaller than in the non-window group, even after considering the difference in numbers of window-side and non-window-side beds. Although it is assumed that this imbalance is due to the differences in hospitalization period and bed turnover rate between the groups, it cannot be denied that patient assignment was influenced by unmeasured confounders. Second, the diagnostic accuracy of delirium was presumed as not completely accurate because delirium development referring to medical charts was retrospectively abstracted. In retrospective studies on delirium, the chart abstraction method by Inouye et al. was generally used with a sensitivity and specificity of 73 and 84%, respectively (33). In the present research, however, diagnosis by this method may have had diminished accuracy since the reviewers were not delirium specialists. To enhance diagnostic accuracy, however, whether the abstracted "delirium" was accompanied by intervention was additionally assessed, and so "delirium with event" was abstracted as a more reproducible and specific outcome. Although this assessment method likely could not sufficiently abstract mild or hypoactive delirium, it was considered that "delirium with event" would be more suitable as the main outcome presuming that non-specialists of delirium basically observe, manage, and treat severe or hyperactive delirium cases more frequently than mild or hypoactive cases in general wards (45). The association of the window group was also evaluated with "delirium" defined by the chart abstraction method, and again no significant relationship was found, implying high reproducibility of this research. Lastly, the external validity of this research is limited due to its design as a single-center study at a university hospital. It is unclear whether the results can be applied to inpatients at other centers, especially those at long-term hospitals or nursing homes, because their characteristics and hospitalization environments differ considerably. Although the negative results in all subgroup analyses in this study partially support the external validity of the findings, additional multi-center studies will be necessary.

In summary, this study revealed no significant association between admission to a window-side bed and delirium development in older patients with a medical disease in a general ward. Clinically, the ideal bed placement of patients at higher risk of delirium may be more optimally decided by factors other than window-side location. Larger multi-center studies are warranted to refine and validate results.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Shinshu University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

DA has full access to all of the data in this research and takes responsibility for the integrity of the data and the accuracy of the data analysis. DA designed the study and drafted the article with support from YY and MH. DA and YY checked the medical charts and assessed the incidence of delirium. MH, KH, YY, and YK revised the article critically for important intellectual content and gave final approval of the submitted version. There are no contributors who should be listed other than the authors. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.744581/full#supplementary-material

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# Intrinsic Capacity vs. Multimorbidity: A Function-Centered Construct Predicts Disability Better Than a Disease-Based Approach in a Community-Dwelling Older Population Cohort

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**Objective:** This study aimed to assess the status of intrinsic capacity (IC)—a novel function-centered construct proposed by the WHO and examine whether impairment in IC predicts subsequent 1-year activities of daily living (ADL) disability better than a disease-based approach, i. e., multimorbidity status.

**Methods:** This study included data of community-dwelling older adults from the Beijing Longitudinal Study on Aging II aged 65 years or older who were followed up at 1 year. Multivariate logistic regressions were performed to estimate the odds of ADL disability at baseline and 1-year follow-up.

**Results:** A total of 7,298 older participants aged 65 years or older were included in the current study. About 4,742 older adults were followed up at 1 year. At baseline, subjects with a higher impairment in IC domains showed higher odds of ADL disability [adj. odds ratio (OR) = 9.51 for impairment in ≥3 domains, area under the curve (AUC) = 0.751] compared to much lower odds of ADL disability in subjects with a higher number (≥3) of chronic diseases (adj. OR 3.92, AUC = 0.712). At 1-year follow-up, the overall incidence of ADL disability increased with the impairment in IC domains higher than the increase in multimorbidity status. A higher impairment in IC domains showed higher odds of incidence ADL disability for impairment in 2 or ≥3 IC domains (adj. OR 2.32 for impairment in ≥3 domains, adj. OR 1.43 for impairment in two domains, AUC = 0.685). Only subjects who had ≥3 chronic diseases had higher odds of 1-year incident ADL disability (adj. OR 1.73, AUC = 0.681) that was statistically significant.

**Conclusion:** Our results imply that a function-centered construct could have higher predictability of disability compared to the multimorbidity status in community older people. Our results need to be confirmed by studies with longer follow-up.

**Keywords: ICOPE, integrated care, comorbidity, aging, older Chinese**

## INTRODUCTION

The disability-free life expectancy has not increased at the same pace as the life expectancy in humans (1). There is an increasing notion in geriatrics that the traditional disease-centered approach may be inadequate to meet the healthcare needs of older adults (2, 3). Strategies that promote "healthy aging" could assist in reducing the burden of disability and dependency in old age. The WHO defines healthy aging as the process of maintaining functional ability that enables well-being in old age (1, 4). Healthy aging is determined by intrinsic capacity (IC) and the environment (i.e., extrinsic factors) of an individual. The WHO introduced the concept of IC through its ambitious and innovative care plan known as the Integrated Care for Older Person (ICOPE) (4), which has a great potential to improve geriatric care even in settings without adequate geriatric medicine expertise. IC is defined as the composite of all physical and mental capacities of an individual. In other words, maintaining IC throughout life may serve as a meaningful approach to avoid dependency in old age by achieving optimal functional ability. Early detection and prevention of disability or dependency may be needed to maintain autonomy in old age.

Older adults with one or more chronic diseases or having multimorbidity are known to be at increased risk of disability (5). There is a high prevalence of multimorbidity in community-dwelling older adults (5, 6). A complex and persisting interplay between the aging process and disease is known to exist; hence, approaches based on the mere treatment of diseases may be inadequate to avoid the disability cascade. Strategies, such as enhancing or maintaining IC throughout life, could play an important role in improving the lives of older adults. However, research on IC is limited. There is very little evidence to confirm that this novel construct could serve its purpose as signified by the WHO ICOPE approach. Prior studies have shown IC to be able to predict poor health outcomes in nursing home residents (7) and to predict loss of functions in the English Longitudinal Study of Aging (ELSA) cohort (8). Two cross-sectional studies in China have shown IC to be associated with various adverse events in older adults (9, 10). Another study also attempted to validate the IC construct in a Chinese population, but the study population was from a single community (11). Moreover, it remains yet to be confirmed if this function-centered construct could be better than the traditional disease-centered approach in determining future disability in a representative community-dwelling older population, particularly in the Chinese population, which bears the largest aging population of the world.

We aimed to estimate the status of IC and examine whether impairment in IC predicts subsequent 1-year disability in a representative community-dwelling Chinese older population. We hypothesized that a function-centered construct, such as IC, could predict disability better than a disease-based approach, i.e., multimorbidity status.

## METHODS

### Study Participants

This study participants were from the Beijing Longitudinal Study on Aging II (BLSA II), a representative community-dwelling older population cohort. The details on study design and cohort profile have been previously described (12, 13). In brief, 10,039 adults aged 55 years and older were selected using a multistage-randomized cluster sampling method from three urban districts and one rural county in the Beijing region. Participants were interviewed face to face by trained clinicians. For this current analysis, 7,298 subjects aged 65 years and older were included (**Figure 1**). The research and ethics committee of Xuanwu Hospital of Capital Medical University approved this research, and each participant provided written informed consent.

## MEASURES

### Intrinsic Capacity

According to the WHO ICOPE guideline (14), IC included five domains: locomotion, vitality, sensory (hearing and vision), cognition, and psychological capacity. We selected commonly used and well-validated scales for each domain, and all the scores were dichotomized as 1 = "impaired" and 0 = "not impaired."

#### Locomotion

Locomotion was evaluated by the Tinetti score (15), which is also generally used to assess mobility, balance, gait, and predict falls in older people. The Tinetti score consists of 13 maneuvers and the score ranges from 0 to 26 (higher is better). The Tinetti test score $<24$ was considered as an impairment in locomotor capacity.

#### Vitality

Vitality was assessed using the Mini-Nutritional Assessment (MNA) scale (16), MNA is composed of 18 items with a maximum score of 30 (higher is better). MNA score $<24$ was considered as an impairment in vitality.

#### Sensory

The sensory capacity domain included vision and hearing impairments. Participants were asked if they experienced any recent decline in vision and hearing. A positive answer to a recent decline in vision or hearing impairments was considered as an impairment in sensory capacity. Self-reported hearing loss has been suggested to be useful where audiometry is not available (17), and self-reported vision impairment has been used as a measure of visual loss in prior IC studies (9).

#### Cognition

Cognition was evaluated using the Mini-Mental State Examination (MMSE) (18). MMSE test is composed of 11 items with a maximum score of 30 (higher is better). MMSE score $<24$ was considered as having an impairment in cognition.

#### Psychology

The psychological domain was evaluated using the 15-item Geriatric Depression Scale (GDS-15), which identifies depressive symptoms in older people with scores varying from 0 to 15 (higher is worse) (19). GDS-15 score $\geq 8$ was considered as having a psychological impairment.

### Multimorbidity Status

Self-reported history of chronic diseases was collected using a single question "Have you been ever diagnosed

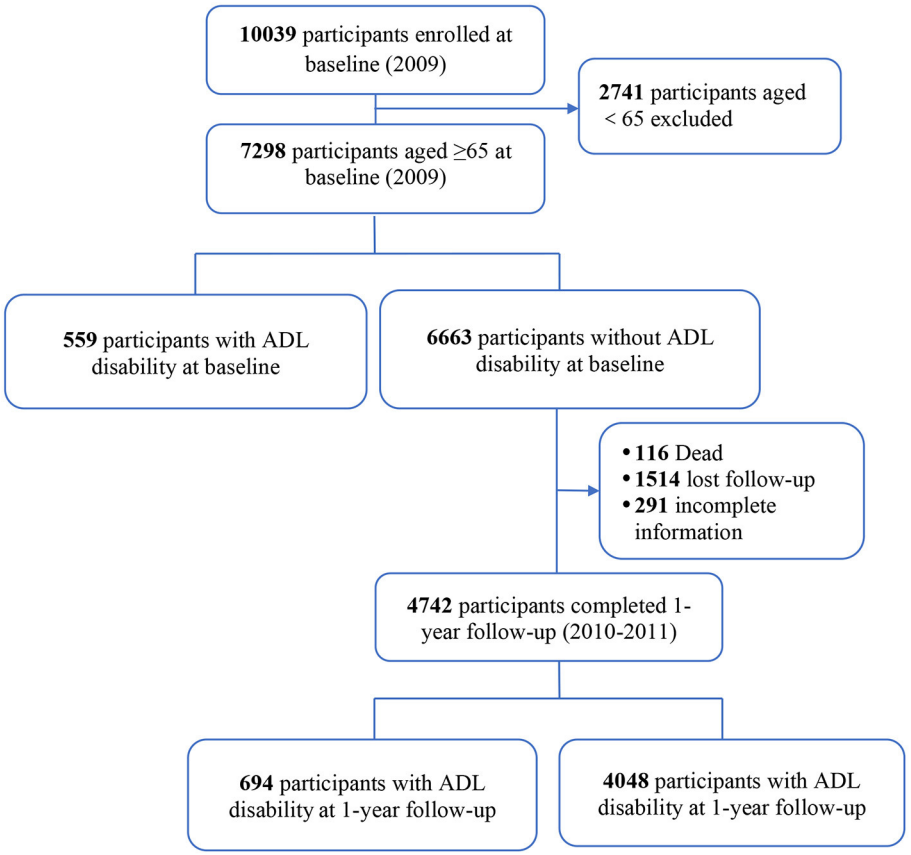


**FIGURE 1 |** Flowchart of the study population.

with any of the following diseases by a doctor?" For our current study, we included six chronic diseases that were most common among the study population including hypertension, diabetes mellitus, cardiovascular disease (CVD), stroke, tumor, and chronic obstructive pulmonary (COPD). The total number of chronic diseases was categorized into four groups: 0, 1, 2, and ≥3 in our analysis.

## Other Covariates

Other covariates included three age groups (65–74, 75–84, and ≥85 years), sex (female and male), education (middle school or below vs. higher education), and marital status (currently married vs. others).

## Outcome Variable

### Disability

Disability was assessed using the Barthel Index for basic activities of daily living (ADL) (20). Barthel index included 10 daily living tasks (feeding, bathing, grooming, dressing, bowels, bladder, toilet use, transfers, mobility, and climbing stairs). Subjects who had limitations in at least one task were considered as having ADL disability.

## Statistical Analyses

For continuous variables, arithmetic means *t*-tests were used to compare between groups. For categorical variables $\chi^2$ test was used to compare the groups. The prevalence of IC impairment was estimated by the proportion of subjects who had an impairment in at least one domain of IC at baseline. The chi-square tests were used to describe the associations between demographic characters and other subgroups with categories of IC impairment. We used logistic regressions to estimate the odds of ADL disability at baseline and 1-year incident ADL disability. Comparisons were made according to IC impairment and multimorbidity. Adjustments were made for age, sex, educational level, and marital status in the regression models. Education and marital status were included to consider the interaction between the environment and IC. Furthermore, these factors are also directly associated with the development and maintenance of IC. To assess the logistic model discrimination, c-statistics for the area under the curve (AUC) were calculated. The Hosmer–Lemeshow goodness-of-fit statistic was used to assess model calibration.

All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and all *p*-values are two-tailed. A *p*-value of $<0.05$ was considered as being statistically significant.

**TABLE 1 |** Characteristics of the study participants at baseline.

| Characteristics | Baseline ($n$ = 7,298) *N* (%) | One-year follow-up ($n$ = 4,742) *N* (%) |
|---|---|---|
| Mean age (SD) | 74.2 (± 5.5) | |
| **Age group** | | |
| 65–74 y | 4,266 (58.5) | 2,940 (62.0) |
| 75–84 y | 2,785 (38.1) | 1,692 (35.7) |
| ≥85 y | 247 (3.4) | 110 (2.3) |
| **Sex** | | |
| Female | 4,447 (60.9) | 2,849 (60.1) |
| Male | 2,851 (39.1) | 1,893 (39.9) |
| **Education** | | |
| Middle school or below | 3,348 (45.9) | 2,079 (56.1) |
| High school or above | 3,944 (54.1) | 2,660 (43.9) |
| **Married** | | |
| Yes | 5,586 (76.5) | 3,741 (78.9) |
| No | 1,712 (23.5) | 1,001 (21.1) |
| **Comorbidity** | | |
| ≥2 Chronic diseases | 2,620 (35.9) | 1,656 (35.0) |
| <2 Chronic diseases | 4,653 (63.8) | 3,073 (65.0) |
| **Number of chronic diseases** | | |
| 0 | 2,031 (27.8) | 1,318 (27.9) |
| 1 | 2,622 (35.9) | 1,755 (37.1) |
| 2 | 1,822 (25.0) | 1,182 (25.0) |
| ≥3 | 798 (10.9) | 474 (10.0) |
| **Impairment in intrinsic capacity (IC) domains** | | |
| Locomotion | 807 (11.1) | 342 (7.2) |
| Vitality | 2,533 (34.7) | 1,501 (31.7) |
| Sensory | 2,390 (32.8) | 1,483 (31.3) |
| Cognition | 1,338 (18.4) | 709 (15.0) |
| Psychology | 806 (11.8) | 454 (10.3) |
| **Activities of daily living (ADL) disability** | | |
| Yes | 559 (7.7) | 603 (12.7) |
| No | 6,663 (91.2) | 4,139 (87.3) |

# RESULTS

## Study Population

Of the 7,298 subjects aged 65 years and over enrolled in this study (**Figure 1**), we followed 6,663 participants without ADL disability for 1 year. At the 1-year follow-up visit, 4,742 (71.2%) subjects completed the study and 116 (1.7%) had died, and 1,514 (22.7%) lost to follow-up and 291 (4.4%) had incomplete data. Subjects who lost to follow-up had similar characteristics as those who were followed at 1 year (**Supplementary Material 1**).

## Baseline Characteristics

The characteristics of the study participants at baseline are presented in **Table 1**. At baseline, the mean age of the included 7,298 participants was 74.2 (±5.5) years, 60.9% were female, 45.9% had middle school or lower education, and 76.5% were currently married. About 35.9% had one of the six chronic diseases, 25% had two chronic diseases, and 10.9% had three or more chronic diseases. The proportion of IC impairment according to its individual domains was 11.1% in locomotion, 34.7% in vitality, 32.8% in sensory, 18.4% in cognition, and 11.8% in psychology.

## Prevalence of IC Impairment at Baseline With Risk Factors

**Table 2** shows the global prevalence (i.e., impairment in at least one domain) of IC impairment and as categorized by the number of impairments in IC domains at baseline. Of the 7,298 participants, 4,709 (64.5%) had an impairment in at least one domain of IC. Among them, 34.5% had an impairment in only one domain, 19.9% in two domains, and 10.1% in three or more IC domains. The prevalence of the IC impairment increased with age (≥85 years had the highest decline of 80%) was higher in females, in individuals with low education, and who were currently unmarried. Individuals with any of the chronic diseases also showed impairments in IC.

## Prevalence of ADL Disability at Baseline

The overall prevalence of ADL disability at baseline was 7.7% and increased with age, higher in females, in individuals with low education, and those who were currently unmarried (**Figure 2**). The ADL disability increased with the number of chronic diseases (15.7% in ≥3 chronic diseases vs. 6.1% with one chronic disease). Similarly, individuals with IC impairment had higher ADL disability (10.6 vs. 2.6% with no IC impairment). Disability increased with impairments in multiple domains of IC (30.4% in ≥3 domains vs. 6.1% with impairment in one domain). Locomotion was the domain with the highest rate of ADL disability (30.9%), and vitality was the domain with the lowest rate of ADL disability (11.6%).

## Association of IC Impairment and Multimorbidity Status With ADL Disability at Baseline

Subjects with a higher number of chronic diseases had higher odds of ADL disability [adj. odds ratio (OR) 3.92, 95%CI = 2.92–5.27 for ≥3 chronic diseases vs. adj. OR 1.38, 95%CI = 1.06–1.82 for one chronic disease]. The AUC for the unadjusted model of multimorbidity was 0.63 and 0.712 for the adjusted model (**Table 3**).

Impairments in multiple domains of IC showed higher odds of ADL disability (adj. OR 9.51, 95%CI = 7.01–13.02 for impairments in ≥3 domains vs. adj. OR 2.26, 95%CI = 1.69–3.06 for impairment in one domain). The association remained equally significant even after including adjustment for chronic diseases. The AUC for the unadjusted model of IC was 0.719 and 0.767 for the fully adjusted model.

## Incident ADL Disability at 1-year Follow-Up

At 1-year follow-up, 694 (14.6%) new onset of ADL disability was detected. The incidence of ADL disability increased with age was higher in females, in individuals with lower education, and those who were currently unmarried (**Figure 3**). The overall incidence of ADL disability showed an increasing trend with impairments in multiple domains of IC (30.4% in ≥3 domains

**TABLE 2 |** Prevalence of overall IC impairment and as categorized by the impairment in intrinsic capacity (IC) domains at baseline.

| Characteristics | IC impairment, *N* (%) | *p* | Number of impairment in IC domains | | | | *p* |
|---|---|---|---|---|---|---|---|
| | | | 0, *N* (%) | 1, *N* (%) | 2, *N* (%) | ≥3, *N* (%) | |
| Overall | 4,709 (64.5) | – | 2,589 (35.5) | 2,519 (34.5) | 1,454 (19.9) | 736 (10.1) | – |
| **Age group** | | | | | | | |
| 65–74 y | 2,589 (58.7) | <0.001 | 1,762 (41.3) | 1,497 (35.1) | 743 (17.4) | 264 (6.2) | <0.001 |
| 75–84 y | 2,006 (72.0) | | 779 (28.0) | 943 (33.9) | 665 (23.9) | 398 (14.2) | |
| ≥85 y | 199 (80.6) | | 48 (19.4) | 79 (32.0) | 46 (18.6) | 74 (30.0) | |
| **Sex** | | | | | | | |
| Female | 2,996 (67.4) | <0.001 | 1,451 (32.6) | 1,488 (33.5) | 991 (22.3) | 517 (11.6) | <0.001 |
| Male | 1,713 (60.1) | | 1,138 (39.9) | 1,031 (36.2) | 463 (16.2) | 219 (7.7) | |
| **Education** | | | | | | | |
| ≤Middle school | 2,449 (73.1) | <0.001 | 899 (26.9) | 1,142 (34.1) | 837 (25.0) | 470 (14.0) | <0.001 |
| >Middle school | 2,257 (57.2) | | 1,687 (42.8) | 1,375 (34.9) | 616 (15.6) | 266 (6.7) | |
| **Married** | | | | | | | |
| Yes | 3,449 (61.7) | <0.001 | 2,137 (38.3) | 1,937 (34.7) | 1,047 (18.7) | 465 (8.3) | <0.001 |
| No | 1,260 (73.6) | | 452 (26.4) | 582 (34.0) | 407 (23.8) | 271 (15.8) | |
| no | 4,641 (64.4) | | 2,564 (35.6) | 2,487 (34.5) | 1,434 (19.9) | 720 (10.0) | |
| **Comorbidity** | | | | | | | |
| ≥2 Chronic diseases | 1,876 (71.6) | <0.001 | 744 (28.4) | 891 (34.0) | 610 (23.3) | 375 (14.3) | <0.001 |
| <2 Chronic diseases | 2,817 (60.5) | | 1,836 (39.5) | 1,618 (37.8) | 839 (18.0) | 360 (7.7) | |
| **Number of chronic diseases** | | | | | | | |
| 0 | 1,159 (57.1) | <0.001 | 872 (42.9) | 670 (33.0) | 347 (17.1) | 142 (7.0) | <0.001 |
| 1 | 1,658 (63.2) | | 964 (36.8) | 948 (36.2) | 492 (18.8) | 218 (8.3) | |
| 2 | 1,291 (70.9) | | 531 (29.1) | 647 (35.5) | 406 (22.3) | 238 (13.1) | |
| ≥3 | 585 (73.3) | | 213 (26.7) | 244 (30.6) | 204 (25.6) | 137 (17.1) | |

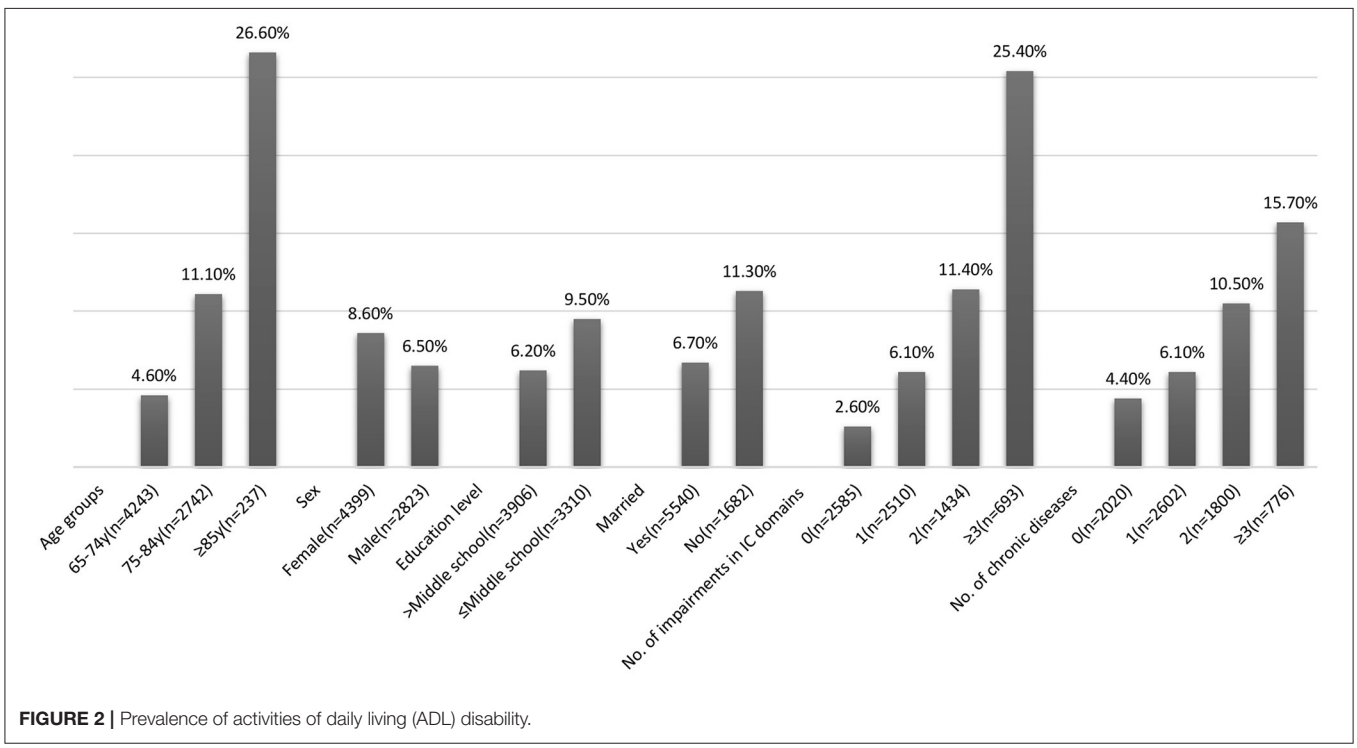


**FIGURE 2 |** Prevalence of activities of daily living (ADL) disability.

**TABLE 3 |** Logistic regression to determine the odds of activities of daily living (ADL) disability at baseline.

| | ORs for the prevalence of ADL disability (95% CI) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Unadjusted** | ***p*** | **Model 1** | ***p*** | **Model 2** | ***p*** | **Model 3** | ***p*** |
| **Age group** | | | | | | | | |
| 65–74 y | Ref | | Ref | | Ref | | Ref | |
| 75–84 y | 2.61 (2.16, 3.15) | 0.586 | 2.02 (1.66, 2.47) | 0.460 | 2.30 (1.89, 2.80) | 0.135 | 1.95 (1.60, 3.23) | 0.165 |
| ≥85 y | 7.60 (5.48, 10.45) | <0.001 | 4.79 (3.34, 6.81) | <0.001 | 7.23 (5.10, 10.16) | <0.001 | 5.13 (3.57, 7.33) | <0.001 |
| **Sex** | | | | | | | | |
| Male | Ref | | Ref | | Ref | | Ref | |
| Female | 1.35 (1.12, 1.62) | 0.001 | 1.22 (0.99, 1.49) | <0.062 | 1.33 (1.09, 1.63) | <0.006 | 1.22 (0.99, 1.50) | 0.061 |
| **Education** | | | | | | | | |
| High school or above | Ref | | Ref | | Ref | | Ref | |
| Middle school or below | 1.59 (1.33, 1.89) | <0.001 | 0.986 (0.82, 1.20) | 0.885 | 1.28 (1.06, 1.54) | 0.010 | 1.04 (0.85, 1.26) | 0.722 |
| **Married** | | | | | | | | |
| Yes | Ref | | Ref | | Ref | | Ref | |
| No | 1.79 (1.48, 2.14) | <0.001 | 1.14 (0.93, 1.40) | 0.203 | 1.24 (1.01, 1.52) | 0.037 | 1.14 (0.93, 1.40) | 0.209 |
| **No. of chronic diseases[a]** | | | | | | | | |
| 0 | Ref | | / | | Ref | | / | |
| 1 | 1.43 (1.10, 1.87) | <0.001 | / | | 1.38 (1.06, 1.82) | <0.001 | 1.34 (1.02, 1.77) | 0.001 |
| 2 | 2.58 (1.99, 3.36) | <0.001 | / | | 2.36 (1.81, 3.10) | 0.002 | 2.07 (1.58, 2.72) | 0.014 |
| ≥3 | 4.10 (3.08, 5.48) | <0.001 | / | | 3.92 (2.92, 5.27) | <0.001 | 3.16 (2.33, 4.29) | <0.001 |
| **No. of impairments in IC domains[b]** | | | | | | | | |
| 0 | Ref | | Ref | | / | | Ref | |
| 1 | 2.48 (1.86, 3.34) | <0.001 | 2.26 (1.69, 3.06) | <0.001 | / | | 2.17 (1.62, 2.94) | <0.001 |
| 2 | 4.93 (3.69, 6.65) | <0.001 | 4.23 (3.15, 5.74) | <0.001 | / | | 3.84 (2.86, 5.22) | <0.001 |
| ≥3 | 12.99 (9.69, 17.61) | <0.001 | 9.51 (7.01, 13.02) | <0.001 | / | | 8.24 (6.07, 11.31) | <0.001 |
| **AUC** | 0.630[a]/0.719[b] | | 0.751 | | 0.712 | | 0.767 | |

*ADL, activities of daily living; OR, odds ratio; No., number; IC, intrinsic capacity; AUC, area under the curve.*

*[a]Multimorbidity status: Chronic diseases include: hypertension, coronary artery disease, diabetes mellitus, stroke, tumor, and chronic obstructive pulmonary.*

*[b]Intrinsic capacity status: IC includes five domains: locomotion, vitality, sensory, cognition, and psychological.*

*Model 1: Adjustment for age, sex, education, and marriage to determine the odds of disability based on IC impairment.*

*Model 2: Adjustment for age, sex, education, and marriage to determine the odds of disability based on multimorbidity status.*

*Model 3: Adjustment for multimorbidity status, age, sex, education, and marriage to determine the odds of disability based on IC impairment.*

vs. 13.7% with one domain) and the number of chronic diseases (20.9% in ≥3 chronic diseases vs. 12.4% with one chronic disease).

## Association of IC Impairment and Multimorbidity Status With 1-year Incident ADL Disability

Only subjects who had ≥3 chronic diseases had a significant odds of 1-year incident ADL disability (adj. OR 1.73, 95%CI = 1.30–2.30). Impairments in multiple domains of IC showed higher odds of incidence ADL disability; however, significance was observed only for impairments in 2 or ≥3 domains (adj. OR 2.32, 95%CI = 1.72–3.11 for impairments in ≥3 domains, adj. OR 1.43, 95%CI = 1.14–1.80 for impairments in two domains). The association remained equally significant even after including adjustment for chronic diseases. The AUC for the fully adjusted model of IC impairment was 0.691 and 0.681 for the model of multimorbidity status (**Table 4**).

# DISCUSSION

In this study, we investigated the association of IC impairment and multimorbidity with disability using a representative sample of community-dwelling older adults in China. Our findings showed IC impairment to be associated with higher odds of disability both cross-sectionally and at 1-year follow-up compared to multimorbidity. Odds of disability increased with impairment in multiple domains of IC compared to the increase in the number of diseases. These findings support our hypothesis that a function-centered approach could provide better prognostic information on the process of disability beyond that contributed by the presence or absence of multiple chronic diseases in older adults.

Our study showed the global prevalence of IC impairment to be 64.5%, which is in agreement with a previous study (in Chinese) of relatively healthy inpatient population (10), and slightly higher than another study from a longitudinal cohort (9). IC impairment was the highest in the vitality domain (34.7%), followed by sensory (32.8%), cognition (18.4%),

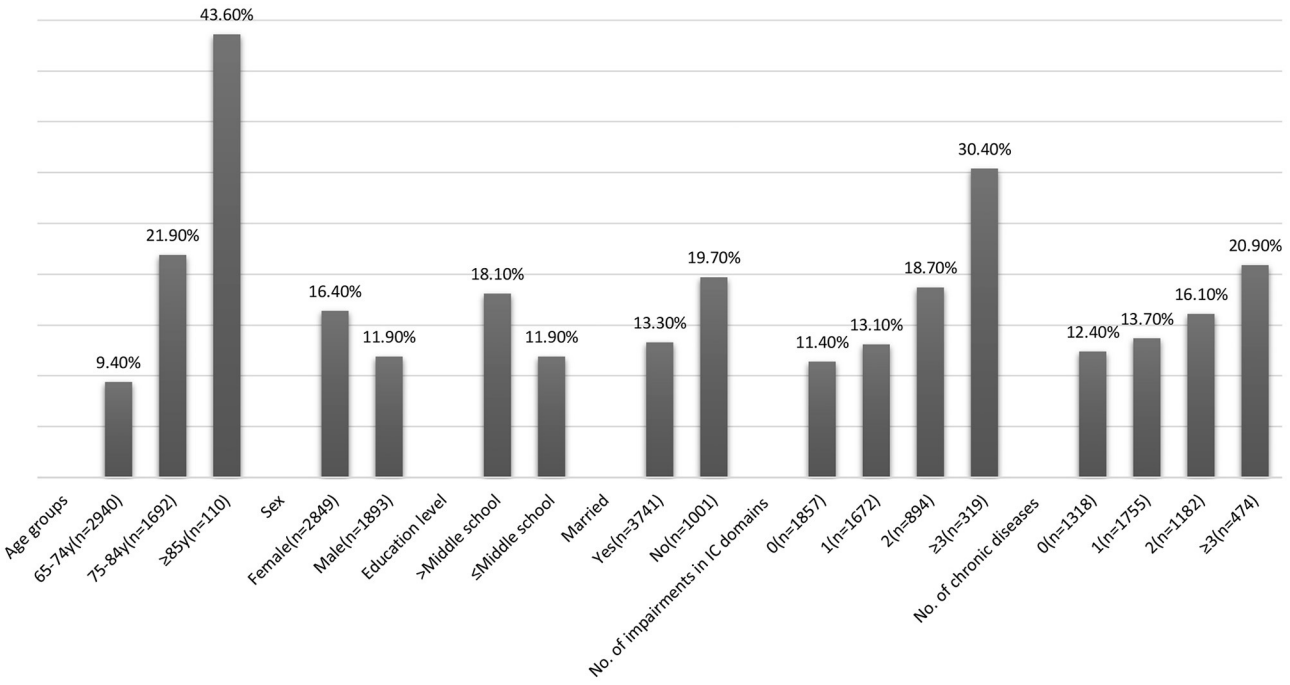


**FIGURE 3 |** Incidence of activities of daily living (ADL) disability at 1-year follow-up.

psychological (11.8%), and locomotion (11.1%). However, impairment according to individual domains of IC differed in the past studies. One of the reasons for such difference is the variations in the methods used for assessing individual domains of IC. Although the WHO ICOPE care plan has suggested certain screening methods for each domain of IC, the overall concept of IC remains to be validated in multiple populations (21). Hence, many previous studies and including our current study have used tools that are different from what the WHO has recommended but which equally capture the spectrum of each domain. For instance, vitality is a measure of physiologic factors (such as energy balance and metabolism) contributing to the IC of an individual. We have used MNA to assess vitality, whereas several techniques such as gait speed and grip strength may be equally effective in assessing vitality. It is undeniable that there is a necessity for robust and uniform approaches to measure IC (21), in particular, if we were to expect implementing IC in clinical settings soon (i.e., avoiding proliferation leading to ambiguities).

The WHO healthy aging framework divides the decline in IC into three periods: a period of relatively high and stable function and capacity, a period of declining capacity, and a period of significant loss in capacity and function indicated by dependency and disability (14). Our study supports this concept showing older individuals who have impairments in multiple domains of IC to have a higher prevalence of ADL disability (representing a state of significant loss in capacity). Indeed, longitudinal data with multiple follow-ups are needed to fully explore the trajectory of IC. The prevalence of the disability was also higher in those with impairments in multiple domains of IC compared to those with a higher number of diseases or multimorbidity. Our study also showed that subjects with the IC impairment had a higher odds of being disabled. Subjects who had impairments in three or more domains had almost 10 times higher risk of being disabled compared to those without any impairments in IC domains. However, the correlation between comorbidity status and ADL disability was not as stronger. Subjects with three or more diseases had about four times higher odds of being disabled. A similar trend was observed even while considering a 1-year incident disability. However, higher odds of incident ADL disability were associated with the presence of three or more chronic diseases and impairments in over two domains of IC. It should also be noted that although the AUCs for both models (i.e., IC and multimorbidity) were almost similar demonstrating uniform performance of the models. However, higher odds ratios in the model with IC impairment showed better predictability of ADL disability compared to the model with multimorbidity. These findings are in accordance with our hypothesis.

The relation between multimorbidity, IC, and disability was also highlighted in a previous study conducted in the ELSA cohort (8). The authors demonstrated that although multimorbidity too predicted incident disability, IC was far more superior, many of the personal characteristics contributing to the loss of function was mediated through IC including multimorbidity. Such findings could be explained through a recent theory that IC could be influenced not only by the environment but also through the level of physiologic reserve of an individual (22). Individuals with lower physiologic reserve or with impairments in IC (22) could experience poor recovery once exposed to stressors and as a result of the continuum of the aging process and diseases and may be vulnerable to being

**TABLE 4 |** Logistic regression to determine the odds of activities of daily living (ADL) disability at 1-year follow-up.

| | ORs for the incidence of ADL disability (95% CI) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Unadjusted** | ***p*** | **Model 1** | ***p*** | **Model 2** | ***p*** | **Model 3** | ***p*** |
| **Age group** | | | | | | | | |
| 65–74 y | Ref | | Ref | | Ref | | Ref | |
| 75–84 y | 2.72 (2.30, 3.23) | 0.956 | 2.58 (2.16, 3.08) | 0.870 | 2.64 (2.22, 3.16) | 0.631 | 2.52 (2.11, 3.02) | 0.678 |
| ≥85 y | 7.50 (5.03, 11.14) | <0.001 | 6.92 (4.56, 10.45) | <0.001 | 7.84 (5.18, 11.79) | <0.001 | 7.05 (4.64, 10.65) | <0.001 |
| **Sex** | | | | | | | | |
| Male | Ref | | Ref | | Ref | | Ref | |
| Female | 1.45 (1.22, 1.72) | <0.001 | 1.49 (1.24, 1.80) | <0.001 | 1.54 (1.28, 1.86) | <0.001 | 1.49 (1.23, 1.80) | <0.001 |
| **Education** | | | | | | | | |
| High school or above | Ref | | Ref | | Ref | | Ref | |
| Middle school or below | 1.63 (1.39, 1.92) | <0.001 | 1.17 (0.98, 1.39) | 0.091 | 1.14 (0.94, 1.39) | 0.009 | 1.18 (0.99, 1.41) | 0.066 |
| **Married** | | | | | | | | |
| Yes | Ref | | Ref | | Ref | | Ref | |
| No | 1.60 (1.33, 1.92) | <0.001 | 1.09 (0.89, 1.33) | 0.391 | 1.26 (1.06, 1.50) | 0.182 | 1.10 (0.90, 1.34) | 0.337 |
| **No. of chronic diseases[a]** | | | | | | | | |
| 0 | Ref | | / | | Ref | | / | |
| 1 | 1.12 (0.91, 1.39) | 0.028 | / | | 1.08 (0.87, 1.35) | 0.063 | 1.07 (0.86, 1.33) | 0.097 |
| 2 | 1.35 (1.71, 1.69) | <0.001 | / | | 1.21 (0.96, 1.53) | 0.086 | 1.17 (0.93, 1.48) | 0.794 |
| ≥3 | 1.86 (1.41, 2.44) | <0.001 | / | | 1.73 (1.30, 2.30) | <0.001 | 1.64 (1.23, 2.18) | 0.001 |
| **No. of impairments in IC domains[b]** | | | | | | | | |
| 0 | Ref | | Ref | | / | | Ref | |
| 1 | 1.18 (0.96, 1.44) | 0.002 | 1.07 (0.88, 1.32) | 0.014 | / | | 1.07 (0.87, 1.31) | 0.0195 |
| 2 | 1.79 (1.44, 2.24) | <0.001 | 1.43 (1.14, 1.80) | <0.001 | / | | 1.40 (1.11, 1.76) | <0.001 |
| ≥3 | 3.41 (2.58, 4.50) | <0.001 | 2.32 (1.72, 3.11) | <0.001 | / | | 2.23 (1.66, 2.99) | <0.001 |
| **AUC** | 0.549[a]/0.589[b] | | 0.685 | | 0.681 | | 0.691 | |

*ADL, activities of daily living; OR, odds ratio; No., number; ref, reference; IC, intrinsic capacity; AUC, area under the curve.*

*[a]Multimorbidity status: Chronic diseases include: hypertension, coronary artery disease, diabetes mellitus, stroke, tumor, and chronic obstructive pulmonary.*

*[b]Intrinsic capacity status: IC includes five domains: locomotion, vitality, sensory, cognition, and psychological.*

*Model 1: Adjustment for age, sex, education, and marriage to determine the odds of disability based on IC impairment.*

*Model 2: Adjustment for age, sex, education, and marriage to determine the odds of disability based on multimorbidity status.*

*Model 3: Adjustment for multimorbidity status, age, sex, education, and marriage to determine the odds of disability based on IC impairment.*

disabled, which is also in line with our results. Some studies on the subject have also shown IC impairment to be associated with disability including in the community-dwelling older population (9, 11) and hospitalized patients (7, 10).

Population aging has led to the emergence of geriatric medicine, particularly in countries such as China (23). Geriatricians have begun to advocate that now is the time to put an end to the disease-based approach (3) and initiate implementation of a function-based approach such as frailty to improve the care needs of older adults (2). Frailty, which is a geriatric syndrome characterized by reduced homeostasis and increased vulnerability to stressors (24), undeniably has stressed the need to focus on functions rather than treating a single disease. For example, China, which has the highest number of older people worldwide, has already enough studies on frailty (12, 25) to justify the need to prevent disability and maintain autonomy in old age (26). However, population aging is a positive aspect of human progress and instead of focusing on health deficits (or negative health attributes) such as the lauded concept of frailty, greater consideration is being given to the concepts that capture positive health attributes and empower older adults such as IC. Our findings have proven a function-centered approach (driven by positive health attributes) such as IC can effectively predict disability in older people better than disease-based approaches such as multimorbidity. The construct of IC holds a great potential to transform geriatric care worldwide including in regions without well-established geriatric medicine.

Our study has several limitations. Some of the recommended assessment methods were not available in our study cohort; hence, we used alternative methods to measure the IC domains. Nevertheless, the methods we used should equally capture the magnitude of all of these domains. Some of the subjects who lost to follow-up could be the ones who were already dependent; hence, the incidence rate of disability may have been underestimated. We used six chronic diseases that were most common in our study population to assess the severity of multimorbidity. However, different chronic diseases might not have the same weight while considering the effect of multimorbidity on disability (e.g., cardiovascular conditions vs. tumors and others or combined), hence could have influenced our findings. Furthermore, self-reported history of disease is also another limitation of our study, which might have impacted the multimorbidity status of the study population. Nevertheless, there are several strengths of this study. Our study was performed

in a representative sample of the community-dwelling older population in the Beijing region. This sample included both urban and rural populations hence could better represent the Chinese aging scenario sample. To our knowledge, this is the first study to specifically compare IC (according to impairments in multiple domains) and multimorbidity (stratified by the number of diseases) in predicting future disability.

In conclusion, our findings imply that a function-centered construct could be more useful in predicting future disability beyond the traditional disease-based approach. Nevertheless, our findings need to be confirmed in future studies with much longer follow-ups. The WHO ICOPE care plan for older people is centered around the construct of IC. This care plan is proposed to help older people in achieving healthy aging, i.e., enable them to be independent and perform tasks that they value the most. The ICOPE approach highlights the need to reform geriatric care from a disease-centered approach to a function-centered approach, which has been justified from our study. This reform should commence from the very base level of healthcare, and priority should be given to the evaluation of IC instead of just treating disease in primary care while examining an old patient. Moreover, public health strategies to maintain IC individuals throughout life course should be developed that are easy to implement and are cost-effective.

## DATA AVAILABILITY STATEMENT

The datasets generated from this study may be available upon reasonable request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xuanwu Hospital of Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

JZ, JC, PC, and LM responsible for study design. JZ and JC responsible for manuscript preparation. JZ, ZZ, JC, YC, and LM responsible for acquisition of subjects and data, and analysis and interpretation of data. JC and PC critically reviewed the article. All authors approved the final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.753295/full#supplementary-material

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# Prognostic Significance of Ultrasound Findings of Acute Acalculous Cholecystitis for Elderly Long-Term Bedridden Patients

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**Background:** Acute acalculous cholecystitis (AAC) is characterized by the development of cholecystitis in the gallbladder without gallstones or with small gallstones unrelated to inflammatory diseases. This disease is not rare in the elderly bedridden patients with co-morbidities and prone to develop life-threatening gangrene or perforation of gallbladder. Early imaging is essential for detecting and effectively treating AAC. This study aimed to evaluate the use of ultrasound diagnostic criteria for the diagnosis and prognosis of elderly long-term bedridden patients with suspected AAC.

**Methods:** We retrospectively studied 374 elderly bedridden patients with clinical manifestations of AC at the acute stage of the disease. Gallbladder anomalies were found in 92 patients by ultrasound examination, which correlated with the duration time of clinical manifestations, complications, as well as therapeutic prognosis. The major and minor ultrasound criteria of AAC were made according to the Tokyo Guidelines 2018. Ultrasound results were thought to be AAC positive when they met two major criteria or one major and two minor criteria.

**Results:** Forty-three (46.7%) of the 92 patients presented with AAC (+) test results based on the ultrasound criteria, with a higher incidence of complications (27.9%) than AAC (–) patients (0%; $P < 0.001$). The median length of symptoms (8 vs. 4 days, $P < 0.001$) and duration of antibiotic therapy (13 vs. 5 days, $P < 0.001$) were longer in the AAC (+) group.

**Conclusions:** The ultrasound-based AAC (+) group often had a worse prognosis than the AAC (–) group. Therefore, patients from the AAC (+) group should receive a follow-up ultrasound examination to detect disease progression early.

**Keywords: acute acalculous cholecystitis, elderly, gallbladder, long-term bedridden patients, ultrasound**

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## INTRODUCTION

In recent years, the average life expectancy has been steadily increasing in many countries (1). For example, China has experienced a significant increase in life expectancy, with the older population growing at a rate of 5% per year. In fact, it is expected that there will be more than 74 million individuals above 80 years of age by 2040 (2). This patient population represents a clinical challenge as older individuals are at greater risk of presenting with an episode of acute cholecystitis (AC) due to the organ dysfunction and weakened immune system, and up to 6% of older patients experience severe AC (3). Acute acalculous cholecystitis (AAC) is a gallstone-free necrotizing inflammation of the gallbladder. Approximately 5–10% of all cases of AC were AAC, which tends to be more intricate with poor prognosis compared with acute calculous cholecystitis (ACC) (4, 5) as the comorbidities such as hypertension, coronary heart disease and diabetes in elder with AAC may anonymize the progress the disease. However, the gallbladder necrosis rate of AAC is about 40–60%, while the gallbladder perforation rate is as high as 5–15%. AAC is associated with a high mortality (30% in most studies; range 10–90% with early or late diagnosis, respectively) (6).

Given the high morbidity and mortality of gallbladder gangrene and perforation that develops from ischemia in a short period of time, early and accurate diagnosis is essential. The manifestations of AAC differ from the population of patients. In children, AAC is the most frequent form of acute cholecystitis (about 50–70%), which mainly results from infectious diseases (such as parasite, Epstein-Barr virus and hepatitis A virus infections). Thus, supportive care (analgesia, rehydration) is usually the primary treatment, together with antibiotic therapy, regular clinical evolution and sonographic monitor (7). While in adults, AAC is often secondary to severe trauma, post resuscitation, different kinds of shock, major surgery, long-term fasting, parenteral nutrition and severe infection, and open or laparoscopic cholecystectomy or percutaneous cholecystostomy are usually the primary treatment besides antibiotic therapy (8). However, in the elder, most patients have a more significant burden of comorbidities, especially for long-term bedridden patients; moreover, some severe patients cannot communicate their symptoms due to anesthetized, intubated, and/or unconscious situation. Furthermore, the unspecific symptoms and laboratory examinations also impede the diagnosis of AAC, including fever, pain in right upper quadrant, leukocytosis, and increased hepatic enzyme levels (9, 10). Susceptible factors in long-term bedridden elderly (such as long-time of enteral fasting, mostly parenteral nutrition, high rate of ventilator use) contribute to the worse prognosis by prolonging time of cholestasis and induce severe complications, which refer to gallbladder perforation, gallbladder hemorrhage, perigallbladder abscess.

Although various composite approaches are applied in clinical work to diagnose AAC, imaging technology is vital for the ultimate diagnosis of AAC. Ultrasound is strongly recommended by the Tokyo Guidelines 2018 (TG13) as the preferred imaging examination for the morphological diagnosis of AC given that it is easy-to-use, easy-to-repeat at the bedside, and provides a real-time assessment (11). Ultrasound is of greater significance in elderly patients with long-term bedridden as this group of people rely more on bedside ultrasound for follow-up, to provide timely information of disease changes. Moreover, ultrasound of the gallbladder is believed to be the most accurate method for diagnosing AAC in critically ill patients (6).

In long-term bedridden elderly patients, however, disease characteristics, comorbidities, and poor functional status augment the rates of misdiagnosis and missed diagnosis by ultrasound. In these patients, AAC often represents further progression of multiple systemic failures. To the best of our knowledge, the occurrence of AAC is rarely investigated as a medical complication in elderly long-term bedridden patients. Although such cases are frequently encountered clinically, only several case reports are available (12, 13). Moreover, no studies have specifically demonstrated the prognostic value of the current ultrasound criteria for AAC in this patient group.

Given the fact that the patient population included represents a clinical challenge as older individuals are at greater risk of presenting with an episode of acute cholecystitis (AC) due to the organ dysfunction and weakened immune system. Up to 6% of older patients experience severe AC, with little systematic information on further study of the elder with AAC. What's more, the relationship between diagnostic criteria, severity classification and treatment indications for the vulnerable subgroup is still limited. Therefore, the aim of the current study was to investigate the necessity of ultrasound in early AAC diagnosis and prognosis improvement in this specific population to improve clinical management and treatment planning.

## MATERIALS AND METHODS

### Research Participants

This retrospective cohort study was performed in Chinese PLA General Hospital. The study protocol was officially approved by ethics committee of Chinese PLA General Hospital, Beijing (No. S2021-230-01, obtained on March, 2021) and conformed to the Declaration of Helsinki. According to the Ethics Committee, our study is retrospective research, therefore, patient consent statements were waived and each patient had signed the standard consent statement before ultrasound. Our study population was selected from 374 elderly bedridden patients (bed rest time $\geq$ 20 days, mean bedtime 92.35 $\pm$ 29.47 days) with multiple comorbidities (diabetes, cardiac disease, obstructive pulmonary diseases, and renal insufficiency) and clinical findings supportive for AC (fever, right upper quadrant abdominal pain, increased abnormal liver function indicators, and tenderness), who underwent ultrasound examinations from January 1, 2018 to May 1, 2020.

Ultrasound is used as the method for diagnosing AAC in our analysis, subsequent imaging (CT or MRI) or pathological examinations are auxiliary tools if there is still uncertainty about the diagnosis. Patients with AC were included into our study initially and patients with cholecystolithiasis/choledocholithiasis, ACC, history of cholecystectomy, malignant tumor, liver disease, and those with uncertain results of cholecystectomy

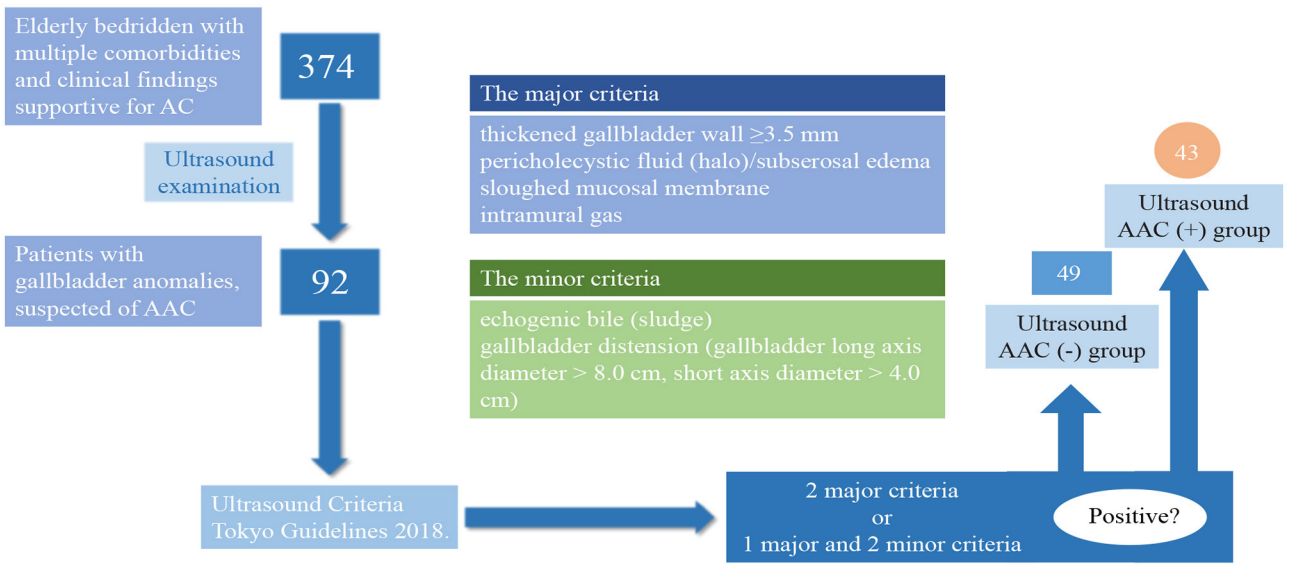


**FIGURE 1 |** AAC ultrasonic examination criteria and a flowchart detailing the patient flow.

were excluded out of analysis totally. The exclusion criteria included patients with hepatobiliary malignancy, concomitant acute cholangitis or common duct stones, chronic cholecystitis, and nonspecific gallbladder wall thickening associated with acute pancreatitis, hepatitis, pyelonephritis, peritonitis, ascites, hypoalbuminemia, congestive heart failure, or chronic renal failure.

The diagnosis criteria of AAC are recommended by the Tokyo Guidelines 2018 (TG13) as follows. A. Local signs of inflammation etc. (1) Murphy's sign, (2) RUQ mass/pain/tenderness. B. Systemic signs of inflammation etc. (1) Fever, (2) elevated CRP, (3) elevated WBC count. C. Imaging findings. Imaging findings characteristic of acute cholecystitis. On the premise of excluding acute hepatitis, other acute abdominal diseases, and chronic cholecystitis and no calculus found, suspected diagnosis of AAC is made on the condition of one item in A + one item in B and definite diagnosis is made on the condition of one item in A + one item in B + C (11).

Among the 374 patients, 282 patients were excluded from our analysis for above reasons, which will be described in detail later and a total of 92 patients suspected of AAC were included in the analysis which were divided into AAC (+) and AAC (–) groups according to TG13.

## Ultrasound Protocol

Ultrasound examinations were carried out with a PHILIPS CX50 portable ultrasound machine (PHILIPS Medical System, Bothell, WA, USA) equipped with a curved transducer (3–7 MHz). Three certified ultrasound technicians with more than 5 years of work experience performed the examination using a standardized ultrasound examination protocol. The ultrasound examination procedures, including standard image acquisition and measurements, were strictly recorded, and each examiner followed the same protocol to minimize the information bias between examiners. The ultrasound images stored in the PACS system were reviewed and recorded by two independent observers.

AAC ultrasonic examination criteria and a flowchart detailing the patient flow are shown in **Figure 1**. Patients were considered AAC (+) if at least two major criteria or one major with two minor criteria were identified from the ultrasound examination. The major criteria included: a thickened wall of the gallbladder (≥ 3.5 mm), pericholecystic fluid (halo)/ subserosal edema, a sloughed mucosal membrane, and intramural gas. The minor criteria included: echogenic bile (sludge) and gallbladder distension (gallbladder long axis diameter > 8.0 cm, short-axis diameter > 4.0 cm) (6). The typical ultrasonic manifestations of AAC are shown in **Figure 2**.

In addition, we reviewed the clinical course, including symptoms, complications, treatment, ultrasound imaging findings, and outcome (mortality) in all the patients. The duration of ultrasound follow-up in our study was at least 2 weeks since the initial clinical suspicion.

Our analysis brought into only complications associated with cholecystitis acquired during subsequent imaging (CT or MRI) or pathological examinations, including gallbladder perforation, gallbladder abscess, gallbladder bleeding, and cholecystitis-associated septicemia.

All the databases and files were retrospectively reviewed with approval from the local ethics committee.

## Data Analysis and Statistics

SPSS Statistics version 18.0 (IBM Corp., Armonk, N.Y., USA) was used for the statistical analysis. Categoric variables are presented as numbers and percentages; continuous variables are

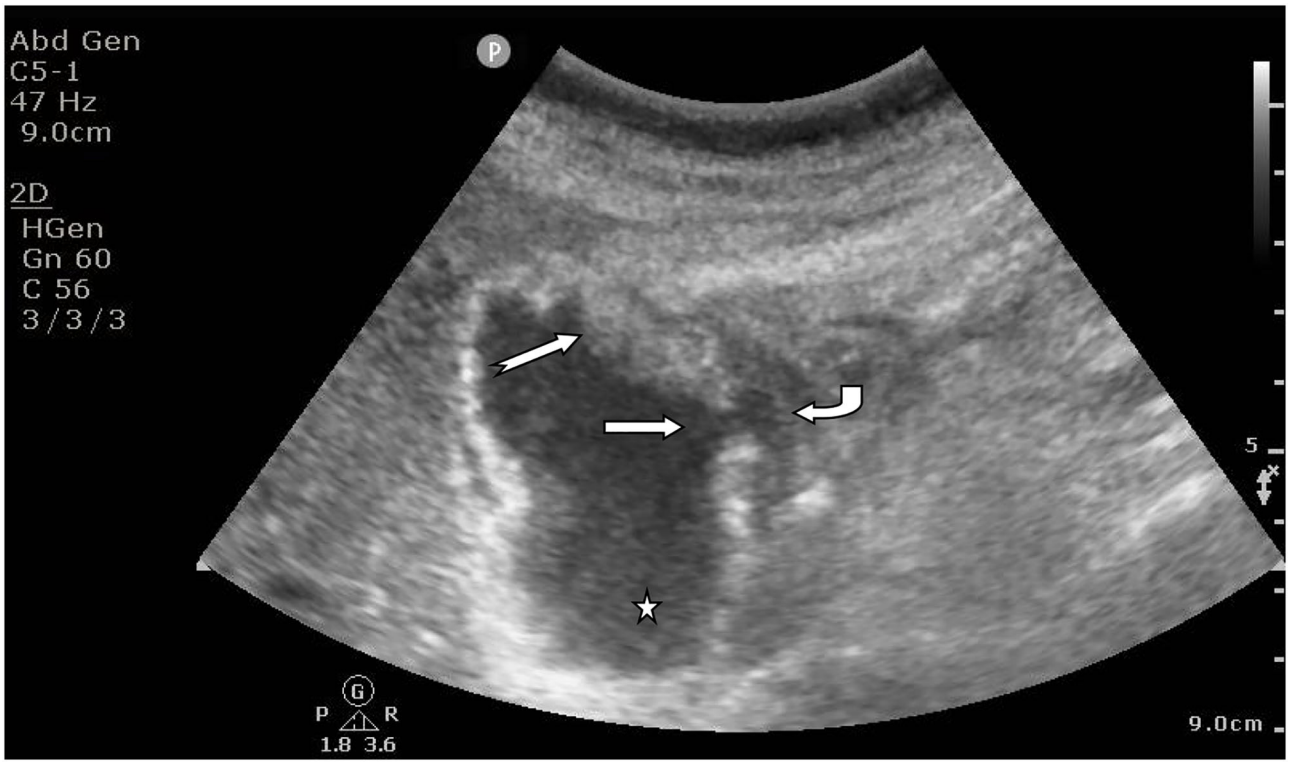


**FIGURE 2 |** Supine examination of a male patient showed increased gallbladder volume, interrupted continuity of the gallbladder wall, perforation (straight arrows), uneven thickening of the gallbladder wall with mucosal layer abscission (swan-tail arrows), cholestasis (pentagonal star), and effusion around the gallbladder (curved arrows).

summarized using means ± standard deviations. The Wilcoxon rank-sum test and chi-square test or Fisher's exact test were used to compare the duration of symptoms and the complication rates between the groups, respectively. A $P < 0.05$ was considered statistically significant.

# RESULTS

## Clinical Characteristic

Among the 374 patients, 97 with cholecystolithiasis/choledocholithiasis confirmed by imaging data, 59 with ACC, 45 with a history of cholecystectomy, 39 with liver disease that may cause symptoms of right upper abdominal pain, 30 with a malignant tumor, and 12 with uncertain results of cholecystectomy were excluded. Therefore, a total of 92 patients suspected of AAC were included in the analysis.

The clinical characteristics were shown in **Table 1**. Their mean age was 81.92 ± 19.68 (58–101) years, and 69.57% were men. There were nine patients (9.78%) with a history of recent major surgery, 15 (16.30%) with prolonged enteral fasting, 28 (30.43%) with parenteral nutrition, 25 (27.17%) on mechanical ventilation, and 15 (16.30%) under sedation.

## Ultrasound Examination

Patients were divided into ultrasound AAC (+) and ultrasound AAC (–) groups. Symptom duration, complications, and gallbladder-associated mortality were compared between the two groups.

Among these 92 patients, 43 cases (46.74%) were diagnosed by ultrasound as AAC (+) and 49 (53.26%) as AAC (–). Additionally, among these 92 patients, ultrasound examinations revealed mucosal layer shedding in the gallbladder wall in 15 cases (16.30%), effusion around the gallbladder in 45 (48.91%), gallbladder distension in 55 (59.78%), thickening of the gallbladder wall in 65 (70.65%), and sludge in 80 (86.96%). No gas in the gallbladder wall was found in any of the ultrasound examinations. The ultrasound characteristics of the AAC (+) and AAC (–) patients are shown in **Table 2**.

## Complications

Complications occurred in 12 patients from the AAC (+) group, including four cases of gallbladder perforation, three of gallbladder bleeding, three of pericholecystic abscess, and two of septicemia. The ultrasound diagnosis of the AAC (+) group was significantly related to the occurrence of complications (27.91%). In contrast, no complications occurred in the AAC (–) group ($P = 0.005$, **Table 3**).

**TABLE 1 |** Patients' demographic and clinical characteristics.

| | Overall patients $n = 374$ | Included patients $n = 92$ |
|---|---|---|
| Age, years, mean (SD) | 84 (12) | 82 (8.0) |
| Gender, male (%) | 214 (57.22) | 64 (69.57) |
| **Co-morbidities, *n* (%)** | | |
| Diabetes mellitus | 132 (35.29) | 32 (33.70) |
| Cardiac disease | 107 (28.61) | 30 (32.61) |
| COPD and/or asthma | 61 (16.31) | 14 (15.22) |
| Chronic renal failure | 49 (13.10) | 9 (9.78) |
| Biliary disease history | 198 (52.94) | 44 (47.83) |
| **Clinical presentation** | | |
| RUQ pain/ mass /tenderness | 223 (59.63) | 65 (70.65) |
| Fever | 179 (47.86) | 40 (43.48) |
| Vomiting | 163 (43.58) | 39 (42.39) |
| Jaundice | 55 (14.33) | 26 (28.26) |
| Hypotension[a] | 23 (6.15) | 9 (9.78) |
| **Laboratory data** | | |
| WBC ($10^9$/L) | 12.5 (8.8, 16.2) | 12.3 (8.5, 15.7) |
| Platelets ($10^9$/L) | 191 (152, 250) | 194 (159, 250) |
| CRP (mg/L) | 119.6 (98.9, 129.4) | 125.3 (108.7, 130.1) |
| Albumin1 (g/L) | 35 (27, 38) | 34 (28, 39) |
| Bilirubin1 ($\mu$mol/L) | 59 (37, 92) | 67 (44, 96) |
| ALT (IU/L) | 127(66, 251) | 139 (85, 266) |
| AST (IU/L) | 159 (76, 359) | 180 (89, 370) |
| ALP (IU/L) | 200 (127, 334) | 214 (117, 340) |
| GGT (IU/L) | 258 (127, 446) | 286 (159, 523) |
| INR | 1.15 (1.10, 1.30) | 1.18 (1.10 1.30) |
| **Radiological investigations** | | |
| US scan, *n* (%) | 374 (100) | 92 (100) |
| CT scan, *n* (%) | 162 (43.32) | 35 (38.04) |
| MRI scan, *n* (%) | 83 (22.19) | 14 (15.22) |
| **AC grade** | | |
| Grade I | 89 (23.80) | 24 (26.09) |
| Grade II | 178 (47.59) | 43 (46.74) |
| Grade III | 107 (28.61) | 25 (27.17) |

[a]*Hypotension was defined as systolic blood pressure of < 90 mmHg.*
*COPD, Chronic obstructive pulmonary disease; RUQ, Right upper quadrant; WBC, White blood cell; CRP, C-reactive protein; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; GGT, Gamma-glutamyl transferase; INR, International normalized ratio; US, Ultrasound; CT, Computed tomography; MRI, Magnetic resonance imaging; AC, Acute cholecystitis. All cases were classified into Grade I (mild), Grade II (moderate), and Grade III (severe) AC according to Tokyo 13/18 Guidelines.*

**TABLE 2 |** Summary of ultrasound imaging characteristics in acute acalculous cholecystitis (AAC) positive and negative groups.

| Characteristic | Total, *n* | Positive[a] | Negative[b] | Chi square | *P* |
|---|---|---|---|---|---|
| **Ultrasound Diagnosis, *n* (%)** | | | | | |
| **Wall thickness ≥ 3.5 mm** | | | | 19.486 | 0.000 |
| No | 27 | 3 (11.11) | 24 (88.89) | | |
| Yes | 65 | 40 (61.54) | 25 (38.46) | | |
| **Pericholecystic fluid** | | | | 11.092 | 0.001 |
| No | 47 | 14 (29.79) | 33 (70.21) | | |
| Yes | 45 | 29 (64.44) | 16 (35.56) | | |
| **Sloughed mucosa** | | | | 20.423 | 0.000 |
| No | 77 | 28 (36.36) | 49 (63.64) | | |
| Yes | 15 | 15 (100.00) | 0 (0.00) | | |
| **Intramural gas** | | | | | |
| No | 92 | 25 (27.17) | 67 (72.83) | | |
| **Hydrops** | | | | 19.242 | 0.000 |
| No | 37 | 7 (18.92) | 30 (81.08) | | |
| Yes | 55 | 36 (65.45) | 19 (34.55) | | |
| **Echogenic bile/sludge** | | | | 2.620 | 0.106 |
| No | 12 | 3 (25.00) | 9 (75.00) | | |
| Yes | 80 | 40 (50.00) | 40 (50.00) | | |
| Total | 92 | 43 (46.74) | 49 (53.26) | | |

[a]*Ultrasound AAC (+) group,* [b]*Ultrasound AAC (-) group.*

In addition, ultrasound examinations revealed that pericholecystic effusion (11 of 45 patients, 24.44%), gallbladder distension (12 of 55 patients, 21.81%), and sludge (12 of 80 patients, 15.00%) were related to the occurrence of complications (**Table 4**). In the AAC (+) group, sloughed mucosa was observed in 15 cases (34.89%), although only four of these presented with complications ($P > 0.99$).

We also analyzed and compared hospital outcomes of AAC patients who with and without complications (**Table 5**). There was no difference in ultrasound-guided percutaneous cholecystostomy (US-PC) between two groups (12 vs. 10; $p = 0.961$). However, 12 AAC patients with complications (100%) underwent US-PC. 4 AAC patients were treated with Cholecystectomy, which were all came from the complications group. There was no patient with complications underwent antibiotics only. The length of ICU stay and follow-up time were comparable between two groups (24.5 vs. 12.9, 33.5 vs. 19.1, $p < 0.05$). (There were two deaths in the complications group, but there was no correlation with gallbladder pathology).

## Follow-Up Ultrasound Examination and Treatment

In the follow-up ultrasound examinations of the 43 patients in the AAC (+) group, 15 (34.88%) still presented as AAC (+). For these 15 patients, the ultrasound follow-up frequency was two to three times in the first 2 weeks and once a week in the following weeks until the symptoms disappeared or until death (mean follow-up time: 21.63 ± 12.67 weeks, range: 4–36 weeks). Another 28 patients (65.12%) in the positive group presented with negative findings for AAC in the follow-up ultrasound examinations after treatment. Of note, during the follow-up, only one AAC (+) patient worsened; in this case, a cholecystoduodenal fistula was identified by ultrasound in time for treatment, and the patient recovered after treatment. There were two deaths in the AAC (+) group, although there was no correlation with gallbladder pathology. In the AAC (-) group, 19 (38.78%) of the 49 patients were diagnosed as AAC (+) on follow-up diagnostic tests within 1 week. Among these 19

**TABLE 3 |** Summary of complications by ultrasound in acute acalculous cholecystitis (AAC) positive and negative groups.

| Complications | Total, *n* | Positive[a] | Negative[b] | Chi square | *P* |
|---|---|---|---|---|---|
| **Ultrasound Diagnosis, *n* (%)** | | | | | |
| **Perforation** | | | | | 0.044 |
| No | 88 | 39 (44.32) | 49 (55.68) | | |
| Yes | 4 | 4 (100) | 0 (0.00) | | |
| **Gallbladder hemorrhage** | | | | | 0.098 |
| No | 89 | 40 (44.94) | 49 (55.06) | | |
| Yes | 3 | 3 (100.00) | 0 (0.00) | | |
| **Pericholecystic abscess** | | | | | 0.098 |
| No | 89 | 40 (44.94) | 49 (55.06) | | |
| Yes | 3 | 3 (100.00) | 0 (0.00) | | |
| **Septicemia** | | | | | 0.216 |
| No | 90 | 41 (45.56) | 49 (54.44) | | |
| Yes | 2 | 2 (100.00) | 0 (0.00) | | |
| **Total** | | | | 15.726 | 0.000 |
| No | 80 | 31 (38.75) | 49 (61.25) | | |
| Yes | 12 | 12 (100.0) | 0 (0.00) | | |

[a] *Ultrasound AAC (+) group,* [b] *Ultrasound AAC (-) group.*

**TABLE 4 |** Summary of patient characteristics and ultrasound features by complication status.

| Characteristic | No | Yes | Chi square | *P* |
|---|---|---|---|---|
| **Any Complication, n (%)** | | | | |
| **Wall thickness ≥ 3.5 mm** | | | | |
| No | 27 (100.00) | 0 (0.00) | | 0.016 |
| Yes | 53 (81.54) | 12 (18.46) | | |
| **Pericholecystic fluid** | | | | |
| No | 46 (97.87) | 1 (2.13) | 10.095 | 0.001 |
| Yes | 34 (75.56) | 11 (24.44) | | |
| **Sloughed mucosa** | | | | |
| No | 69 (89.61) | 8 (10.39) | | 0.103 |
| Yes | 11 (73.33) | 4 (26.67) | | |
| **Intramural gas** | | | | |
| No | 87 (94.57) | 5 (5.43) | | |
| **Hydrops** | | | | |
| No | 37 (100.00) | 0 (0.00) | | 0.001 |
| Yes | 43 (78.18) | 12 (21.82) | | |
| **Echogenic bile/sludge** | | | | |
| No | 12 (100.00) | 0 (0.00) | | 0.354 |
| Yes | 68 (88.00) | 12 (15.00) | | |
| **Ultrasound diagnosis of AAC** | | | | |
| Positive | 31 (72.09) | 12 (27.90) | 15.726 | 0.000 |
| Negative | 49 (100.00) | 0 (0.00) | | |
| **Results of two imaging diagnoses** | | | | |
| Both negative | 29 (100.00) | 0 (0.00) | | 0.016 |
| Any positive | 51 (80.95) | 12 (20.83) | | |
| Total | 80 (86.96) | 12 (13.04) | | |

**TABLE 5 |** Treatment and outcomes of AAC patients with complications vs. without complications.

| | Total (*n* = 43) | Complications (*n* = 12, 27.91%) | Without complications (*n* = 31,72.09%) | *p*-value |
|---|---|---|---|---|
| **Treatment** | | | | |
| US-PC | 22 (68.75) | 12 (100.00) | 10 (32.26) | NS |
| Cholecystectomy[a] | 4 (100.0) | 4 (100.0) | 0 (0.0) | <0.05 |
| Antibiotics only | 17 (30.4) | 0 (0.0) | 17(54.8) | <0.05 |
| **Outcomes** | | | | |
| ICU cases | 10 (23.3) | 6 (50.0) | 6 (19.4) | NS |
| Length of ICU stay, days | 16.5 (5.6–18.5) | 24.5 (14.0–47.3) | 12.9 (4.9–18.3) | <0.05 |
| Mortality (Indirectly associated with AAC) | 2 (4.7) | 2 (16.7) | 0 (0.0) | <0.05 |
| Follow-up time (weeks) | 21.6 (4.0–36.5) | 33.5 (8.7–40.6) | 19.1 (4.0–25.3) | <0.05 |

*US-PC, Ultrasound-guided percutaneous cholecystostomy; ICU, intensive care unit.* [a] *Ultrasound and pathologic findings were congruent in these patients.*

patients, 11 cases (57.89%) were diagnosed upon ultrasound re-examination, five (26.31%) with CT, and three (15.79%) with MRI. Following treatment, all of the patients in the AAC (-) group were experienced a complete disappearance of fever and right upper quadrant pain.

Significant alleviation or resolution of cholecystitis manifestations occurred in 17 of 43 patients in the AAC (+) group and 39 of 49 in the AAC (–) group following treatment with antibiotics. Twenty-two AAC (+) patients and 10 AAC (–) patients underwent ultrasound-guided percutaneous cholecystostomy (US-PC). Only four AAC (+) patients received cholecystectomy at the initial occurrence.

The median length of symptoms (eight vs. four days, $P < 0.001$) and duration of antibiotic therapy (13 vs. 5 days, $P < 0.001$) were longer in the AAC (+) group (**Table 6**).

## DISCUSSION

It has been suggested that the changes in the gallbladder observed with AAC may be a manifestation of systemic illnesses, such as intercurrent infections, metabolic disorders, vascular problems, injuries, and malignancies (14–17). Importantly, AAC mainly occurs in patients with debilitating conditions, and the development of AAC in elderly patients has already been reported with a male predominance (18). In our study, the proportion of older men was as high as 69.57% (64/92). All 92 patients suspected of AAC presented with systemic diseases of varying severity (9.78% stayed in the intensive care unit for significant surgery, 16.30% were on prolonged enteral fasting, 30.43% on parenteral nutrition, 27.17% on mechanical ventilation, and 16.30% under sedation). AAC poses significant diagnostic challenges in this group because it is generally a secondary event that occurs in acute-developed patients with complications. Therefore, the sensitivity and specificity of clinical

**TABLE 6 |** Summary of clinical treatment and the duration of cholecystitis symptoms and antibiotic therapy by ultrasound follow-up.

| Items | Ultrasound Diagnosis | | Total, *n* |
|---|---|---|---|
| **Treatment** | **Positive[b] (*n* = 43)** | **Negative[c] (*n* = 49)** | |
| US-PC | 22 (68.75) | 10 (31.25) | 32 |
| Cholecystectomy[a] | 4 (100.00) | 0 (0.00) | 4 |
| Antibiotics | 17 (30.36) | 39 (69.64) | 56 |
| **Duration time** | | | ***P* value** |
| Length of symptoms, median (IQR[d]), days | 8 (4–15) | 4 (1–6) | <0.001 |
| Antibiotic duration, median (IQR[d]), days | 13 (10–17) | 5 (4–8) | <0.001 |

*US-PC, Ultrasound-guided percutaneous cholecystostomy. [a]Ultrasound and pathologic findings were congruent in these patients, [b]Ultrasound AAC (+) group, [c]Ultrasound AAC (-) group, [d]The median interval (IQR).*

manifestations are reduced, and an abdominal ultrasound is requested for the first time.

In our study, the analysis was carried out using only the ultrasound criteria. The use of ultrasound in AAC has been well described in case series studies (6, 11). Although ultrasound diagnostic criteria and its diagnostic rate in the diagnosis of AAC varies among studies, diagnosing AAC based on identifying major and minor criteria is considered to be reasonably sensitive (19, 20). The role of diagnostic ultrasound in patients with AAC is twofold; namely, it is used to clarify the diagnosis in suspected cases based on clinical and laboratory results as well as to detect complications. Our results suggest that ultrasound diagnosis of AAC (+) in long-term bedridden elderly patients is predictive of a higher incidence of complications and a longer duration of clinical manifestation. The complication rate in the AAC (+) group was 27.91% in this study [compared with 0% in the AAC (–) group]. The higher incidence rate observed in our study agrees with previous studies (18–20). For example, a previous study found that among 94 patients with hematological malignancies and clinical manifestations, the AAC (+) group often presented with a higher rate of complications and mortality (20.9%) than the AAC (–) group (0%; $P < 0.001$) (21).

Although the pathogenesis of AAC is largely unknown, it is most likely related to bile stasis, necrosis, and ischemia of the gallbladder wall (22). In our study, all the AAC patients were found to have atherosclerotic vascular-associated diseases, such as hypertension, diabetes, apoplexy, or ischemic heart disease. Studies have shown that atherosclerosis and increased blood viscosity in the elderly aggravates the ischemia of the gallbladder wall (23). In particular, diffuse damage to the wall of small blood vessels, which narrows and obstructs the arterial lumen, and damage to gallbladder mucosa significantly increase the risk of AAC (16, 24–26). In fact, a study by Mungazi et al. (27) confirmed that systemic arteriosclerosis can cause a blood flow obstruction in the gallbladder wall, and that this gallbladder blood flow disorder makes patients more prone to gallbladder gangrene and gallbladder perforation. It is noteworthy that although gallbladder enlargement and cholestasis were considered secondary criteria, they were found in all 12 patients with complications in our study. This is likely caused by an excessive increase in gallbladder volume and increased pressure in the gallbladder cavity that produces gallbladder ischemia, leading to gangrene and gallbladder perforation, among other complications. Previous studies have shown that bile stasis due to slow or incomplete gallbladder contraction can alter the chemical composition of bile through increasing the level of stimulators, such as lysophosphatidylcholine, which predisposes the patient to mucosal injury (28). Increased intraluminal pressure resulted from bile stasis is believed to compromise gallbladder perfusion pressure, which may get exacerbated by vasoactive drugs and hypotension in critically ill patients (28, 29). Among bedridden elder patients, risk factors for gallbladder dysmotility and bile stasis are common, such as mechanical ventilation, fasting, total parenteral nutrition, and continuous enteral feeding. Inflammation of the gallbladder wall is further aggravated in these patients, and edema of the gallbladder mucosa occurs, which affects the gallbladder vein reflux and increases the risk of gallbladder perforation (17, 30). As there is no consensus and guidelines on the management of senile cholecystitis at home and abroad. Treatment is generally based on past experience, diagnostic laboratory, clinical, and imaging examinations. Operations are not adopted in our study due to many high-risk factors such as old age, bedridden and comorbidities. Percutaneous transhepatic gallbladder drainage or conservative treatment are applied to patients to relieve clinical manifestations according to patient conditions.

In this study, ultrasound monitoring of the gallbladder contributed to the diagnosis of AAC. In addition, ultrasound examination was instrumental in monitoring abnormalities as well as for efficacy evaluation in elderly long-term bedridden patients. We considered these two aspects in our study because the use of ultrasound is well worth concerns to broaden therapy options and decrease complications in AAC. An ultrasound follow-up in highly suspicious cases facilitates early diagnosis and prompts treatment, reducing complications and mortality. In this study, 38.78% of patients in the AAC (–) group were diagnosed as AAC (+) during the first week of ultrasound or CT follow-up review, indicating the rapidly progressing nature of AAC. Nevertheless, these patients often presented with early or mild AAC manifestations with fewer complications and a shorter disease course, leading to a negative diagnosis during the initial ultrasound examination. On the other hand, the lower complication rate and shorter duration of symptoms may also attribute to early diagnosis and treatment in this group. This is in line with a study by Thampy et al. (21), which found that 41% of the patients in the AAC (–) group had positive results for AAC within 1 week of the follow-up ultrasound. Due to the rapid progression of AAC, the role of ultrasound follow-up has been emphasized for the early detection of progressed AAC as well as to prevent misdiagnoses and missed diagnoses (19). Ultrasound can also be used as an adjunct treatment in AAC patients. In our study, only four of the 92

patients underwent a cholecystectomy, which is not surprising considering that elderly patients are often poor candidates for surgery because of concomitant medical problems. Thirty-two cases [22 AAC (+) and 10 AAC (–)] received percutaneous transhepatic gallbladder drainage (PTGBD) for AC in our study. Importantly, ultrasound could be used to guide the PTGBD procedure, increasing its safety and efficacy, or to constantly monitor its therapeutic efficacy.

Our study is not without limitations. First, as a retrospective study, patient randomization was not possible. Moreover, due to the relatively small patient sample size, it was not possible to perform a subgroup analysis, and conclusions on the relationship between the laboratory test results and imaging examinations in patients with gallbladder abnormalities could not be made. Second, most of the patients (56 of 92) had no pathologic confirmation. This is mainly because the patients in the acute stage were critically ill, and all of them were older patients who typically do not tolerate a cholecystectomy well. Finally, since we aimed to assess the prognostic importance of ultrasound, the ultrasound results of the gallbladder after treatment were not well assessed. In the future, more prospective studies are required to further investigate these unsolved issues.

Despite the above limitations, ultrasound is recommended as a pivotal diagnostic technique for the early discovery and treatment of gallbladder diseases in long-term bedridden elderly patients. The appropriate application of ultrasound may be instructive in determining the most appropriate and effective therapeutic schedule for individual patients.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Chinese PLA General Hospital, Beijing. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

YL, MY, GX, and JL: concept and design and supervision. QL, LS, CC, YQ, and ZY: acquisition, analysis, or interpretation of data and drafting of the manuscript. QL, LS, and CC: statistical analysis. MY, GX, and JL: obtained funding. The corresponding author attests that all listed authors meet authorship criteria. All authors critical revision of the manuscript for important intellectual content and read and approved the final draft of the manuscript.

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# Prevalence of Insomnia Symptoms in Older Chinese Adults During the COVID-19 Pandemic: A Meta-Analysis

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**Background:** The ongoing COVID-19 pandemic has disproportionately affected the sleep health of older adults, but the limited number of studies on insomnia symptoms of older Chinese adults differed in terms of screener of insomnia, sample size, and prevalence, making mental health planning for this population difficult. This meta-analysis estimated the prevalence of insomnia symptoms in older Chinese adults during the COVID-19 pandemic.

**Methods:** Both Chinese (CNKI, Wanfang, VIP) and English (PubMed, EmBase, PsycInfo) databases were systematically searched to identify cross-sectional studies containing data on the prevalence of insomnia symptoms in older Chinese adults during the pandemic. Risk of bias (RoB) of included studies was assessed with the Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data.

**Results:** Nine studies with a total of 27,207 older Chinese adults were included. RoB scores of these studies ranged between zero and six. The pooled prevalence rates of insomnia symptoms and moderate and severe insomnia symptoms were 24.6% [95% confidence interval (CI): 19.5–30.5%] and 11.1% (95% CI: 7.2–16.9%), respectively. In subgroup analysis, significantly higher prevalence rates were observed in studies defining insomnia symptoms as "Insomnia Severity Index (ISI) $\geq$ 8" than in those defining them as "ISI $\geq$ 15" (32.6 vs. 15.6%, $P < 0.001$) and in older adults living in the COVID-19 epicenter than in those living in other places (35.2 vs. 23.3%, $P = 0.006$).

**Conclusion:** Nearly one out of every four older Chinese adults suffered from insomnia symptoms during the pandemic. Mental health services for this population during the pandemic should include supportive activities aimed at improving mental well-being, periodic assessment of insomnia symptoms, and psychiatric assessment and treatment when necessary.

**Keywords: insomnia, older adults, prevalence, COVID-19, China**

## INTRODUCTION

Sleep disturbances and insomnia are common among older adults. In China, 35.9% of the adults aged 60+ years suffer from sleep disturbances and 24.4–26.8% report insomnia symptoms in the most recent month, as defined by a cut-off score on the Pittsburgh Sleep Quality Index (PSQI) (1–3). Factors associated with late-life insomnia symptoms included no spouses, mental health problems, major medical conditions, negative life events, and acute or recurring psychosocial stressors (2–5).

The ongoing COVID-19 pandemic is a significant source of stress in people's lives and has profound negative effects on the mental health of people of all ages (6). Because the majority of COVID-19-related hospitalizations and deaths occur in persons aged 65+ years, older adults have been disproportionately affected by the pandemic (7). The current public health guidelines recommend older adults to keep social distancing with others and stay indoors as much as possible, which in turn result in elevated levels of loneliness and social isolation in older adults (8, 9). Importantly, in the context of the pandemic, older adults are confronted with disruptions to the daily routines due to lockdown, difficulties in timely accessing to needed healthcare services due to the overwhelmed hospitals, financial loss due to economic recession, separation from family members due to mass quarantine, and difficulties in using digital technologies due to lack of internet access and no smartphones (10–12); these associated stressors may further exacerbate the mental health of older adults.

Mental health problems, including insomnia, are a common stress reaction following exposure to a traumatic event such as the COVID-19 pandemic. Nevertheless, compared to the numerous studies on mental health and sleep problems in general adults during the pandemic (13–15), relatively fewer have investigated sleep problems in the elderly population.

China has the world's largest population of older adults and Chinese adults aged 65+ years will rise to 366 million in 2050, accounting for 26.1% of the total population (16). Since the reform and open door policy in 1978, substantial socioeconomic–cultural changes have challenged the mental health of older adults in China. For example, the massive rural-to-urban migration of young labors has resulted in millions of left-behind older adults, the one-child policy and the erosion of the traditional family structure have resulted in millions of empty-nest elders, and the diminishing traditional value of filial piety has reduced adult children's willingness to care for older adults (17, 18). Therefore, the ongoing pandemic coupled with the rapid aging may have a much more devastating toll on the mental and sleep health of older Chinese adults.

In China, a few population-based studies have examined the prevalence of insomnia symptoms in older adults amid the pandemic, but these studies varied in terms of screener of insomnia [i.e., PSQI vs. Insomnia Severity Index (ISI)], sample size (i.e., from 35 to 13,964), and prevalence (i.e., from 13.1 to 42.9%) (19–23). Importantly, nearly all available data on older adults are derived from whole population-based studies, where elderly-specific prevalence data are not easily accessed (i.e., only shown in the main text). To facilitate the development of mental health policy aimed at preventing or reducing insomnia in older Chinese adults during the pandemic, it is necessary to systematically review available studies and estimate the prevalence of insomnia symptoms.

## MATERIALS AND METHODS

This systematic review and meta-analysis was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (**Supplementary Table 1**). Two authors (QQ Zhang and L Li) independently searched literature, extracted data, and performed risk of bias (RoB) assessment. Any disagreements were resolved by a discussion with the correspondence author (BL Zhong). In this study, after cross-checking, no discrepancies were found in the results of literature search, study selection, and data extraction. However, the two authors had different RoB scores on two included studies. After discussion with the correspondence author, consensus was achieved across the three authors.

### Literature Search

Potential studies published between January 1, 2020 and September 17, 2021 were searched in both Chinese and English databases: Chinese National Knowledge Infrastructure (CNKI), Wanfang data, VIP Information, PubMed, EmBase, and PsycInfo. The search terms were as follows: (sleep disturbance OR sleep quality OR insomnia OR sleep problem OR sleep disorder OR sleep symptom) AND (epidemiology OR cross-sectional study OR prevalence OR rate) AND (coronavirus disease 2019 OR 2019-n-CoV OR severe acute respiratory syndrome coronavirus 2 OR COVID-19 OR COVID) AND (China OR Chinese). To avoid missing studies that focused on the general population but simultaneously presented elderly-specific data in their main texts, we had no restrictions on participants in the search strategy. Further manual search was performed among the references of selected papers and related reviews.

### Study Selection

Studies were included if they satisfied the following criteria: (a) participants were general older Chinese adults aged 50 years or above; (b) reported the prevalence of insomnia symptoms, as measured by validated screeners of insomnia, including PSQI, ISI, and Athens Insomnia Scale (AIS); and (c) cross-sectional or cohort studies (only the baseline data were extracted) that were conducted in China during the COVID-19 pandemic. We also included general population-based studies that separately presented elderly-specific prevalence of insomnia symptoms. We excluded studies focusing on special populations such as COVID-19 patients, older adults living in long-term care facilities, and old patients with major medical conditions, as well as those using unstandardized assessments of insomnia. Currently, there is no agreement on the cut-off age to define older adults (24). Following several previous studies (25, 26), older adults were defined as those aged 50 years or older in the present study. Because PSQI has been widely used as a screener of insomnia and it has comparable accuracy for insomnia screening compared to

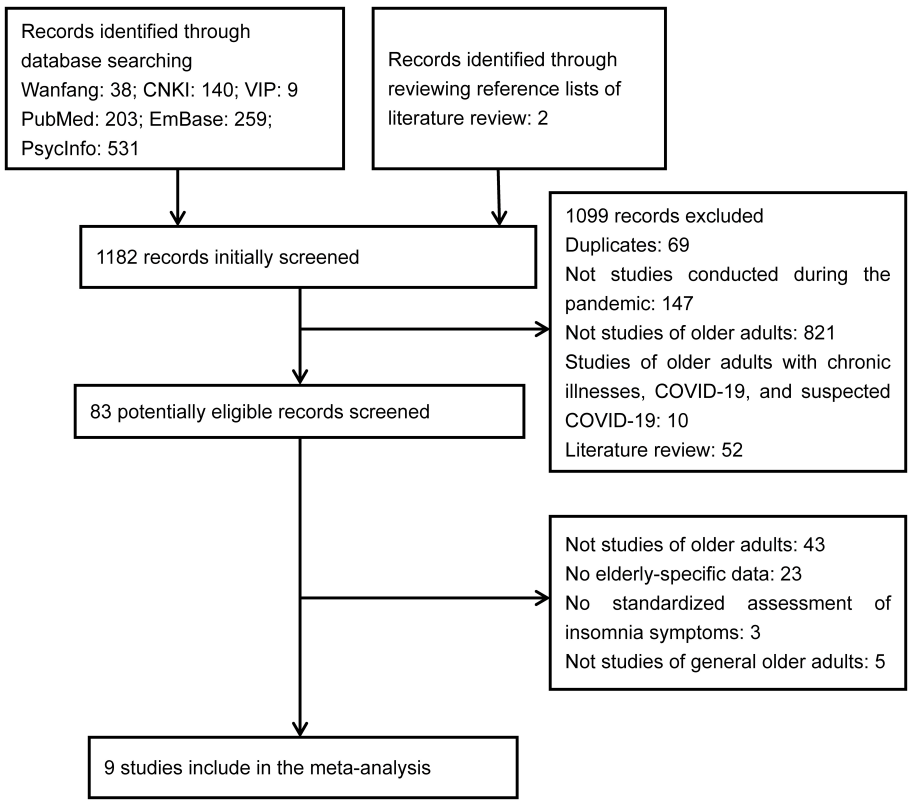


**FIGURE 1 |** Flowchart of study inclusion.

ISI and AIS (27, 28), PSQI is suitable for detecting the presence of insomnia symptoms.

## Data Extraction

We extracted the following information from included studies: first author, publication year, study site, survey date, survey method, sampling approach, sample size, insomnia screener, and rate of insomnia symptoms.

## RoB Assessment of Included Studies

The Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data ("JBI checklist" hereafter) was used to measure the level of RoB of included studies (29). The JBI checklist has nine methodology domains: sample frame, sampling, sample size, description of participants and setting, sample coverage of the data analysis, validity of the instrument for assessing the outcome, standardization and reliability of the instrument for assessing outcome, statistical analysis, and response rate (30). Each domain has four options (yes, no, unclear, or not applicable) and one point is assigned to a "yes" answer. The total RoB score ranges between zero and nine, with a higher score denoting a lower RoB.

## Statistical Analysis

We used the "metaprop" package of R, version 4.0.2, to conduct the meta-analysis. $I^2$ statistic was used to test heterogeneity between studies and, when there was evidence of heterogeneity ($I^2 > 50\%$), a random-effect model was used to generate the pooled estimate of prevalence. Subgroup analysis was used to explore the source of heterogeneity in the prevalence estimate of insomnia symptoms. Publication bias was assessed by funnel plots, Egger's regression model, and Begg's test. Because Generalized Linear Mixed Models (GLMMs) perform better than the conventional two-step approach when small-sample studies are included for synthesizing proportions, GLMMs were used to directly model event counts without data transformation within studies (31). Sensitivity analysis was conducted by removing each study individually to assess the robustness of pooled estimate of prevalence. $P < 0.05$ (two-sided) was deemed as statistically significant.

# RESULTS

Altogether, nine studies with 27,207 older Chinese adults were eligible and included (**Figure 1**) (19–23, 32–35). All studies assessed the presence of insomnia symptoms in convenient samples of older adults during the outbreak period of COVID-19 in China. With regard to the insomnia screener, five studies used ISI, three used PSQI, and one used AIS. Detailed characteristics of included studies are displayed in **Table 1**.

**TABLE 1 |** Characteristics of studies included in the meta-analysis.

| References | Site | Survey date | Sampling method | Sample size | Males, *n* (%) | Age of older adults, years | Survey method | Assessment of insomnia | No. of old adults with insomnia symptoms (prevalence, %) | Score of risk of bias |
|---|---|---|---|---|---|---|---|---|---|---|
| Gao et al. (34) | Ya'an, China | February 10–15, 2020 | Convenience sampling | 37 | Not reported | ≥55 | Online self-administered questionnaire | PSQI > 7 | 9 (24.3) | 3 |
| Liu et al. (20) | Mainland China | February 1–10, 2020 | Convenience sampling | 231 | 120 (51.9) | ≥51 | Online self-administered questionnaire | PSQI > 7 | 53 (22.9) | 5 |
| Wang et al. (32) | Mainland China | February 2–18, 2020 | Convenience sampling | 1,209 | Not reported | ≥50 | Online self-administered questionnaire | ISI ≥ 8 | 432 (35.7) | 4 |
| Wang et al. (33) | Mainland China | February 4–18, 2020 | Convenience sampling | 781 | Not reported | ≥50 | Online self-administered questionnaire | PSQI > 7 | 190 (24.3) | 5 |
| Wang et al. (23) | Mainland China | February 10–17, 2020 | Convenience sampling | 7,177 | Not reported | ≥50 | Online self-administered questionnaire | ISI ≥ 15 | 940 (13.1) | 5 |
| Wang et al. (22) | Mainland China | March 16–29, 2020 | Convenience sampling | 13,964 | Not reported | ≥50 | Online self-administered questionnaire | ISI ≥ 15 | 2,576 (18.4) | 5 |
| Zhang et al. (21) | Wuhan, China | February 1–5, 2020 | Convenience sampling | 35 | Not reported | ≥51 | Online self-administered questionnaire | ISI ≥ 8 | 15 (42.9) | 3 |
| Zheng et al. (19) | Mainland China | February 28–March 11, 2020 | Convenience sampling | 3,730 | 1,704 (45.7) | ≥50 | Online self-administered questionnaire | ISI ≥ 8 | 1,027 (27.5) | 6 |
| Zhou et al. (35) | Mainland China | February 23–March 1, 2020 | Convenience sampling | 43 | Not reported | ≥60 | Online self-administered questionnaire | AIS ≥ 6 | 12 (27.9) | 3 |

*ISI, Insomnia Severity Index; PSQI, Pittsburgh sleep Quality Index; AIS, Athens Insomnia Scale.*

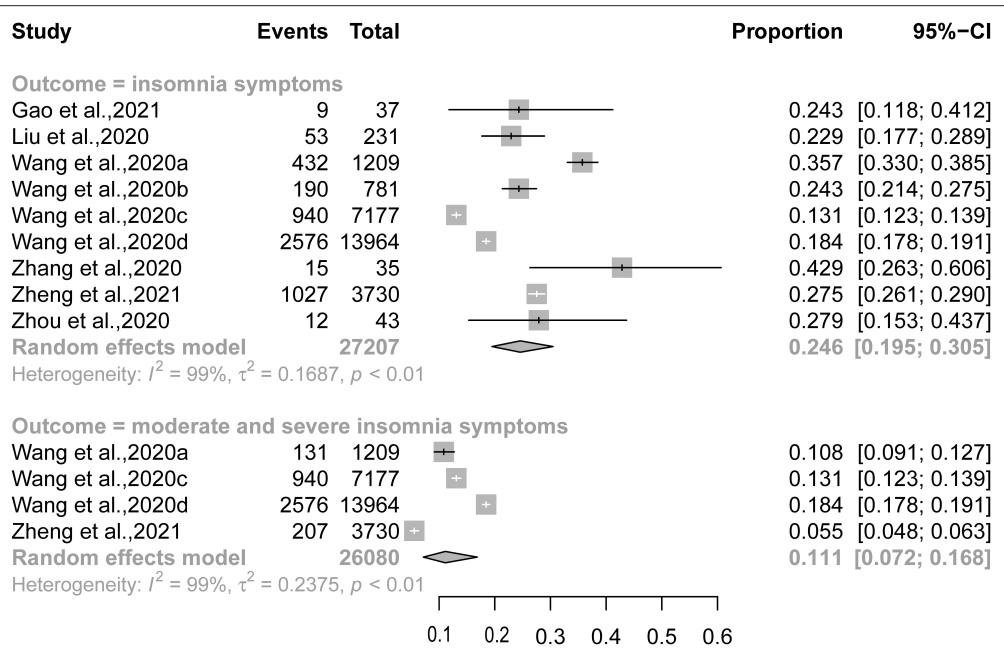


**FIGURE 2 |** Forest plot of the prevalence of insomnia symptoms and moderate and severe insomnia symptoms.

**TABLE 2 |** Subgroup analysis of the source of heterogeneity of included studies.

| Characteristics | | No. of studies | Sample size | No. of older adults with insomnia symptoms | Heterogeneity, $I^2$ (%) (*P*) | Pooled prevalence (95%CI), % | Q | *P* |
|---|---|---|---|---|---|---|---|---|
| Assessment | ISI | 5 | 26,115 | 4,990 | 99.3 (<0.001) | 25.1 (17.0, 35.4) | Rreference | |
| | PSQI | 3 | 1,049 | 252 | 0.00 | 24.0 (21.5, 26.7) | 0.050 | 0.824 |
| | ASI | 1 | 43 | 12 | Not applicable | 27.9 (16.6, 43.0) | 0.120 | 0.733 |
| Cut-off score of ISI | ≥8 | 3 | 4,974 | 1,474 | 93.8 (<0.001) | 32.6 (26.8, 39.0) | Rreference | |
| | ≥15 | 2 | 21,141 | 3,516 | 99.0 (<0.001) | 15.6 (12.3, 19.7) | 22.640 | <0.001 |
| Site | Hubei, China | 2 | 182 | 64 | 10.4 (<0.001) | 35.2 (28.6, 42.4) | Rreference | |
| | Others | 8 | 27,025 | 5,190 | 98.7 (<0.001) | 23.3 (18.5, 28.7) | 7.470 | 0.006 |
| Age, years | ≥50 | 7 | 27,127 | 5,233 | 98.9 (<0.001) | 24.5 (18.6, 31.4) | Rreference | |
| | ≥55 | 2 | 80 | 21 | 0.00 | 26.3 (17.8, 36.9) | 0.090 | 0.760 |
| Risk of bias score | 0–3 | 3 | 115 | 36 | 37.1 (<0.001) | 31.3 (23.4, 40.4) | Rreference | |
| | 4–6 | 6 | 27,092 | 5,218 | 99.1 (<0.001) | 22.9 (17.5, 29.2) | 2.650 | 0.104 |
| Sample size | <506 | 4 | 346 | 89 | 51.5 (<0.001) | 27.0 (20.1, 35.2) | Rreference | |
| | ≥506 | 5 | 26,861 | 5,165 | 99.3 (<0.001) | 22.9 (16.7, 30.5) | 0.620 | 0.431 |

*ISI, Insomnia Severity Index; PSQI, Pittsburgh sleep Quality Index; AIS, Athens Insomnia Scale.*

The JBI checklist scores of the studies ranged between three and six. No studies were rated as low RoB (JBI checklist score = 9) (**Table 1**). The three most common limitations were inappropriate way of sampling of participants (9/9), unclear response rates of study samples (9/9), and unclear information for assessing whether the outcome was measured in a standard, reliable way for all participants (9/9).

The pooled prevalence rates of insomnia symptoms and moderate and severe insomnia symptoms were 24.6% [95% confidence interval (CI): 19.5–30.5%] and 11.1% (95% CI: 7.2–16.9%), respectively (**Figure 2**). Results of subgroup analysis showed that significantly higher prevalence rates were observed in studies defining the presence of insomnia symptoms as "ISI ≥ 8" than in those defining it as "ISI ≥ 15" (32.6 vs. 15.6%, $P < 0.001$) and in studies carried out in the COVID-19 epicenter than in those in other places (35.2 vs. 23.3%, $P = 0.006$) (**Table 2**).

The Egger's test ($t = 0.97$, $P = 0.365$) and Begg's test ($z = 0.210$, $P = 0.835$) revealed no statistically significant publication bias (**Figure 3**).

When each study was excluded sequentially, the pooled estimate of prevalence (22.9–26.6%) and heterogeneity across studies ($I^2$: 97.7–98.8%) were not altered significantly, suggesting that no outlying study could influence the overall results of the meta-analysis (**Figure 4**).

## DISCUSSION

This meta-analysis quantitatively summarized studies estimating the prevalence of insomnia symptoms in older adults in China amid the COVID-19 pandemic. We found an overall prevalence rate of 24.6% of insomnia symptoms in older Chinese adults and significantly higher rates in studies defining insomnia symptoms as "ISI ≥ 8" (vs. "ISI ≥ 15") and older adults living within the COVID-19 epicenter (vs. other places). In addition, 11.1% of the older Chinese adults had moderate and severe insomnia symptoms.

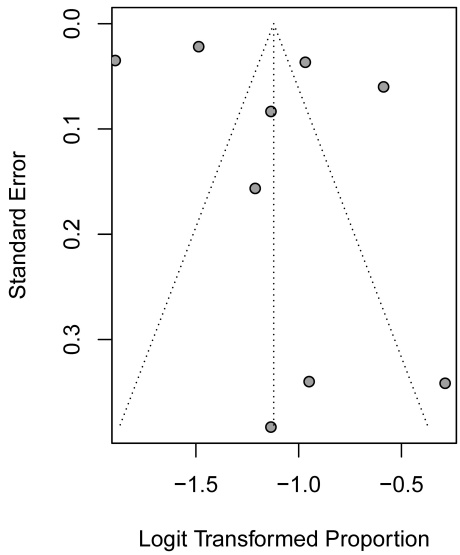


**FIGURE 3 |** Funnel plot of publication bias among the nine included studies.

Compared to the 24.4–26.8% prevalence of insomnia symptoms among older Chinese adults during the non-pandemic era (1–3), a comparable prevalence of insomnia symptoms (24.6%) was found in older Chinese adults during the pandemic. This estimate seems to not support the additional risk of insomnia exerted by the pandemic. Nevertheless, we argue that the result from this direct comparison should be considered cautiously owing to the significant heterogeneity in the methodologies of included studies. For example, most studies included in this meta-analysis defined older adults as those aged 50 years or over but the aforementioned 24.4–26.8% prevalence estimate was derived from studies of older adults aged 60 years or above (1–3). Because there is evidence showing an increasing prevalence of insomnia symptoms with increasing age in older Chinese adults (3, 36), our study should have a higher prevalence estimate of insomnia symptoms in older adults during the pandemic if older adults were those aged 60+ years. Moreover, as displayed in **Table 2**, when studies were limited to those defining the presence of insomnia symptoms as "ISI ≥ 8," the synthesized prevalence rose to 32.6%, which is higher than the aforementioned 24.4–26.8% prevalence in older adults during the non-pandemic era (1–3). In addition, in our study the 11.1% prevalence of moderate and severe insomnia symptoms indicates that nearly a half (11.1/24.6%) of the older adults with insomnia symptoms are severe enough for clinical attention. These data may suggest an elevated risk of insomnia symptoms in older Chinese adults during the COVID-19 pandemic.

In the literature, living in Hubei or its capital city, Wuhan, was significantly associated with insomnia symptoms in both the general population and older adults in China (19, 37). In accordance with these studies, our meta-analysis confirmed the significantly higher risk of insomnia symptoms in older adults living within the COVID-19 epicenter than in those living in other places. This may be ascribed to the longer duration of mass quarantine, more strict social distancing measures, and higher

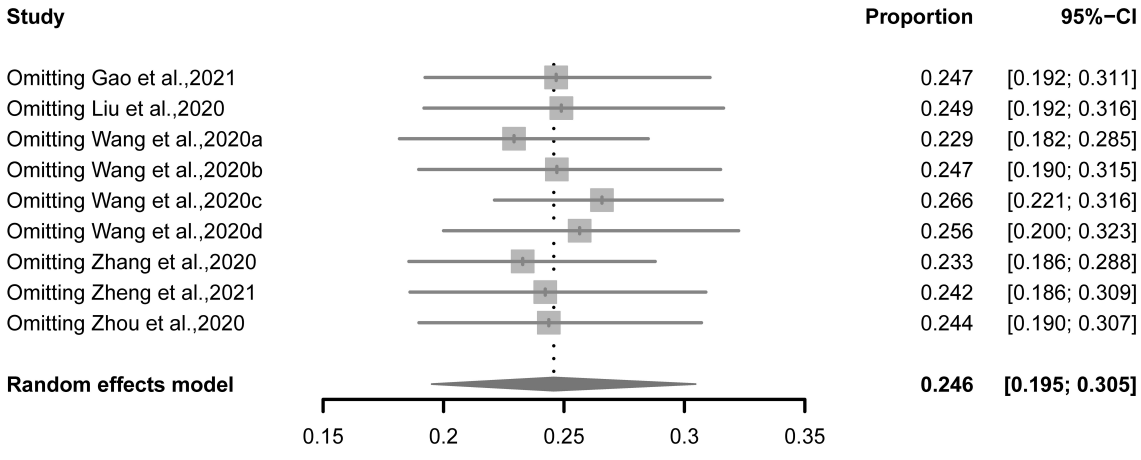


**FIGURE 4 |** Sensitivity analysis of the pooled estimate of prevalence in this meta-analysis.

risk of exposure to COVID-19 in persons living within than those living outside the epicenter (12).

The strength of this meta-analysis is the large sample size of older Chinese adults, which provided an overall and reliable estimate of the prevalence of insomnia symptoms in this vulnerable population during the COVID-19 pandemic. Nevertheless, this study also has a few limitations. First, none of the included studies were of low RoB. Because subgroup analysis according to RoB score revealed a trend toward lower prevalence of insomnia symptoms in studies with higher RoB scores (**Table 2**), we may have overestimated the true prevalence of insomnia symptoms in older Chinese adults during the pandemic. However, as we argued above, several included studies used strict criteria to define the presence of insomnia symptoms (i.e., "ISI $\geq$ 15"), so we may have underestimated the true prevalence of insomnia symptoms. Given the two limitations, it is difficult to evaluate the extent and direction of bias in the prevalence estimate. Second, since sex-specific and region-specific (i.e., urban vs. rural) prevalence data were available in only one of the included studies (19), this meta-analysis was not able to provide data on the demographic characteristics of insomnia symptoms in older adults. Third, all of the included studies were conducted during the COVID-19 outbreak period, so it remains unclear whether the sleep quality of older adults improves or worsens during the post-outbreak period. Finally, data on healthcare services utilization of older adults with moderate and severe insomnia symptoms are important for mental health policy-making during the pandemic, but the included studies had no such data.

In summary, nearly one out of every four older Chinese adults suffered from insomnia symptoms, suggesting a high level of mental healthcare need in this population in the context of COVID-19 pandemic. Insomnia has been associated with a range of physical and mental morbidities and elevated mortality in older adults (4). Given the high prevalence of insomnia symptoms, mental health services for this population during the pandemic should include supportive activities aimed at improving mental well-being, periodic assessment of insomnia symptoms to ensure early recognition of older adults who meet the diagnostic criteria for clinical insomnia, and psychiatric assessment and treatment when necessary. The higher prevalence rate of insomnia symptoms in older adults living in the epicenter indicates that more mental health resources should be assigned to older adults in the COVID-19 epicenter. In addition, prospective studies are warranted to understand the longitudinal changes of insomnia symptoms in older Chinese adults during the post-outbreak era.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

Q-QZ: acquisition and analysis of data for the study, drafting the paper, and interpretation of data for the study. LL: design and acquisition of data for the study. B-LZ: drafting the paper, revising the paper for important intellectual content, and interpretation of data for the study. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.779914/full#supplementary-material

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# Unveiling the Burden of Interactions Among Clinical Risk Factors for 1-Year Mortality in Hospitalized Older Patients

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**Background:** Hospitalized older patients are particularly exposed to adverse health outcomes.

**Objective:** In this study, we aimed at investigating the prognostic interactions between disability in basic activities of daily living (BADL), cognitive impairment, low handgrip strength, anticholinergic cognitive burden (ACB), and depression on 1-year mortality.

**Setting and Subjects:** Our series consisted of 503 older patients discharged from acute care hospitals.

**Methods:** Disability in at least one BADL, ACB, depression, cognitive impairment, and low handgrip strength was considered in the analysis. One-year mortality was investigated by Cox regression analysis and prognostic interactions among study variables were assessed by survival tree analysis.

**Results:** Basic activities of daily living disability, ACB, cognitive impairment, and low handgrip strength were significantly associated with 1-year mortality. Survival tree analysis showed that patients with BADL disability and high ACB carried the highest risk of poor survival [hazard ratio (HR): 16.48 (2.63–74.72)], followed by patients with BADL disability and low ACB (HR: 8.43, 95% CI: 1.85–38.87). Patients with cognitive impairment and no BADL disability were characterized by a lower but still significant risk of mortality (HR: 6.61, 95% CI: 1.51–28.97) and those with high ACB scores and good cognitive and functional performance (HR: 5.28, 95% CI: 1.13–24.55).

**Conclusion:** Basic activities of daily living dependency, cognitive impairment, and ACB score were the three main predictors of 1-year mortality among patients discharged from acute care hospitals; the interaction between BADL dependency and ACB score was

found to significantly affect survival. Early identification of such high-risk patients may help tailor targeted interventions to counteract their detrimental effects on prognosis.

**Keywords: hospitalized older patients, anticholinergic burden, functional impairment, cognitive impairment, handgrip, depression**

## INTRODUCTION

Hospitalized older individuals represent a complex and extremely heterogeneous portion of the geriatric population, exposed to a constant burden associated with multimorbidity, polypharmacy, and acute diseases, which may affect the overall quality of life and prognosis (1, 2).

Several prospective studies have identified multiple predictors of increased risk of death in this population (3, 4). Dependency in performing basic activities of daily living (BADL) is recognized as a contributor to mortality and recurrent hospitalization among older patients with chronic diseases (5, 6). Cognitive impairment is a well-known predictor of poor outcomes in geriatric populations, in terms of mortality (7). Depressive symptoms also are highly prevalent among older hospitalized patients (8) and may exert relevant negative functional and prognostic implications (9). Low handgrip strength is a dynamic indicator of poor muscle function (10) and the main risk factor for diagnosing sarcopenia (11); it was shown to predict mortality, disability, and physical functioning (12). Finally, medication burden was also associated with increased mortality (13), and exposure to anticholinergic medications is an important predictor of poor outcomes among hospitalized older patients (14–17).

Interestingly, the above risk factors are known to affect each other. For example, BADL dependency may increase the risk of depression (18). Additionally, low handgrip strength may favor the development of further physical and cognitive disability (19, 20). Anticholinergic cognitive burden (ACB) was also found to predict both functional and cognitive impairment among older patients discharged from acute care hospitals (14). Moreover, ACB impact on mortality was greater among patients with low handgrip strength, depressive symptoms, and functional dependency. Finally, depression was found to predict functional impairment among both community-dwelling and hospitalized older patients (21, 22).

Despite this bulk of evidence, BADL dependency, ACB, depression, low handgrip or cognitive impairment have only been considered as individual potentially interacting variables in the former studies (14, 15, 17, 23, 24). For this reason, we aimed at providing a comprehensive investigation of the prognostic interactions involving all these risk factors simultaneously present in a population of older patients discharged from acute care hospitals, to identify the most clinically relevant combinations and improve prognostic stratification.

## MATERIALS AND METHODS

This study used data from a multicenter prospective observational study carried out in seven geriatric and internal medicine wards of Italian acute care hospitals. All patients consecutively admitted to participating wards between June 2010 and May 2011 were asked to participate in the study. Exclusion criteria included age <65 years and unwillingness to participate in the study. After obtaining written informed consent, all participants were assessed within the first 24 h from hospital admission and followed up until discharge. Study researchers collected information about demographic and clinical characteristics, cognitive and functional status, and medication intake before, during hospitalization, and at discharge. Medications were coded according to the Anatomical Therapeutic and Chemical classification (25). After discharge, patients were reassessed at 3, 6, and 12 months. All ethics committees at participating institutions approved the study.

Overall, 1,120 patients were enrolled in the study. Patients with incomplete baseline data ($N = 3$) and those who died during hospitalization ($N = 39$) were excluded from our analysis. Patients with missing data for cognitive impairment ($N = 218$), depression ($N = 129$), and handgrip ($N = 100$), and those with incomplete follow-up data ($N = 131$) were also excluded, leaving a final sample of 503 patients to be included in the analysis.

Patients excluded from the study were older (83.1 ± 7.3 vs. 79.4 ± 7.0; $p < 0.001$) and had higher prevalence of BADL disability (52.7 vs. 21.1%, $p < 0.001$), cognitive impairment (69.4 vs. 41.3%, $p < 0.001$), depression (46.1 vs. 35.0%, $p = 0.005$), and higher average ACB score (1.4 ± 1.4 vs. 1.2 ± 1.1, $p < 0.001$) compared to those included in the study.

### Exposure Variables

Comprehensive geriatric assessment and medication data were collected at the time of discharge. Cognitive impairment was defined as having age- and education-adjusted Mini-Mental State Examination < 24 (26); the number of BADL (27) was calculated at discharge, and an analytic variable was created to identify patients with dependency in at least 1 BADL. Depression was defined as having a 15-item Geriatric Depression Scale (28) score > 5. Exposure to anticholinergic medications was quantified by calculating the anticholinergic cognitive burden (ACB) score (29), which was chosen because it was externally validated (30) and considered more accurate in the evaluation of central anticholinergic burden compared with other tools (31). The exposure variable based on the calculation of ACB score at discharge was categorized as follows: low-medium anticholinergic burden (ACB = 0 or 1 and ACB = 0 for patients taking no anticholinergic medications) and high anticholinergic burden (ACB score 2 or more). Muscle strength was assessed by handgrip strength, measured by calibrated hand dynamometer (North Coast Hydraulic Hand Dynamometer, North Coast Medical Inc, Morgan Hill, CA, USA), as previously described (32). According to the revised European Working Group on Sarcopenia in Older People 2 criteria, low muscle strength was

classified as handgrip <27 kg in men and <16 kg in women (11). Finally, the cumulative number of exposure variables (low handgrip, BADL disability, depression, cognitive impairment, and high ACB) was also calculated and included in the analysis.

## Outcome

The outcome of this study was 1-year mortality. Data on living status during follow-up were obtained by interviewing the patients and/or their formal and/or informal caregivers. The time from the day of the study enrollment through the day of death was used as the time to failure variable for the model. Survivors were censored at the end of the follow-up. About patients who died during the follow-up period, date and place of death were retrieved by relatives or caregivers. The municipal registers were consulted when neither patients or relatives nor caregivers could be contacted.

## Covariates

Age, sex, number of diseases at discharge, and number of medications prescribed at discharge were considered as potential confounders in the analysis. Selected diagnoses known to affect prognosis in older patients (hypertension, heart failure, coronary artery disease, atrial fibrillation, peripheral artery disease, diabetes mellitus, chronic obstructive pulmonary disease, chronic kidney disease, stroke, dementia, and cancer) were also included in the preliminary analysis. Finally, to account for the continuity of exposure to anticholinergic drugs during the first follow-up period after discharge, the ACB score at 3-month follow-up was also considered as a potential confounder in the analysis.

## Statistical Analysis

First, we compared survivors and patients who died during follow-up concerning study variables and covariates. Continuous variables were reported as mean ± SD. Categorical variables were expressed as several cases (percentage). To compare the characteristics of the patients, according to 1-year survival status, we used the Student's *t*-test for continuous variables and the chi-squared test for categorical ones.

The relative risk of 1-year mortality related to either single study risk factors (low handgrip, cognitive impairment, functional impairment, depression, and ACB score) or the cumulative number of risk factors was then investigated by Cox regression analysis. We fitted three different Cox regression models: model A, adjusted for age and sex; model B, adjusted for age, sex, number of medications, and number of diagnoses; and model C, adjusted for age, sex, number of medications, and individual diagnoses associated with mortality in the preliminary analysis (heart failure, hypertension, coronary artery disease, cancer, and atrial fibrillation) instead of several medications. Models B and C were also repeated after adjusting for ACB score at 3-month follow-up.

Risk factors significantly associated with mortality in adjusted models were further analyzed by Venn diagram to investigate their overlapping. Therefore, to improve the graphical representation of the predictive models, we fitted a survival tree model based on study risk factors significantly associated with mortality in Cox regression models. Survival trees represent peculiar non-parametric alternatives to (semi)parametric models; they are characterized by extreme flexibility that allows automatic detection of certain types of interactions without specifying them beforehand. In this study, the splitting criterion was based on a node deviance measure between a saturated model log-likelihood and a maximized log-likelihood as proposed by Leblanc and Crowley (33), with further node adjustment to simplify the graphical representation of the fitted survival tree. To assess the performance of the fitted survival tree, the leaf node membership was added as a categorical variable in a Cox regression model using the node with the best survival as the reference category. Bootstrap model validation (1,000 resamplings) was performed to limit the bias of the estimates. The accuracy performance of the model was assessed by calculating the C-index value and 95%CI for leaf node membership and compared with that of individual study risk factors or the cumulative number of risk factors.

We also planned to perform sensitivity analyses. To account for potential residual confounding, survival tree analysis was repeated including patients with available data for risk factors significantly associated with the outcome. C-index of leaf node membership was separately calculated for each survival tree. Finally, survival tree analysis was repeated by forcing into survival tree analysis risk factor(s) not significantly associated with mortality.

Analyses were performed using SPSS (version 26.0, SPSS, Chicago, IL, USA) and rpart (34) and partykit (35) packages of R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

# RESULTS

Baseline characteristics according to 1-year mortality are depicted in **Table 1**. Among the 503 study participants (mean age: 79.4 ± 7.0, 50.7% women), 69 patients (13.7%) died during the follow-up. Patients who died were older, malnourished, and had a higher number of medications and diagnoses, a greater prevalence of heart failure, coronary artery disease, cancer, and atrial fibrillation, and a lower prevalence of hypertension. Patients who died during the follow-up were also characterized by a higher prevalence of low handgrip, cognitive impairment, BADL disability, depression, and high anticholinergic burden. ACB medications prescribed at discharge according to 1-year mortality are reported in **Supplementary Table 1**.

Cox regression analysis showed that all study risk factors but depression were associated with increased 1-year mortality in age- and sex-adjusted models, and similar findings were obtained in models B and C after adjusting for potential confounders (**Table 2**). Such results were confirmed after including ACB score at 3-month follow-up in fully adjusted models B and C; the corresponding figures (hazard ratio and 95% CI) for model C including ACB score at 3 months were 4.88 (2.60–9.16) for ACB score, 2.42 (1.48–3.98) for BADL disability, 1.87 (1.12–3.12) for cognitive impairment, 1.78 (1.03–3.01) for low handgrip strength, and 1.31 (0.76–2.26) for depression.

**TABLE 1 |** Demographic and clinical characteristics of discharged patients grouped by 1-year mortality.

| | All patients ($n = 503$) | Survivors ($n = 434$) | Died ($n = 69$) | *P* |
|---|---|---|---|---|
| Age, mean (± SD) | 79.4 ± 7.0 | 78.9 ± 7.0 | 82.8 ± 6.3 | <0.001 |
| Gender, F, *n* (%) | 255 (50.7%) | 225 (51.8%) | 30 (43.5%) | 0.24 |
| Heart failure, *n* (%) | 142 (28.2%) | 109 (25.1%) | 33 (47.8%) | <0.001 |
| Hypertension, *n* (%) | 399 (79.3%) | 356 (82.0%) | 43 (62.3%) | <0.001 |
| CAD, *n* (%) | 158 (31.4%) | 127 (29.2%) | 31 (44.9%) | 0.013 |
| PAD, *n* (%) | 43 (8.5%) | 37 (8.5%) | 6 (8.7%) | 0.989 |
| COPD, *n* (%) | 206 (40.9%) | 171 (39.4%) | 35 (50.7%) | 0.10 |
| CKD, *n* (%) | 243 (50.2%) | 204 (48.6%) | 39 (60.9%) | 0.09 |
| Cancer, *n* (%) | 70 (13.9%) | 43 (9.9%) | 27 (39.1%) | <0.001 |
| Diabetes, *n* (%) | 154 (30.6%) | 132 (30.4%) | 22 (31.9%) | 0.92 |
| Dementia, *n* (%) | 53 (10.5%) | 47 (10.8%) | 6 (8.6%) | 0.745 |
| Cerebrovascular, *n* (%) | 83 (16.5%) | 70 (16.1%) | 13 (18.8%) | 0.697 |
| Atrial fibrillation, *n* (%) | 93 (18.5%) | 72 (16.6%) | 21 (30.4%) | 0.010 |
| Number of medications, mean (± SD) | 7.62 ± 2.79 | 7.47 ± 2.71 | 8.51 ± 3.15 | 0.011 |
| Number of comorbidities, mean (± SD) | 5.3 ± 2.7 | 5.1 ± 2.6 | 6.2 ± 2.8 | 0.003 |
| **Study risk factors** | | | | |
| ACB score | | | | <0.001 |
| 0-1 | 355 (70.6%) | 319 (73.5%) | 36 (52.2%) | |
| 2 or more | 148 (29.4%) | 115 (26.5%) | 33 (47.8%) | |
| BADL disability | 106 (21.1%) | 76 (17.5%) | 30 (43.5%) | <0.001 |
| Cognitive impairment | 208 (41.3%) | 167 (38.5%) | 41 (59.4%) | 0.002 |
| Low handgrip strength | 233 (46.3%) | 191 (44.0%) | 42 (60.9%) | 0.01 |
| Depression | 176 (35.0%) | 147 (33.9%) | 29 (42.0%) | 0.24 |

*p-value from t-test or $\chi^2$ test as appropriate. Data are expressed as mean ± SD or n and percentage as appropriate.*
*BADL, basic activities of daily living; CAD, coronary artery disease; PAD, peripheral artery disease; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease.*

**TABLE 2 |** The Cox regression analysis of study risk factors in relation to 1-year mortality.

| | Model A HR (95%CI) | Model B HR (95%CI) | Model C HR (95%CI) |
|---|---|---|---|
| ACB score 0–1 | – | – | – |
| ACB score 2 or more | 2.17 (1.33–3.43) | 1.66 (1.01–2.80) | 1.61 (1.00–2.71) |
| Low handgrip | 1.85 (1.03–3.33) | 1.77 (1.02–3.18) | 1.72 (1.01–3.20) |
| Cognitive impairment | 1.94 (1.19–3.17) | 1.90 (1.16–3.11) | 2.01 (1.21–3.33) |
| BADL disability | 2.95 (1.79–4.86) | 2.75 (1.65–4.56) | 2.50 (1.53–4.08) |
| Depression | 1.33 (0.80–2.19) | 1.26 (0.76–2.08) | 1.31 (0.76–2.36) |

*Model A: adjusted for age and gender; Model B: adjusted for age, sex, number of medications and number of diagnoses; and Model C: as for model B with heart failure, hypertension, coronary artery disease, cancer, and atrial fibrillation instead of number of diagnoses.*
*ACB, anticholinergic cognitive burden; BADL, basic activities of daily living; HR, hazard ratio.*

Overlapping among study risk factors significantly associated with mortality is shown in **Figure 1**. Low handgrip strength was the most common risk factor in the study population (55.6%), followed by cognitive impairment (41.3%), high ACB (29.4%), and BADL disability (21.1%). In contrast to high ACB, low handgrip, and cognitive impairment, the presence of BADL disability was rarely observed alone and was often observed in combination with other risk factors. The most common combinations of study risk factors involved cognitive impairment and low handgrip strength (13.7%), BADL disability, cognitive impairment, and low handgrip strength (7.7%), low handgrip and high ACB (5.4%), and cognitive impairment, low handgrip, and high ACB (4.6%).

Results obtained by survival tree analysis are reported in **Figure 2**. Node 5 (patients with no BADL disability, no cognitive impairment, ACB score $< 2$, and normal handgrip, $n = 113$) showed the lowest mortality and was considered as the reference

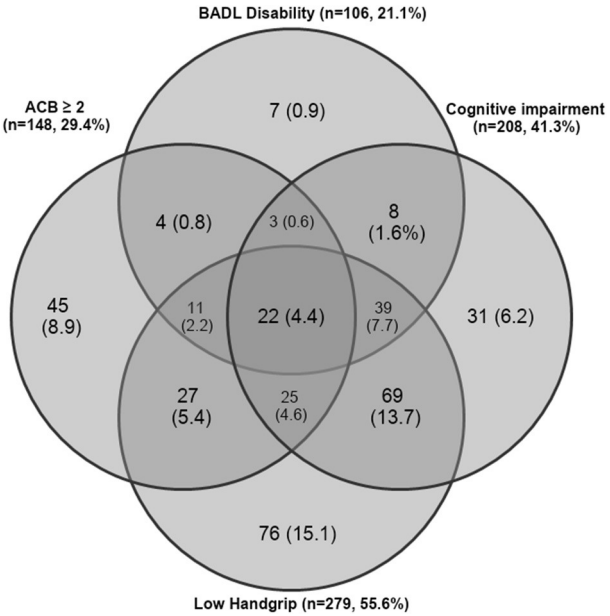


**FIGURE 1 |** Venn diagram showing overlapping of study risk factors significantly associated with mortality in the study population.

category in the Cox regression models. The highest risk of mortality was observed among patients belonging to Node 11 (BADL disability and ACB score of 2 or more) and Node 10 (BADL disability and ACB score $< 2$). Node 5 (no BADL disability, no cognitive impairment, ACB score $< 2$, and normal handgrip), and Node 6 (no BADL disability, no cognitive impairment, ACB score $< 2$, and impaired handgrip) shared the lowest mortality risk, while Nodes 7 and 8 showed intermediate mortality risk profiles. Descriptive features of leaf node groups are reported in **Supplementary Table 2**.

The analysis including the number of risk factors or leaf node membership instead of single study risk factors showed that the coexistence of 2 or more study risk factors was significantly associated with mortality (**Figure 3**). Leaf node membership showed a progressive increase in mortality risk of death starting from Node 6.

The leaf node membership showed a good predictive accuracy (C-index: 0.812; 95% CI: 0.761–0.852), which was significantly better compared to that observed with several risk factors and individual risk factors ($p < 0.05$, see **Table 3**). Similar results were obtained by sensitivity analyses to fit a family of survival trees among patients with available BADL ($n = 807$), cognitive impairment ($n = 670$), handgrip ($n = 621$), and ACB score ($n = 807$) data (**Supplementary Figure 1**). The C-index of single survival trees was substantially comparable to that of the main survival tree (mean C-index 0.81, $p = 0.85$). Forcing the inclusion of depression in the survival tree analysis allowed us to identify a subgroup of patients with BADL disability and depression interacting in predicting 1-year mortality (**Supplementary Figure 2**).

## DISCUSSION

In this prospective cohort study, we used a tree-based risk algorithm to identify the most relevant prognostic interactions among selected risk factors in older patients discharged from acute care hospitals. The survival tree analysis allowed the identification of selected combinations of risk factors leading to different prognostic outcomes concerning 1-year mortality. Patients with BADL disability and ACB score $\geq 2$ (Node 11) were characterized by the highest mortality, followed by individuals with BADL disability and ACB score $< 2$ (node 10). Node 8 (cognitive impairment with no BADL disability) and Node 7 (ACB score of 2 or more with no cognitive impairment and no BADL disability) were characterized by a lower but still significantly increased mortality risk. Finally, cognitive impairment was highly prevalent among high-risk nodes but did not show any other significant interaction with either functional impairment or anticholinergic burden.

Functional impairment was an independent predictor of mortality in this population, thus confirming results from previous studies (36, 37); functional disability may be the expression of both presence and severity of multiple diseases on the health of individual and then characterize individuals at higher risk of poor long-term survival (38). Furthermore, dependency in at least one BADL showed a significant interaction with high ACB.

Patients with BADL disability and ACB of two or more were characterized by a 16-fold increased risk of mortality compared to the reference category, which confirms previous evidence regarding the need of moderating the use of anticholinergic medications in patients with functional impairment (17), even in the setting of this study considering a wider set of potential risk factors compared to the former one (17). The anticholinergic burden may, in fact, contribute to the effects of functional impairment on survival in older patients, by increasing the vulnerability of functionally impaired individuals to adverse drug reactions (39). Moreover, the anticholinergic burden has been shown to impair gait and balance and contribute to the risk of recurrent falls (40), representing a major cause of functional disability and death (14, 16). On the other side, anticholinergic medications may impair physical performance and worsen BADL dependency in patients with pre-existing disabilities, which might exponentially increase the risk of death (41).

Interestingly, depression seems to play a minor prognostic role in this study. Its prognostic weight was detected only in a small proportion of individuals after performing sensitivity analyses. Nevertheless, when forcing depression in the survival tree we could intercept a subgroup of patients with BADL disability and low ACB in which depression played an adjunctive negative prognostic role. At variance, the lack of interaction between BADL disability and either cognitive impairment or handgrip strength suggests that all these risk factors may impact prognosis through independent pathways.

Among patients with BADL independence, cognitive impairment was significantly associated with the outcome (Node 8), thus confirming previous evidence regarding this

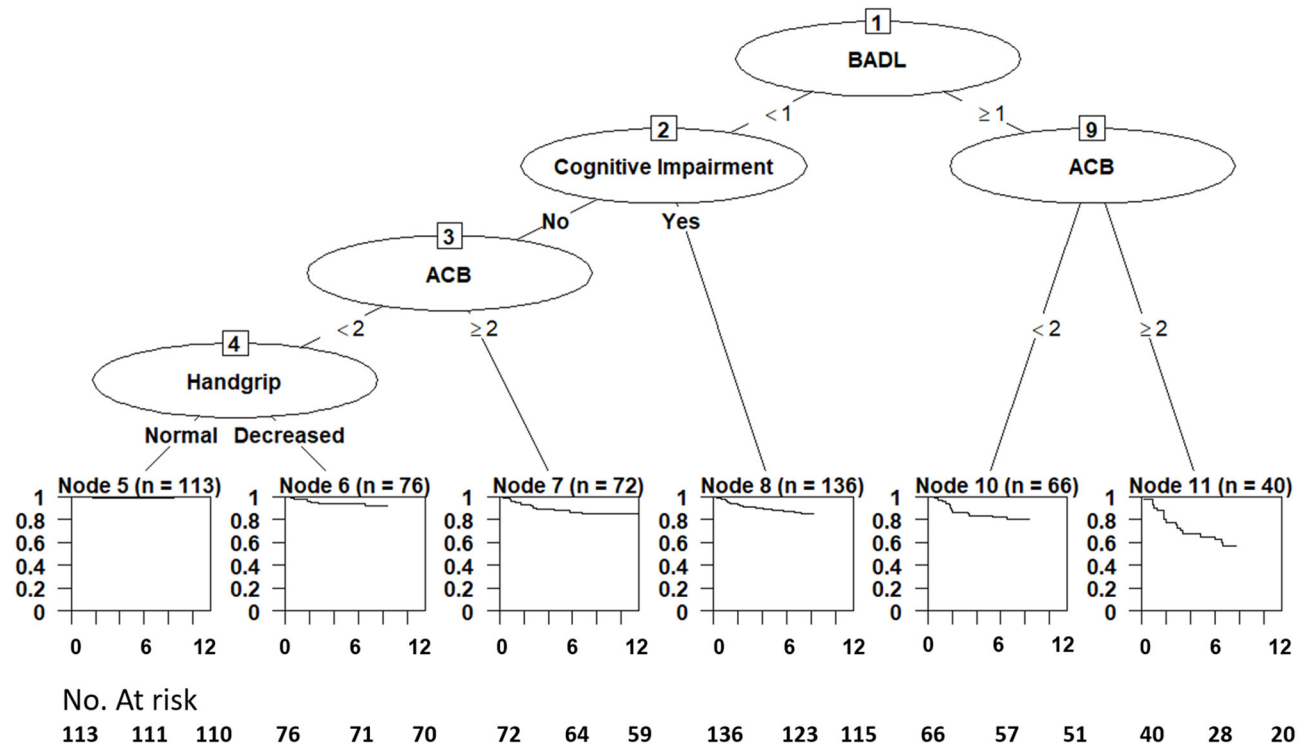


**FIGURE 2 |** Survival tree of study risk factors showing 1-year mortality among patients carrying the leaf node combinations of study risk factors. ACB, anticholinergic cognitive burden; BADL, basic activities of daily living.

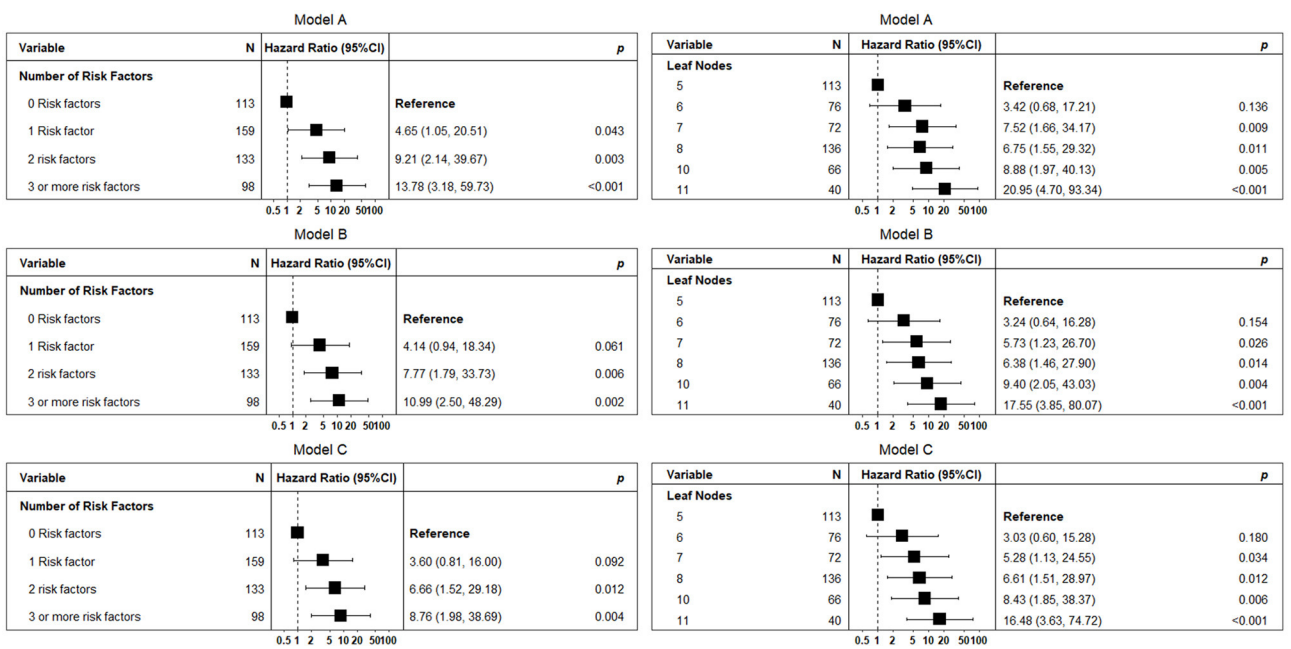


**FIGURE 3 |** A forest plot showing the hazard ratio and 95% CIs associated with several selected risk factors (panel A) and leaf nodes (panel B), with time to 1-year mortality as the dependent variable. Squares represent the hazard ratio and the horizontal bars extend from the lower limit to the upper limit of the 95% CI of the estimate of the hazard ratio. Model A: adjusted for age and sex; Model B: adjusted for age, sex, and number of medications and diagnoses; and Model C: as for model B with heart failure, hypertension, coronary artery disease, cancer, and atrial fibrillation instead of several diagnoses.

**TABLE 3 |** C-index in predicting 1-year mortality obtained by leaf nodes membership from survival tree analysis compared to individual risk factors or number of risk factors.

| | Age and sex-adjusted C-index | *P* value |
|---|---|---|
| Depression | 0.661 (0.611–0.720) | <0.001 |
| Low handgrip | 0.685 (0.626–0.743) | <0.001 |
| Cognitive impairment | 0.701 (0.644–0.754) | <0.001 |
| ACB score ≥ 2 | 0.699 (0.642–0.755) | <0.001 |
| BADL disability | 0.718 (0.661–0.774) | 0.007 |
| Number of risk factors | 0.736 (0.685–0.787) | 0.018 |
| Leaf node membership | 0.812 (0.761–0.852) | – |

*ACB, anticholinergic cognitive burden; BADL, basic activities of daily living.*

association, even in patients without diagnosis of dementia (42, 43); possible explanations for this association include poor adherence to medications, increased drug burden due to the treatment of cognitive disturbances, and increased incidence of frailty (43). Anticholinergic medications carried a less striking, but still important prognostic weight in patients with BADL independence; drugs with anticholinergic properties may increase the risk of cognitive impairment and dementia, falls and mortality (15, 17, 24). Furthermore, these drugs may have cardiovascular and neurological effects (44, 45), such as arrhythmias, syncope, hallucinations, and seizures, that might provoke serious adverse events, ultimately leading to death (46).

In the end, low handgrip strength, despite its high prevalence, did not independently contribute to the risk of mortality in the survival tree analysis, thus suggesting that its detrimental consequences are probably mediated by other factors.

The findings of this study may have several clinical and prognostic implications. The major prognostic interaction involved functional disability and ACB score, consequently, the assessment of functional status and revision of drug treatment during hospital stay should be important cornerstones of clinical evaluation of older hospitalized patients, to identify high-risk patients to whom reserve individualized pharmacological treatments. In this regard, the ACB score may represent a useful index of both pharmacological and mortality risk and may help predict future functional decline (14). A high ACB score was a significant predictor of mortality among patients with preserved functional and cognitive abilities; for this reason, careful and tailored prescribing and regular monitoring of anticholinergic medications should not be scotomized when dealing with these patients.

## Limitations

This study has some limitations. First, given the observational design, confounding by indication is a relevant limitation in the study. Even if results were confirmed in sensitivity analyses, a greater prevalence of depression, cognitive, and functional impairment, and a greater ACB score were found among patients excluded because of missing data. Additionally, missing may have contributed to the observation of a less relevant role of depression and handgrip in survival tree analyses. Second, the results of the survival tree analysis may lack the precision of estimates due to the small sample size, and wide CIs are in keeping with this hypothesis. Third, our results identify variables that by themselves may influence the outcome, and we could not account for illness severity, duration, and management of individual diagnoses. Fourth, the short duration of follow-up does not allow to optimally explore the long-term association between study risk factors and mortality. Additionally, our database included only limited information about the duration of exposure to ACB drugs and we could only calculate the ACB score at a 3-month follow-up. While adjusting the analysis for the ACB score at 3 months did not affect our main findings, we could not fully explore the effect of long-lasting exposure to anticholinergic medications compared with a shorter duration of treatment. Finally, the lack of information about post-acute care utilization did not allow us to investigate the impact of post-discharge care on study results. Finally, our results apply to the population of older patients discharged from acute care hospitals and cannot be generalized to the general older population.

On the other hand, the main strengths of our study are represented by the inclusion of a real-world unselected population of hospitalized older patients, the thorough evaluation of medications, and the use of a comprehensive geriatric assessment to explore the independent effect of several risk factors. In addition, this is the first study that identified several important interactions between previously investigated risk factors taken altogether by using the survival tree approach. Furthermore, the high stability and reproducibility of survival trees in sensitivity analyses and the high level of predictive accuracy estimates add further significance to study findings. If confirmed on larger studies, such interactions may have significant clinical and prognostic implications, which might suggest the need of using appropriate interventions to counteract the negative effects of selected risk factors on outcomes of patients. In this regard, hospital physicians should consider the assessment of ACB and functional and cognitive status as very important components of clinical practice.

## Conclusion and Implications

Functional impairment was the main predictor of 1-year mortality among hospitalized older patients and was found to interact with ACB score. Among patients with preserved functional capacity, cognitive impairment and anticholinergic

burden were the main predictors of 1-year mortality. These findings support the importance of a careful evaluation of functional and cognitive status, and anticholinergic burden among older patients discharged from acute care hospitals to de-prescribe anticholinergic medications whenever possible, especially in patients carrying a selected combination of risk factors.

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Data are available for CRIME study researchers at IRCCS INRCA (www.inrca.it).

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Catholic University of Rome (Project identification code: P/582/CE/2009). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

ACor, VC, LS, FG, ACoz, and AF participated in data analysis, manuscript writing, and revising and manuscript approval. GO, SV, CR, AChe, and ACor participated in data collection and writing, revising, and approving manuscript. FL participated in writing the manuscript, revising it for important intellectual content, and approval. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.771115/full#supplementary-material

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# Role of Age-Related Changes in DNA Methylation in the Disproportionate Susceptibility and Worse Outcomes of Sepsis in Older Adults

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Sepsis, a complex multisystem disorder, is among the top causes of hospitalization and mortality in older adults. However, the mechanisms underlying the disproportionate susceptibility to sepsis and worse outcomes in the elderly are not well understood. Recently, changes in DNA methylation have been shown to be linked to aging processes and age-related diseases. Thus, we postulated that age-related changes in DNA methylation may play a role in the onset and prognosis of sepsis in elderly patients. Here, we performed genome-wide methylation profiling of peripheral blood from patients with sepsis and controls. Among the CpG sites whose methylation changes may contribute to an increase in sepsis susceptibility or mortality, 241 sites that possessed age-related changes in DNA methylation in controls may partly explain the increased risk of sepsis in older adults, and 161 sites whose methylation significantly correlated with age in sepsis group may be the potential mechanisms underlying the worse outcomes of elderly septic patients. Finally, an independent cohort was used to validate our findings. Together, our study demonstrates that age-related changes in DNA methylation may explain in part the disproportionate susceptibility and worse outcomes of sepsis in older adults.

**Keywords: sepsis, DNA methylation, aging, susceptibility, outcomes**

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## INTRODUCTION

Sepsis, a life-threatening organ dysfunction characterized by a dysregulated host response to infection, is a leading cause of morbidity and mortality among critically ill patients (1). The incidence of sepsis is expected to increase, likely due to the aging of our population (2, 3). More than 60% of sepsis occur in patients aged ≥65 years, and about 60% of in-hospital mortality from sepsis occur in this age group (4, 5). Aging has been recognized as the primary risk factor for developing sepsis (6). However, the underlying mechanisms of the disproportionate susceptibility to sepsis and worse outcomes in older adults are not yet fully understood.

Epigenetic alterations, especially changes in DNA methylation, have been found to be associated with a variety of diseases, including sepsis (7–9). Several studies have revealed differential methylation profiles which were correlated with sepsis status both in adults and neonates (10–12). Recently, we found that inhibiting DNA methylation by Decitabine can mitigate the effects of sepsis and improve survival of mice by attenuating the activation of NF-κB signaling pathway and down-regulating inflammatory cytokine levels. (13). In addition, changes in DNA methylation also have emerged as a biomarker of aging (14). Approximately 2–14% of the CpG sites show age-associated changes, either hypermethylation or hypomethylation with increasing age (15). Interestingly, age-related changes in DNA methylation have been linked to various diseases, such as diabetes (16), kidney diseases (17), and cancer (18–20). Nevertheless, age-related DNA methylation changes have not been studied in the context of sepsis.

In this study, we analyzed genome-wide methylation profiling in peripheral blood from sepsis patients and controls to explore sepsis and outcome-related CpG sites. Furthermore, we investigated DNA methylation patterns associated with subject age, and found some age-related DNA methylation changes which may explain in part the increase in sepsis incidence and mortality with age.

# MATERIALS AND METHODS

## Patients and Samples Collection

All the patients included were from the First Affiliated Hospital, College of Medicine, Zhejiang University. Patients diagnosed of sepsis during the intensive care unit (ICU) period were enrolled in the study. Sepsis was defined according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) (21). Detailed information of the selected patients was further inspected by a clinician. Controls were age-matched healthy volunteers. The hospital's Institutional Review Committee on Human Research approved the study protocol and all of the patients provided written informed consent.

Follow-up blood samples were obtained within the first 24h after admission. Peripheral blood was collected into EDTA-containing tubes. DNA was extracted using QIAamp® DNA Mini and Blood Mini kits (AXYGEN).

## DNA Preparation and Genome-Wide Methylation Profiling

The extracted DNA was bisulfite converted by Epitect Bisulfite kit (Qiagen, Germany) according to the manufacturer's instruction, 1–2 ug of genomic DNA was incubated with the bisulfite reactions in a thermocycler following the procedure of recommended bisulfite conversion thermal cycler conditions. The converted DNA could be combined to the column, washed, incubated with a desulfonation buffer, washed again and finally eluted with 20 μl of elution buffer. The quality and purity of the converted DNA was tested by Nanodrop.

Genome-wide methylation profiling was performed using Infinium HumanMethylation 450 BeadChip array (Illumina, San Diego, CA, USA). After whole-genome amplification with 200 ng of input bisulfite-converted DNA, the product was fragmented, purified and applied to the BeadChips using Illumina-supplied reagents and conditions. After extension, the array was stained fluorescently, and scanned with an iSan System (Illumina). Data were analyzed by GenomeStudio Methylation Module V1.8 Software (Illumina). A CpG site was considered to be informative if the sum of the signals for methylated and unmethylated sequence at the CpG site was significantly higher (detection $p$-value $< 0.01$) than signals of the negative control probes on the same array. For each CpG site, the β value reflects the methylation level, which was computed by $\beta = (\max(M, 0))/(|U| + |M| + 100)$. A β value of 0–1.0 indicates the percent methylation from 0 to 100%, respectively.

## Pyrosequencing

The genomic DNA was bisulfite converted from unmethylated cytosine to uracil by using the Epitect Bisulfite kit (Qiagen). Primers were designed by the PyroMark Assay Design 2.0. PCR was performed with the following cycling conditions: initial denaturation step: 95°C for 3 min; 40 cycles of PCR in denaturation step: 94°C for 30 s; annealing step: Tm of primer; extension step: 72°C for 1 min and final extension: 72°C for 7 min. Subsequently, streptavidin coated sepharose beads, PyroMark Gold reagents (Qiagen), Pyrosequencing Vacuum Prep Tool and PyroMark Q96 software (Qiagen) were used for the determination and analysis of DNA methylation according to the manufacturers' instructions.

## Statistical Analyses

All statistical analyses were done using R (http://www.r-project.org/) and GraphPad Prism 8.0 (GraphPad Software, CA, USA). For methylation variable positions (MVPs) identification, we used the R limma package to establish a linear model and calculated the $p$-value. The Benjamini & Hochberg method was used to correct for multiple testing, and false discovery rate [FDR] $< 0.05$ was considered significant Sepsis outcome-related CpG sites were identified using the R limma package, and $p$-value $< 0.05$ was considered statistically significant. Functional enrichment analyses were performed by R package missMethyl (22) and Metascape (https://metascape.org/). The cutoff criteria was set at $p$-value $< 0.05$. We used default function lm() in R to construct the linear regression model, which was fitted by least-squares to DNA methylation of CpG sites and age. An association was considered significant where $p$-value $< 0.05$. Student's $t$-test was used for the comparison between groups in the validation cohort, and $p$-value $< 0.05$ was considered statistically significant.

# RESULTS

## Sepsis-Associated Methylation Variable Positions

To investigate the epigenetic changes in sepsis, genome-wide methylation profiling was performed in peripheral blood from 24 sepsis patients aged 16–88 years, and 12 healthy controls aged 22–84 years (patient characteristics are shown in **Supplementary Table 1**). After quality control, batch correction, and normalization, a total of 8,437 CpG sites which significantly differentially methylated between sepsis and control group were identified as methylation variable positions (MVPs)

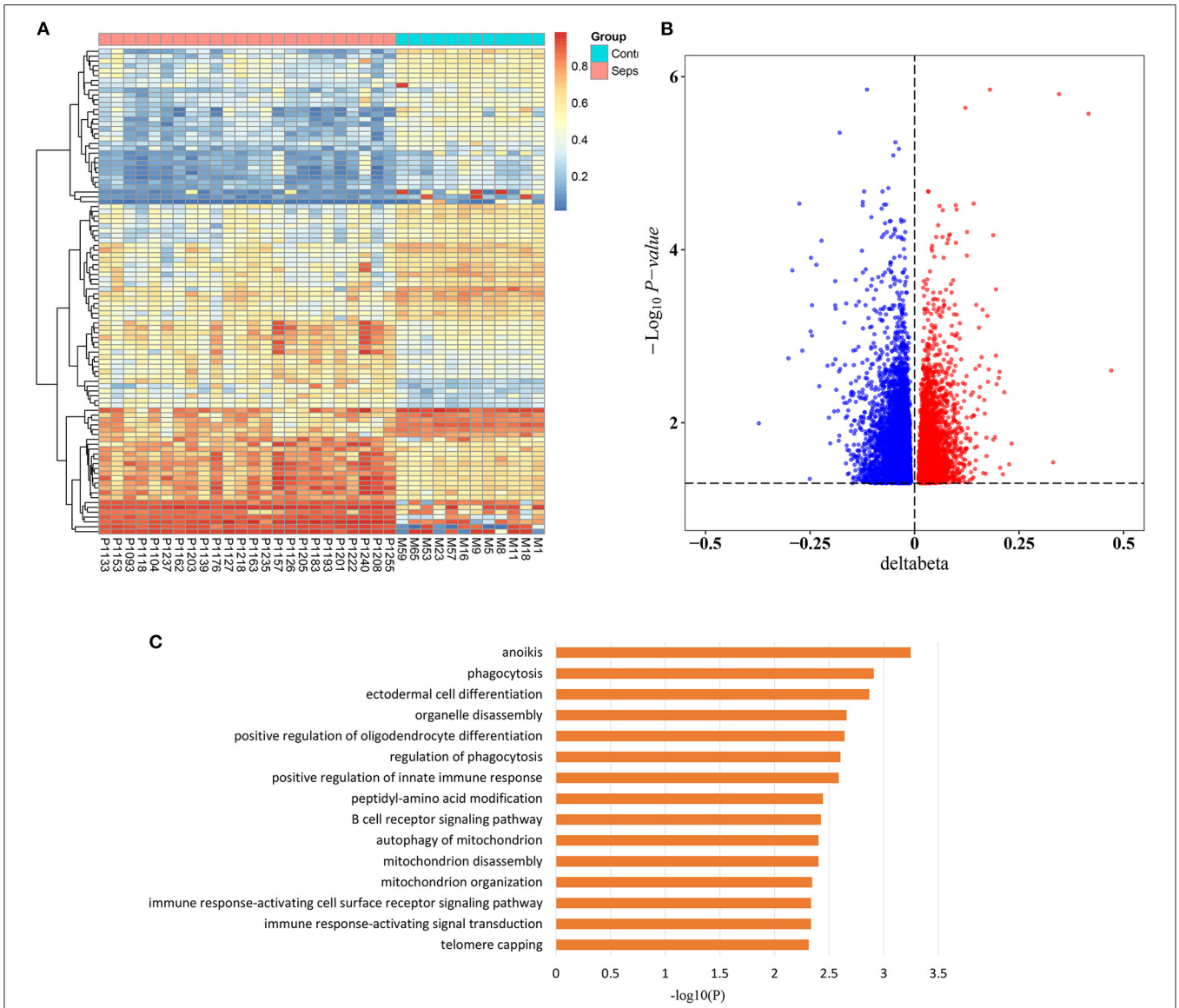


**FIGURE 1 |** Sepsis-associated methylation variable positions. **(A)** Heatmap of top 100 methylation variable positions (MVPs) based on Δβ in sepsis patients and controls (FDR < 0.05). **(B)** Volcano plot of 8,437 MVPs. **(C)** The top 15 significant gene ontology (GO) biological process terms of sepsis-associated MVPs.

(**Supplementary Table 2**). The top 100 MVPs are shown as an expression heatmap (**Figure 1A**). Principal component analysis (PCA) using the methylation data of all MVPs showed samples from sepsis patients and controls separated along the first principal component (**Supplementary Figure 1**). In all MVPs, 3,042 CpG sites (36.1%) were hypermethylated in sepsis patients, while 5,395 (63.9%) sites were hypomethylated (**Figure 1B**). However, only a small part of MVPs have relatively large Δβ (|Δβ|> 0.2), and focusing on those with large Δβ, we found that 6 of 26 differentially methylated genes (23.1%) were previously associated with sepsis in the literature (**Supplementary Table 3**), including ALK, ZEB2, MNDA, AIM2, TLR5, and JUNB. These genes are involved in various stages of sepsis, from immune response, to acute inflammation, to the regulation of apoptosis. More importantly, they have also been found to be implicated in aging process.

In addition, in order to unravel the potential biological function of all sepsis-associated MVPs, we performed Gene Ontology (GO) enrichment analysis (**Figure 1C**). The results demonstrated that they were mainly enriched in phagocytosis, immune response, and mitochondria, which are highly associated with sepsis (23–25).

## Role of Age-Related CpG Sites in the Disproportionate Susceptibility to Sepsis in the Elderly

Considering that sepsis disproportionally affects the elderly, we next divided sepsis patients and controls into two age

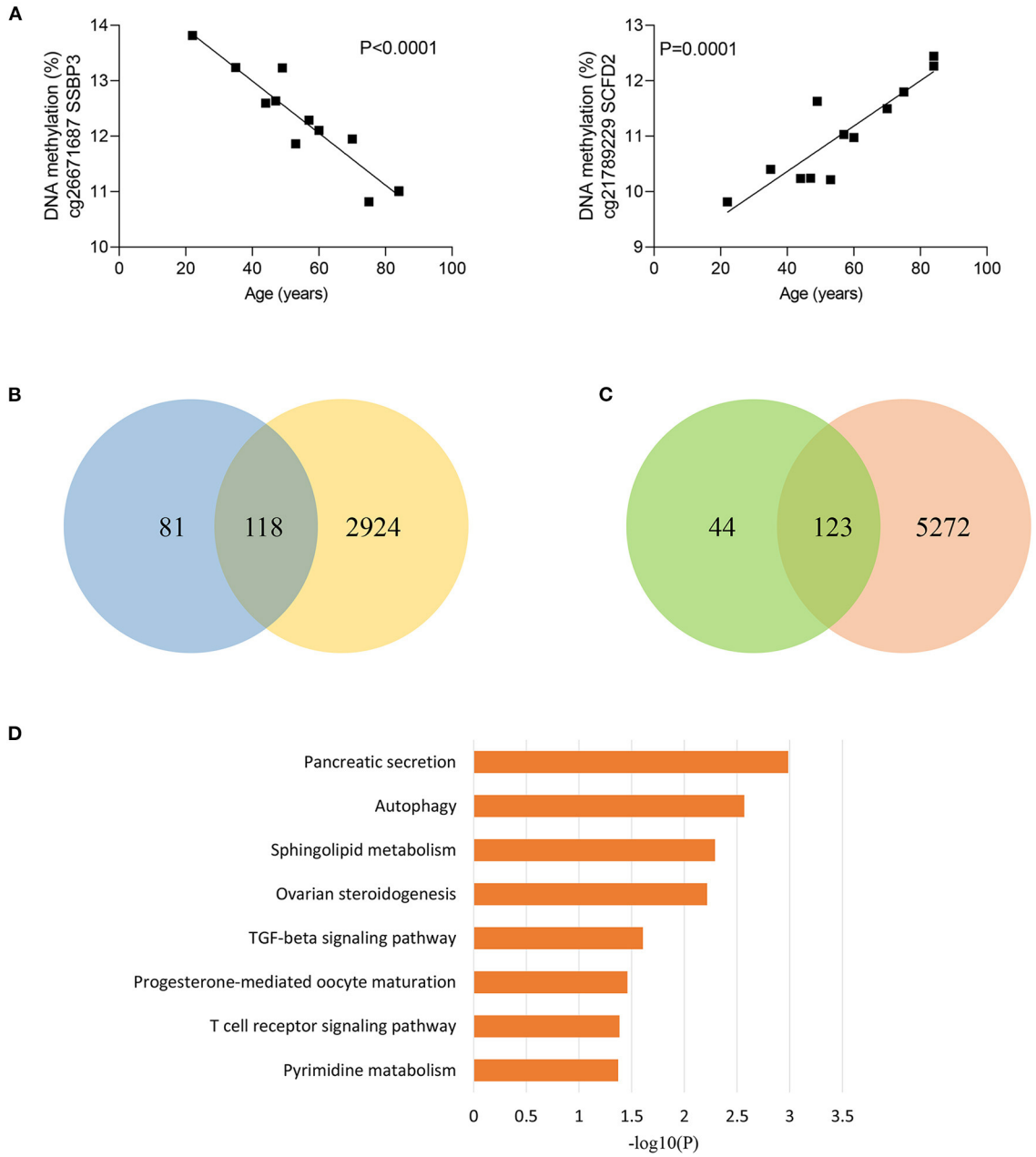


**FIGURE 2 |** Age-related methylation changes in controls. **(A)** Correlation between age and methylation of two CpG sites with the most significant correlations with age in controls as analyzed by linear regression analysis ($n = 12$). **(B)** Venn diagram showing the intersection of the sets of hypermethylated sites with increasing age in controls and hypermethylated sites in sepsis. **(C)** Venn diagram showing the intersection of the sets of hypomethylated sites with increasing age in controls and hypomethylated sites in sepsis. **(D)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of 227 genes distributed by 241 age-related CpG sites.

groups, younger than 50 years and older than 50 years. Of note, 5,87 of 84,37 CpG sites (69.8%) were differentially methylated between sepsis patients and controls under 50 years, while 8,012 of 8,437 CpG sites (95.0%) were differentially methylated between sepsis patients and controls over 50 years, supporting that aging is the primary risk factor for developing sepsis. Then, we tested to determine whether methylation of sepsis-associated MVPs are correlated with age. Then, we performed linear regression analysis and found that methylation of 366 sepsis-associated CpG sites were significantly associated with age in control group (**Supplementary Table 4**). The most significant correlations between DNA methylation and age are

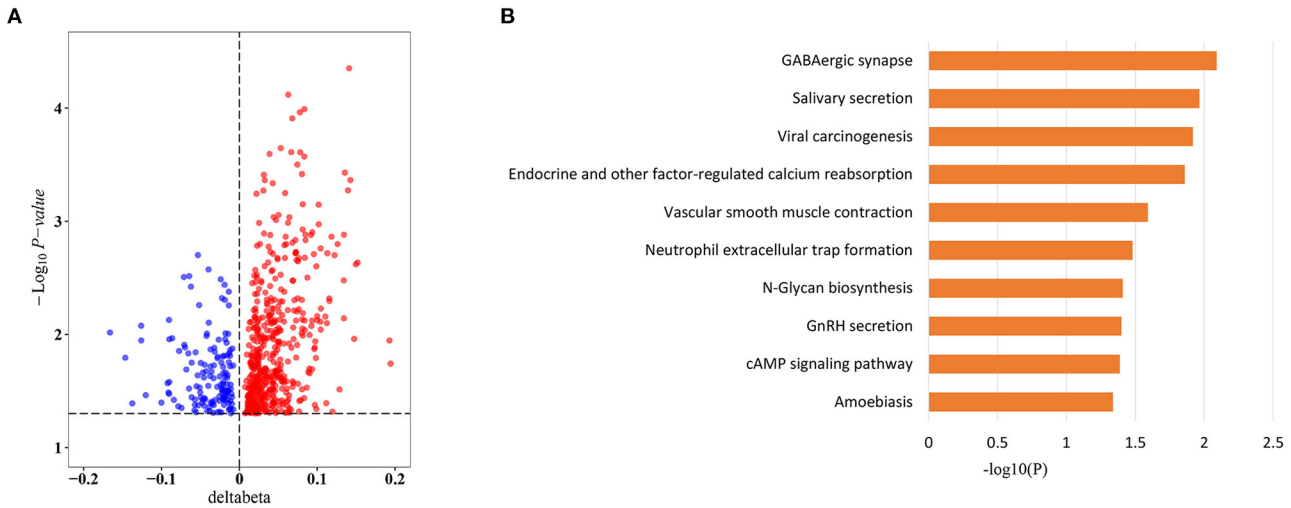


**FIGURE 3 |** Sepsis outcome-related CpG sites. **(A)** Volcano plot of sepsis outcome-related CpG sites ($n = 657$). **(B)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of sepsis outcome-related CpG sites.

presented in **Figure 2A**. Next, we wondered whether sepsis-associated CpG sites were enriched in age-related CpG sites and performed Fisher's exact test. However, there was no statistical significance ($p = 0.097$, **Supplementary Table 5**). Of the 366 age-related CpG sites, 199 sites (54.4%) were hypermethylated with increasing age, and 167 sites (45.6%) were hypomethylated (**Supplementary Figure 2**). Moreover, we observed that 118 of 199 sites (59.3%) hypermethylated with age in control group exhibited an increased DNA methylation in sepsis patients (**Figure 2B**). Similarly, 123 of 167 sites (73.7%) hypomethylated with age in control group exhibited a decreased DNA methylation in sepsis patients (**Figure 2C**). Together, these findings indicated that 241 age-related CpG sites may play a mechanistic role in the disproportionate susceptibility to sepsis in older adults. Furthermore, we verified whether genes distributed by 241 CpG sites were enriched in specific pathways, and found that the TGF-β signaling pathway was the highest-ranked pathway (**Figure 2D**), which is involved in the pathogenesis of sepsis (26).

## Sepsis Outcome-Related CpG Sites

To explore whether sepsis-associated MVPs were associated with sepsis outcomes, we divided sepsis patients into two groups (7 survivors and 17 non-survivors) according to their hospital outcomes. Intriguingly, 657 of 8,437 CpG sites were significantly differentially methylated between two groups (**Supplementary Table 6**), and more CpG sites were hypermethylated in non-survivors, with 514 (78.2%) hypermethylated vs. 143 (21.8%) hypomethylated CpG sites (**Figure 3A**). Next, we investigated which pathways were affected by the methylation changes that were associated with sepsis outcomes. Interestingly, these CpG sites were involved in cAMP signaling pathway (**Figure 3B**), which plays an important role in sepsis-induced organ dysfunction (27, 28).

## Role of Age-Related CpG Sites in the Worse Outcomes of Elderly Septic Patients

Given that elderly septic patients have increased hospital mortality than younger patients (29), we wondered whether methylation of sepsis outcome-related CpG sites were correlated with age in sepsis group. Strikingly, methylation of 163 CpG sites were significantly associated with age in sepsis patients (**Supplementary Table 7**). **Figure 4A** shows the most significant correlations between DNA methylation and age. Among the 163 CpG sites, more CpG sites were hypermethylated with age: 150 (92.0%) hypermethylated vs. 13 (8.0%) hypomethylated CpG sites. Moreover, we observed that 149 of 150 sites (99.3%) hypermethylated with increased age in sepsis group exhibited an increased DNA methylation in non-survivors (**Figure 4B**). A similar observation was made for 12 of 13 hypomethylated CpG site (92.3%) (**Figure 4C**). Together, these data suggested that 161 age-related CpG sites may play a crucial role in the worse outcomes of elderly septic patients. Furthermore, pathway analysis of the genes distributed by 161 CpG sites revealed that the mTOR signaling pathway was the top enriched pathways (**Figure 4D**), which is implicated in immunosuppression following sepsis and aging process (30, 31).

## Validation of the CpG Site in VAC14

Between the above two age-related CpG sets, 9 CpG sites were shared and directionally consistent (**Figures 5A,B**). Among the 9 sites, 2 CpG sites were distributed in VAC14, which is relevant to bacteremia secondary to multiple pathogens (32). Then, we selected one site (cg06542681) with the most significant association between DNA methylation and age in control group for further validation. The pyrosequencing assays were performed to analyze DNA methylation of the CpG site in VAC14 in peripheral blood taken from another cohort of 48 sepsis patients and 48 controls (patient characteristics are

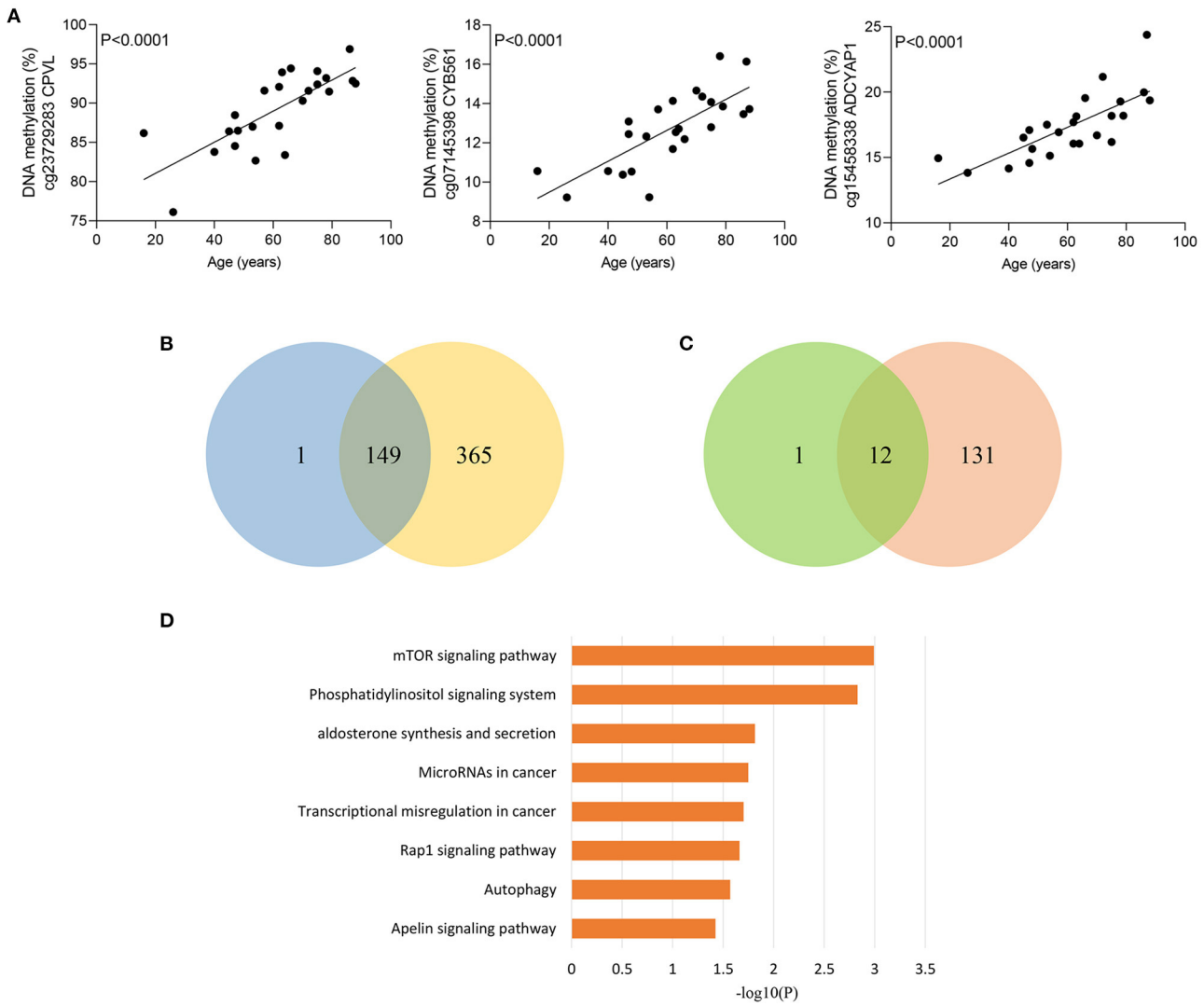


**FIGURE 4 |** Age-related methylation changes in sepsis patients. **(A)** Correlation between age and methylation of three CpG sites with the most significant correlations with age in sepsis patients as analyzed by linear regression analysis ($n = 24$). **(B)** Venn diagram showing the intersection of the sets of hypermethylated sites with increasing age in sepsis and hypermethylated sites in non-survivors. **(C)** Venn diagram showing the intersection of the sets of hypomethylated sites with increasing age in sepsis and hypomethylated sites in non-survivors. **(D)** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of 155 genes distributed by 161 age-related CpG sites.

shown in **Supplementary Table 8**). As expected, this CpG site hypermethylated with increasing age and exhibited an increased DNA methylation both in sepsis patients and non-survivors (**Figures 6A–C**). Overall, our findings supported that age-related changes in DNA methylation may potentially contribute to the disproportionate susceptibility and worse outcomes of sepsis in the elderly.

## DISCUSSION

Our study shows the genome-wide methylation profiling of peripheral blood from adult patients with sepsis and healthy controls, and further provides the first sepsis-specific study of age-related DNA methylation changes. We identified some CpG sites whose methylation changes may contribute to an increase in sepsis susceptibility or mortality. Then, age-related DNA methylation changes wereexamined, which may provide new insights into the disproportionate susceptibility and worse outcomes of sepsis in the elderly. Indeed, 241 CpG sites whose methylation varies with age in controls may contribute to increased risk of sepsis in older adults. In addition, 161 CpG sites whose methylation changes significantly correlated with age in sepsis group may partly explain the poor prognosis of elderly septic patients (**Figure 7**). Together, our findings revealed for the first time that age-related changes in DNA methylation may play a mechanistic role in the increased susceptibility and mortality of sepsis in the elderly.

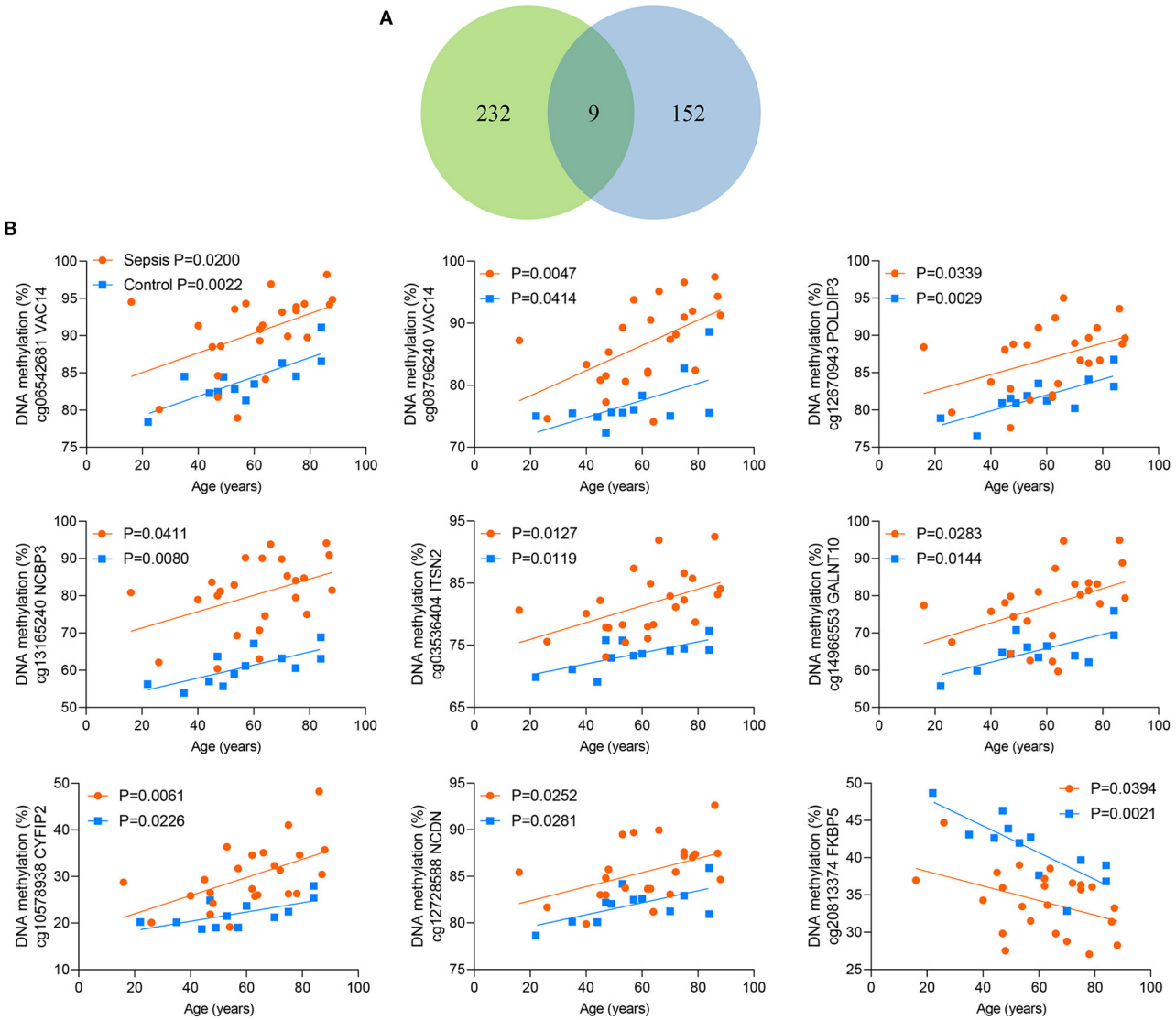


**FIGURE 5 |** Nine shared CpG sites. **(A)** Venn diagram showing the intersection of the sets of 241 age-related CpG sites in controls and 161 age-related CpG sites in sepsis patients. **(B)** Correlation between age and methylation of 9 CpG sites as analyzed by linear regression analysis.

Epigenetic changes have been linked to key phases of sepsis, from the host-pathogen interaction to inflammatory response, to immune suppression, to organ failures (8). Our results demonstrated the existence of DNA methylation changes in sepsis patients vs. controls, supporting that epigenetic alterations may play an important role in the pathogenesis of sepsis. Furthermore, we revealed 657 CpG sites whose methylation changes were related to sepsis prognosis, with significantly more CpG sites hypermethylated in non-survivors. These findings are in line with our previous research that inhibiting DNA methylation may mitigate inflammation and improve survival in sepsis (13).

The current studies show that aging has a major impact on DNA methylation (33). Tens to hundreds of thousands of CpG sites show significant methylation changes (increase or decrease) with age. Several studies of anti-aging interventions demonstrate that the age-related changes in DNA methylation play a mechanistic role in aging (15). Importantly, aging is associated with increased incidence, delayed recovery, and worse outcomes of sepsis (34, 35). Thus, we hypothesized that age-related changes in DNA methylation may play a role in the susceptibility and prognosis of sepsis. Indeed, 241 CpG sites that possessed significant differences in DNA methylation between sepsis patients and controls in a direction consistent with the age-related change, meaning that these 241 age-related CpG sites may be the potential mechanisms underlying the increased risk of sepsis in older adults. Interestingly, genes distributed by these CpG sites were mainly involved in TGF-β

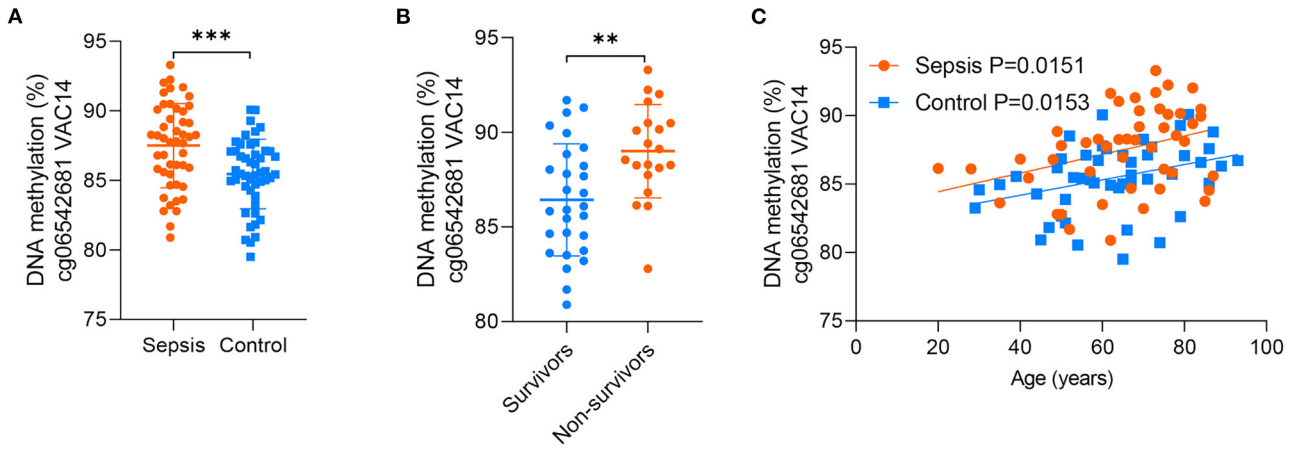


**FIGURE 6 |** Validation of the CpG site in VAC14. **(A)** DNA methylation of the CpG site in VAC14 in peripheral samples from 48 sepsis patients and 48 controls were analyzed by the pyrosequencing assays. **(B)** 48 sepsis patients were divided into two groups (28 survivors and 20 non-survivors) according to their hospital outcomes. **(C)** Correlation between age and methylation of the CpG site in VAC14 as analyzed by linear regression analysis. Data shown are mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$. Comparisons between two groups were done by student's t-test.

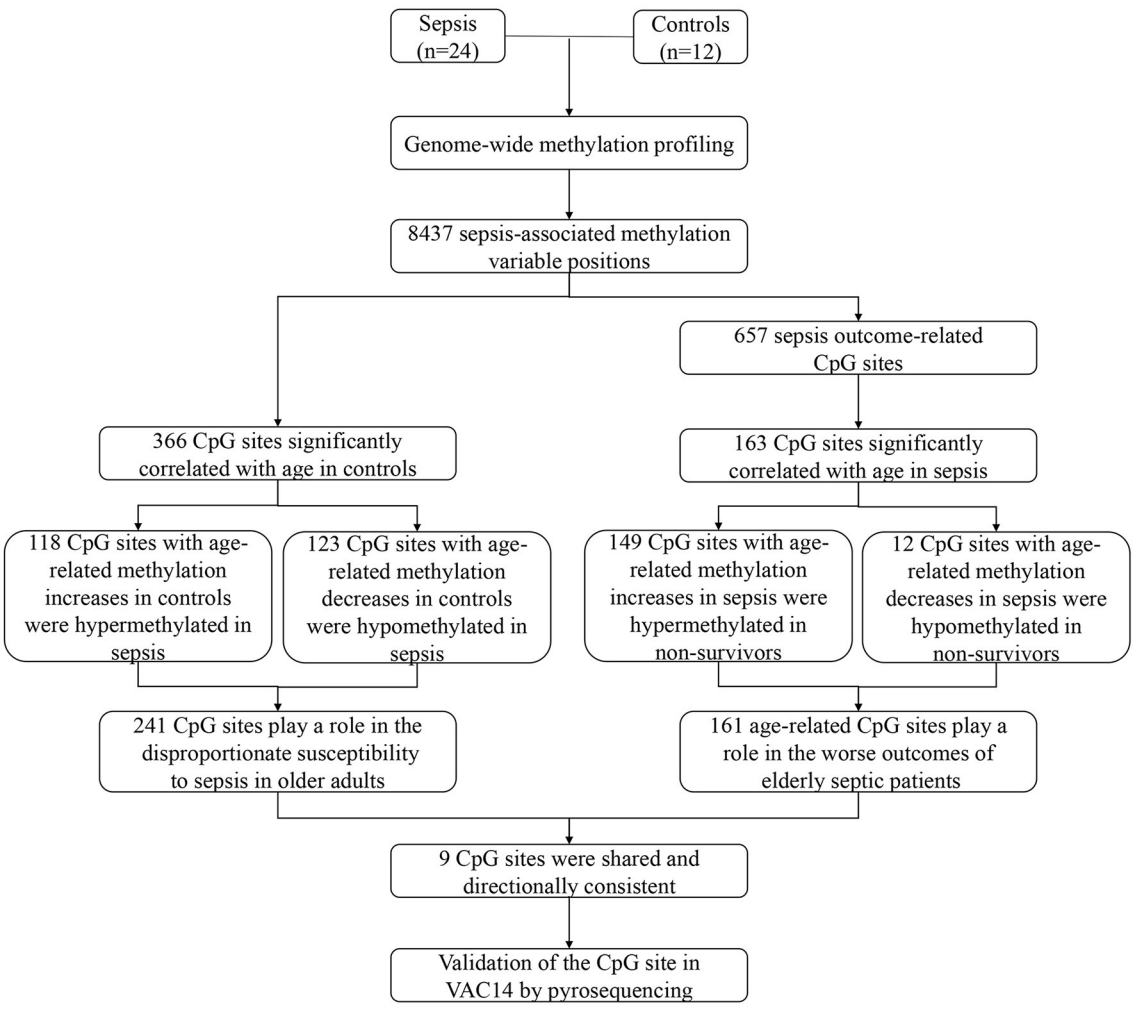


**FIGURE 7 |** Schematic of study.

signaling pathway, which is essential for LPS-induced sepsis (26). Moreover, inhibiting TGF-β pathway can ameliorate sepsis-induced organ dysfunction and increase survival time of septic mice (36, 37). In addition, 161 CpG sites that possessed significant differences in DNA methylation between survivors and non-survivors in a direction consistent with the age-related change, supporting that 161 age-related CpG sites may play a crucial role in the worse outcomes of elderly septic patients. Importantly, the top enriched pathway among these CpG sites distributed genes was mTOR signaling pathway, which plays an important role in immunosuppression following sepsis (30). Similarly, several studies have indicated that inhibiting mTOR signaling pathway can attenuate sepsis-induced organ dysfunction in rats (38, 39). Additionally, mTOR signaling is strongly implicated in aging (31). The mTOR inhibitor rapamycin is now the only known pharmacological intervention that can extend lifespan in all tested animal models (40–43). One possible underlying mechanism is that inhibition of mTOR complex 1 (mTORC1) may stimulate autophagy, which helps clear damaged mitochondria, the accumulation of which are linked to aging and aging-related diseases such as sepsis (40). Accordingly, our findings open new perspectives for reducing the incidence and mortality of sepsis, especially in elderly patients.

Given that DNA methylation in cytokine genes changes upon aging (44–46), we analyzed inflammatory cytokine genes, TNF-α, IL-1β, IL-6, and IL-8, which includes a total of 72 CpG sties. Interestingly, 6 sites (8.3%) were correlated with age in sepsis patients, and the CpG site with the most significant correlation was in TNF-α (**Supplementary Figure 3A**). Moreover, this site was hypermethylated in non-survivors (**Supplementary Figure 3B**), which is consistent with previous studies that increased methylation in TNF gene was associated with poorer survival in pancreatic cancer (47). Thus, our findings indicated that DNA methylation changes in TNF-α gene along with aging may play a role in sepsis progression. However, methylation of TNF have been found to be negative correlated to age in healthy controls (48). We next explored the correlation between methylation of TNF and age in our control group, and a total of 25 CpG sites were included in our methylation data. All of these sites showed negative correlation along with age (minimum $p$-value was found in cg17741993, $p = 0.09$), although there was no statistical significance, which may be attributed to the small sample size.

One potential limitation of our study is the size of the discovery cohort. In order to alleviate this limitation and further verify our findings, we validated the selected CpG site with a larger sample size. Another limitation is that we were unable to test the relation of identified age-related DNA methylation changes with expression of respective nearby annotated genes. Since DNA methylation is often associated with gene expression (49), future studies are needed to explore expression of genes with age-related DNA methylation changes, and further investigated exact mechanisms of these genes in the occurrence and progression of sepsis.

In conclusion, our study revealed for the first time that age-related changes in DNA methylation may play a role in the disproportionate susceptibility and worse outcomes of sepsis in older adults, and provide a new insight into the potential link among sepsis, epigenetic changes, and aging.

## DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the OMIX, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix/release/OMIX904), accession number OMIX904.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

JC, HJ, and XL designed the study. LS, TZ, and QS provided the samples. WZ, YC, and CZ carried out experiments. CW, YW, and FN performed the statistical analysis. XL and LS wrote the manuscript. All authors contributed to the manuscript and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.822847/full#supplementary-material

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# Polypharmacy in Older Adults Undergoing Major Surgery: Prevalence, Association With Postoperative Cognitive Dysfunction and Potential Associated Anesthetic Agents

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**Background:** Polypharmacy, which is defined as the use of 5 or more medications, can exert significant adverse impact on older adult patients. The objective of this study was to determine the prevalence of polypharmacy, and to investigate its association with postoperative cognitive dysfunction (POCD) in older adult patients who underwent elective major surgery at Siriraj Hospital—Thailand's largest national tertiary referral center.

**Methods:** This prospective study included older adult patients aged ≥65 years who were scheduled for elective major surgery during December, 2017 to December, 2019 study period. Patient demographic, sociodemographic, anthropometric, clinical, comorbidity, anesthetic, surgical, and medication data were collected and compared between the polypharmacy and non-polypharmacy groups. Postoperative cognitive dysfunction (POCD) was diagnosed in patients with at least a 2-point decrease in their Montreal Cognitive Assessment score after surgery. Multivariate logistic regression analysis was used to identify independent predictors of POCD.

**Results:** A total of 250 patients (141 males, 109 females) with an average age of 72.88 ± 6.93 years were included. The prevalence of polypharmacy was 74%. Preoperative data showed the polypharmacy group to be more likely to be receiving potentially inappropriate medications, to be scheduled for cardiovascular thoracic surgery, and to have more comorbidities. There was a non-significant trend in the association of polypharmacy and POCD (crude odds ratio (OR): 2.11, 95% confidence interval [CI]: 0.90–4.94; $p = 0.08$). Benzodiazepine, desflurane, or isoflurane administration during surgery were all significantly associated with POCD in univariate analysis. Multivariate

analysis revealed intraoperative benzodiazepine (adjusted OR [aOR]: 2.24, 95% CI: 1.10–4.68; $p = 0.026$) and isoflurane (aOR: 2.80, 95% CI: 1.35–5.81; $p = 0.006$) as two independent variables associated with the development of POCD. Desflurane was found to be a protective factor for POCD with a crude OR of 0.17 (95% CI: 0.03–0.74, $p = 0.019$); however, independent association was not found in multivariate analysis.

**Conclusion:** There was a high prevalence of polypharmacy in this study; however, although close ($p = 0.08$), significant association was not found between polypharmacy and POCD. Benzodiazepine and isoflurane were both identified as independent predictors of the development of POCD among older adult patients undergoing elective major surgery, especially among those classified as polypharmacy.

**Keywords: prevalence, polypharmacy, postoperative cognitive dysfunction, older adult patients, elective major surgery**

## INTRODUCTION

An aging society is defined as >10% of the population aged over 60 years, and an aged society is defined as more than 14% of the population aged over 60 years (1). Most high-income countries and many middle-income countries (including Thailand) have become aging countries. Increasing age is commonly associated with more comorbidities, multiple medications, and deterioration of organ function. Alterations of drug pharmacokinetics and pharmacodynamics among older adults increases the risk of adverse drug reactions, subsequent hospitalization, and increased mortality (2).

Polypharmacy is a global problem that is expected to worsen with advances in medicine and the increasing development and discovery of new drugs. There are several definitions of polypharmacy in the literature, and many studies that reported significant association between polypharmacy and subsequent negative clinical outcomes. The most commonly reported factors are taking five or more prescribed drugs and receiving potentially inappropriate medications (PIMs) (3–5). The likelihood of adverse drug reactions increases commensurate with the number of drugs taken, and rises in adverse drug reactions results in an increased number of hospital admissions (6). Polypharmacy exerts several other detrimental effects on older adults, including delirium and cognitive impairment, which increase medical expenses, morbidity, and mortality (7, 8). The risk increases if one or more prescriptions in a polypharmacy case are drugs defined as PIMs.

As the population ages, there is an increased incidence of anesthesia and surgery among older adults, and it has been established that age-related factors increase the likelihood of postoperative complications (9). Furthermore, polypharmacy was found in the majority of older adult patients undergoing major elective noncardiac surgery, and it was found to be associated with a reduced survival rate and a higher rate of adverse events (10).

Postoperative cognitive dysfunction (POCD) is defined as a decline in cognitive function after anesthesia and surgery. Symptoms of POCD may develop from within 1 week after surgery to months after surgery. In this setting, cognitive function includes learning and memory, verbal ability, perception, attention, executive function, and abstract thinking (11). Meanwhile postoperative delirium (POD) is a sudden change in mental status marked by a disturbance of awareness of the environment and a disruption in attention after surgery. There are three types of POD expression: hypoactive, hyperactive, and mixed. POD may also be involved in the development of POCD. The difference between POD and POCD is that POD diagnosis involves symptom detection, whereas POCD diagnosis requires neuropsychological testing administered pre- and post-operatively. The prevalence of POCD was reported to be as high as 41% among older adult patients after some surgical procedures (12). Understanding the relationship among anesthesia, surgery, and cognitive impairment is essential for guiding clinical practice (13). Several potential causes of POCD have been proposed, including advanced age, prior delirium, preoperative cognitive impairment, low education, and the use of anticholinergic medications prior to surgery (14–18). Data specific to the association between POCD and medications used during the perioperative period are scarce. Previous study has addressed the issue of polypharmacy and cognitive decline, but not in postoperative setting (19). Accordingly, the aim of this study was to determine the prevalence of polypharmacy, and to investigate its association with POCD in older adult patients who underwent elective major surgery at Siriraj Hospital—Thailand's largest national tertiary referral center.

## METHODS

This prospective study was conducted as part of the Siriraj Integrated Perioperative Geriatric Research Network of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (study COA No. 515/2017). We recruited Thai-speaking patients who were aged 65 years or older and scheduled for elective major surgery during the December, 2017 to December, 2019 study period. Major surgery was defined as a procedure potentially lead to organ ischemia, high intraoperative blood loss, high noradrenalin requirements, long operative time, and requiring perioperative blood transfusion.

Operation generates systemic inflammatory response and the need for intermediate or intensive care was also considered as a major surgery. Potential consequences of major surgery include high morbidity and mortality (20). Patients unable to undergo cognitive assessments, having severe visual or auditory dysfunction, having significant psychotic disorders affecting their ability to cooperate, having preoperative delirium, or being bedridden were excluded. We also excluded patients who were unable to attend follow-up visits during the postoperative period. Patient baseline characteristics and intraoperative data were obtained from their electronic medical records.

## Data Collection

Patient demographic data, comorbidities, body mass index (BMI), and current patient medication data were collected preoperatively via patient interview and review of hospital medical records. Intraoperative and postoperative data were also gathered from medical records, including type of surgery, anesthetic technique, anesthetic medications, operative time, and intraoperative and postoperative complications. Polypharmacy was defined as the use of five or more medications preoperatively (3). PIMs were identified according to Beers criteria (5) and assessment by a geriatrician. The Montreal Cognitive Assessment (MoCA)–Thai version was administered by a psychologist to measure each patient's baseline cognitive status before undergoing surgery. It evaluates at eight different cognitive abilities: visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall, and orientation. The maximum MoCA score is 30, with a higher value indicating better performance. The test has been widely used to assess older people with various form of cognitive dysfunction.

During postoperative days 5–9, the same psychologist visited all enrolled participants at the inpatient ward. If they were medically stable (e.g., absence of acute stroke or hypotension), the MOCA test was readministered. POCD was diagnosed according to the previously published recommendation (21) that POCD be defined as a postoperative decrement of cognitive test scoring $\geq$1 standard deviation. However, in the present study, we diagnosed POCD when there was decrease of $\geq$2 points in the postoperative MoCA score compared to the preoperative MoCA score, which was also a previously reported diagnostic method (22).

**TABLE 1 |** Preoperative characteristics of the study population.

| Characteristics | (*N* = 250) |
|---|---|
| Age (years) | 72.88 ± 6.93 |
| <70 years | 75 (30.0%) |
| 70–79 years | 130 (52.0%) |
| ≥80 years | 45 (18.0%) |
| **Gender** | |
| Male | 141 (56.4%) |
| Female | 109 (43.6%) |
| **Education** | |
| < 12 years of education | 219 (88.7%) |
| ≥ 12 years of education | 28 (11.3%) |
| BMI | 24.08 ± 4.10 |
| Underweight (<18.5 kg/m$^2$) | 24 (9.6%) |
| Normal (18.5–24.9 kg/m$^2$) | 131 (52.4%) |
| Overweight (25–29.9 kg/m$^2$) | 75 (30.0%) |
| Obese (≥30.0 kg/m$^2$) | 20 (8.0%) |
| **ASA classification** | |
| II | 60 (24.0%) |
| III | 174 (69.6%) |
| IV | 16 (6.4%) |
| **Site of surgery** | |
| CVT | 141 (56.4%) |
| Non-CVT | 109 (43.6%) |
| Presence of polypharmacy | 185 (74%) |
| Presence of PIMs | 67 (26.8%) |

*Data presented as mean ± standard deviation or number and percentage.*
*SD, standard deviation; BMI, body mass index; ASA, American Society of Anesthesiology; CVT, cardiovascular thoracic surgery; PIMs, potentially inappropriate medications.*

## Sample Size Calculation and Statistical Analysis

The sample size calculation took into account both components of our study objective. The primary objective of this study was to determine the prevalence of polypharmacy. Previous study reported a prevalence of polypharmacy of approximately 29% (23). Cochran's formula was used to calculate the sample size, with the Z value set to 1.96 at a 95% confidence level and the acceptable tolerance (e) set at 8%. Using those estimates, a minimum sample size of 116 patients would be required to satisfy the primary objective. Sample size calculation for secondary objective was also performed for identification of factors associated with POCD using multivariate analysis. We estimated following previous study which discovering 5 factors independently associated with the development of POCD (24). Using the rule of thumb method suggested for 10 events per variable with estimated prevalence of POCD of 20% from the Siriraj Integrated Perioperative Geriatric Research Network database, a minimum of 250 patients would be required. The final sample size for the study was therfore chosen to be 250.

All data analyses were performed using SPSS Statistics version 18 (SPSS, Inc., Chicago, IL, USA). Participant dermograhic, clinical, and intraoperative data were analyzed using descriptive statistics. We divided patients into two groups according to the number of medications they were taking, as follows: the polypharmacy group (five or more drugs) and the non-polypharmacy group (less than five drugs). Categorical data were compared using chi-square test or Fisher's exact test, and the results are given as number and percentage. Continuous data were compared using 2-sample *t*-test for normally distributed data (results shown as mean plus/minus standard deviation), and using Mann-Whitney *U* test for non-normally distributed data (results show as median and range; minimum, maximum). To identify association between polypharmacy and POCD, we

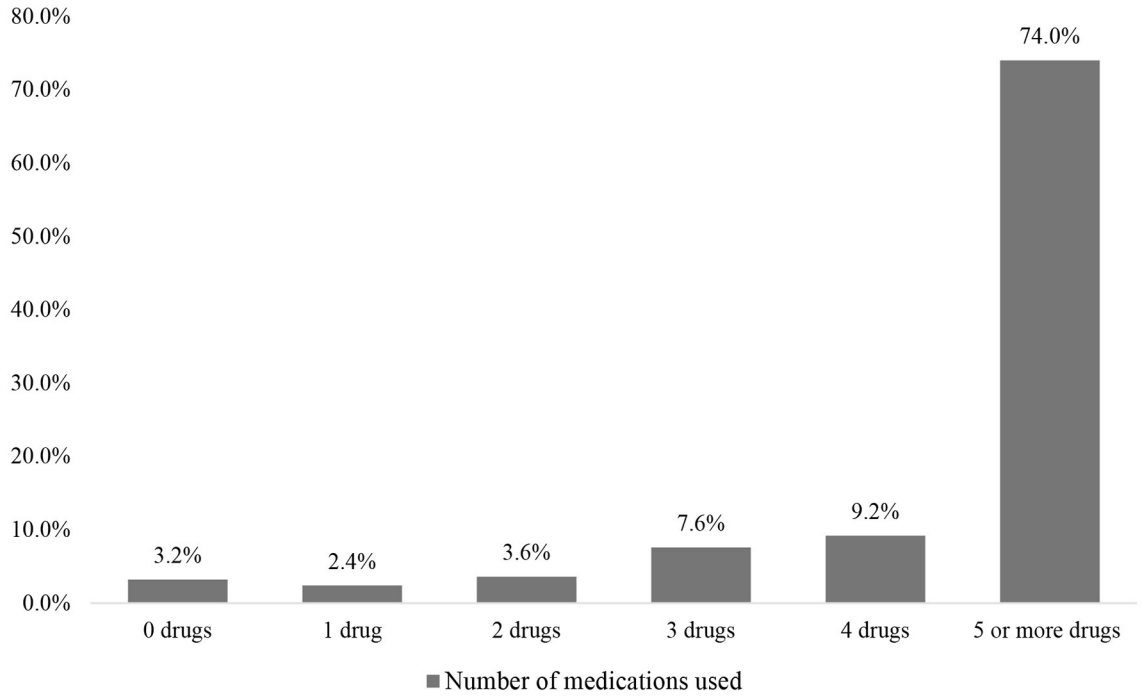


**FIGURE 1 |** The percentage of overall patients stratified by the number of drugs used. The results show the prevalence of polypharmacy (five or more drugs) to be 74% among older adults who underwent major surgery.

divided participants into groups based on the presence or absence of POCD. Evaluated variables with a $p < 0.10$ in univariate analysis were entered into multivariate analysis to identify factors that independently predict POCD. The results of the univariate and multivariate analyses are presented as crude and adjusted odds ratios (respectively) and their respective 95% confidence intervals. A $p < 0.05$ was considered statistically significant for all tests.

## RESULTS

We evaluated 250 participants aged 65 and over who underwent elective major surgery. This study included 141 males and 109 females with an average age of $72.88 \pm 6.93$ years. Concerning the type of surgery, 141 patients underwent cardiovascular thoracic (CVT) surgery, and 109 had non-CVT surgery (**Table 1**). As demonstrated in **Figure 1**, polypharmacy was found in 74% ($n = 185$) of older adult patients who underwent surgery. Moreover, a 31% prevalence of PIMs was found in the polypharmacy group, which was significantly higher than the rate in the non-polypharmacy group (12.3%) ($p = 0.002$).

Patients in the polypharmacy group were significantly more likely to be scheduled for CVT surgery, to have higher disease burden (ASA Physical Status classification of three or above), and to have a higher prevalence of comorbidities, including hypertension, dyslipidemia, and ischemic heart disease/myocardial infarction. Antiarrhythmic drugs, antihypertensive drugs, diabetic drugs, diuretic drugs, and a benzodiazepine-based anxiety reliever were among the most common medications prescribed. In contrast, patients in the non-polypharmacy group had significantly more malignant cancer and alcohol use (**Table 2**). Carvedilol (26.4%), metformin (24.9%), furosemide (25.4%), amlodipine (23.7%), and enalapril (18.4%) were the first five most commonly prescribed drugs for patients in the polypharmacy group (**Figure 2**). Concerning anesthesia-related variables, which are shown in **Table 3**, patients with polypharmacy were significantly more likely to receive general anesthetic procedures (82.7%), inhalation (86.5%), isoflurane inhaler (35.1%), a muscle relaxant with rocuronium (42.2%), analgesic with morphine (55.1%), and blood transfusion (66.9%).

Regarding evaluation for POCD in this study. Only 175 patients were available for MoCA testing after surgery. Reason for data missing were unstable medical conditions death, unwillingness to answer questions, and discharging from the hospital prior to evaluation. Of those, 51 individuals (29.1%) were identified as having POCD. **Table 4** shows variables potentially associated with POCD compared between the non-POCD and POCD groups. Ischemic heart disease/myocardial infarction, receiving antihypertensive drug, CVT surgery, received benzodiazepine during surgery, received isoflurane, received rocuronium muscle relaxant, and received blood product were all factors significantly associated with POCD. Having received desflurane and muscle relaxant reversal were both significantly more common among those found not to

**TABLE 2 |** Preoperative characteristics compared between the non-polypharmacy and polypharmacy groups.

| Characteristics | Non-polypharmacy ($n = 65$) | Polypharmacy ($n = 185$) | *p*-value |
|---|---|---|---|
| Age | 73.32 ± 6.99 | 72.73 ± 6.92 | 0.557 |
| <70 years | 14 (21.5%) | 61 (33.0%) | 0.223 |
| 70–79 years | 38 (58.5%) | 92 (49.7%) | |
| ≥80 years | 13 (20.0%) | 32 (17.3%) | |
| Gender | | | 0.697 |
| Male | 38 (58.5%) | 103 (55.7%) | |
| Female | 27 (41.5%) | 82 (44.3%) | |
| Education | | | 0.424 |
| < 12 years of education | 55 (85.9%) | 164 (89.6%) | |
| ≥ 12 years of education | 9 (14.1%) | 19 (10.4%) | |
| ASA classification | | | <0.001* |
| II | 35 (53.8%) | 25 (13.5%) | |
| III | 29 (44.6%) | 145 (78.4%) | |
| IV | 1 (1.5%) | 15 (8.1%) | |
| **Comorbidities** | | | |
| Hypertension | 32 (49.2%) | 167 (90.3%) | <0.001* |
| Atrial fibrillation | 7 (10.8%) | 25 (13.5%) | 0.569 |
| Congestive heart failure | 2 (3.1%) | 25 (13.5%) | 0.020* |
| Ischemic heart disease/myocardial infarction | 9 (13.8%) | 112 (60.5%) | <0.001* |
| Valvular heart disease | 12 (18.5%) | 46 (24.9%) | 0.293 |
| Peripheral vascular disease | 0 (0.0%) | 6 (3.2%) | 0.344 |
| Dyslipidemia | 32 (49.2%) | 138 (74.6%) | <0.001* |
| Hyperthyroid | 1 (1.5%) | 2 (1.1%) | 1.000 |
| Hypothyroid | 0 (0.0%) | 6 (3.2%) | 0.344 |
| Diabetes mellitus | 8 (12.3%) | 83 (44.9%) | <0.001* |
| Asthma | 1 (1.5%) | 3 (1.6%) | 1.000 |
| COPD | 2 (3.1%) | 3 (1.6%) | 0.607 |
| CKD stage ≥3a | 24 (36.9%) | 82 (44.3%) | 0.299 |
| Malignancy | 26 (40.0%) | 40 (21.6%) | 0.004* |
| Cirrhosis | 1 (1.6%) | 5 (2.7%) | 0.598 |
| Alcohol use | 5 (7.7%) | 4 (2.2%) | 0.040* |
| Current smoker | 1 (1.5%) | 3 (1.6%) | 1.000 |
| **Medication group** | | | |
| Antiarrhythmic drug | 17 (26.2%) | 109 (58.9%) | <0.001* |
| Antidepressant drug | 1 (1.5%) | 10 (5.4%) | 0.297 |
| Antiemetic drug | 0 (0.0%) | 5 (2.7%) | 0.331 |
| Antihypertensive drug | 39 (60.0%) | 172 (93.0%) | <0.001* |
| Benzodiazepine | 3 (4.6%) | 41 (22.3%) | <0.001* |
| Diabetic drug | 4 (6.2%) | 67 (36.4%) | <0.001* |
| Diuretic | 10 (15.4%) | 58 (31.4%) | 0.015* |
| Site of surgery | | | <0.001* |
| Non-CVT | 41 (63.1%) | 68 (36.8%) | |
| CVT | 24 (36.9%) | 117 (63.2%) | |
| Presence of PIMs | 8 (12.3%) | 59 (31.9%) | 0.002* |

*Data presented as mean ± standard deviation or number and percentage.*
*A $p < 0.05$ indicates statistical significance.*
*ASA, American Society of Anesthesiologists; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; CVT, cardiovascular thoracic surgery; PIMs, potentially inappropriate medications.*
*$p < 0.05$.

have POCD. More preoperative polypharmacy was identified in the POCD group than in the non-POCD group; however, the difference between groups did not reach statistical significance (84.3 *vs.* 71.8%, respectively; $p = 0.080$).

Univariate analysis to identify factors that are potentially independently associated with POCD in older adult patients after major surgery is shown in **Table 5**. Those results show that all included variables were significantly associated with POCD, except polypharmacy and age. Univariate analysis revealed only a trend toward significant association between polypharmacy and POCD with a crude odds ratio (OR) of 2.11 (95% CI: 0.90–4.94, $p = 0.08$). The three strongest risk factors for POCD were use of an isoflurane inhaler, undergoing CVT surgery, and receiving blood product during operation with crude ORs of 3.68 (95% CI: 1.85–7.34, $p < 0.001$), 3.35 (95% CI: 1.60–7.01, $p < 0.001$), and 3.04 (95% CI: 1.39–6.63, $p = 0.005$), respectively. Interestingly, receiving desflurane was a protective factor for POCD with a crude OR of 0.17 (95% CI: 0.03–0.74, $p = 0.019$). Exploratory analysis was the carried out to identify independent predictors of POCD. Having all significant factors from univariate analysis in the multivariate model showed the effect of collinearity of the involved factors. The selection of factors to be included was based on the OR, the *p*-value, and the clinical meaningfulness of the factors according to the judgment of the authors. The final models for multivariate analysis are shown in **Table 6**. Since inhaler agents were found to be significantly associated with POCD in univariate analysis, the decision was made to have two models to separately explore the effect of this medication group. After adjusting for polypharmacy, perioperative benzodiazepine use remained an independent risk factor for POCD with an adjusted OR of 2.27 (95% CI: 1.10–4.68, $p = 0.026$); however, the protective effect of desflurane use was not sustained (adjusted OR [aOR]: 0.21, 95% CI: 0.47–0.95; $p = 0.066$). Model B revealed benzodiazepine and isoflurane to both be independently associated with POCD with aORs of 2.11 (95% CI: 1.00–4.43, $p = 0.048$) and 2.80 (95% CI: 1.35–5.81, $p = 0.006$), respectively. Additional analysis was performed to explore the effect of dosage of benzodiazepine between the POCD and non-POCD groups. That analysis revealed no significant difference between groups (**Figure 3**). **Table 7** shows significantly higher benzodiazepine and isoflurane use in the polypharmacy group than in the non-polypharmacy group (both $p < 0.05$).

## DISCUSSION

The prevalence of polypharmacy among older adult patients who underwent major surgery in this study was very high. This study also investigated for correlation between preoperative polypharmacy and POCD, which is a clinical setting for which data remain scarce. The results of this study showed only a trend toward significant association between polypharmacy and POCD ($p = 0.08$). Interestingly, we identified other medications that are commonly used intraoperatively to be both significantly and independently associated with POCD.

Polypharmacy is very common among older adults, which is a subpopulation that frequently has more chronic comorbidities

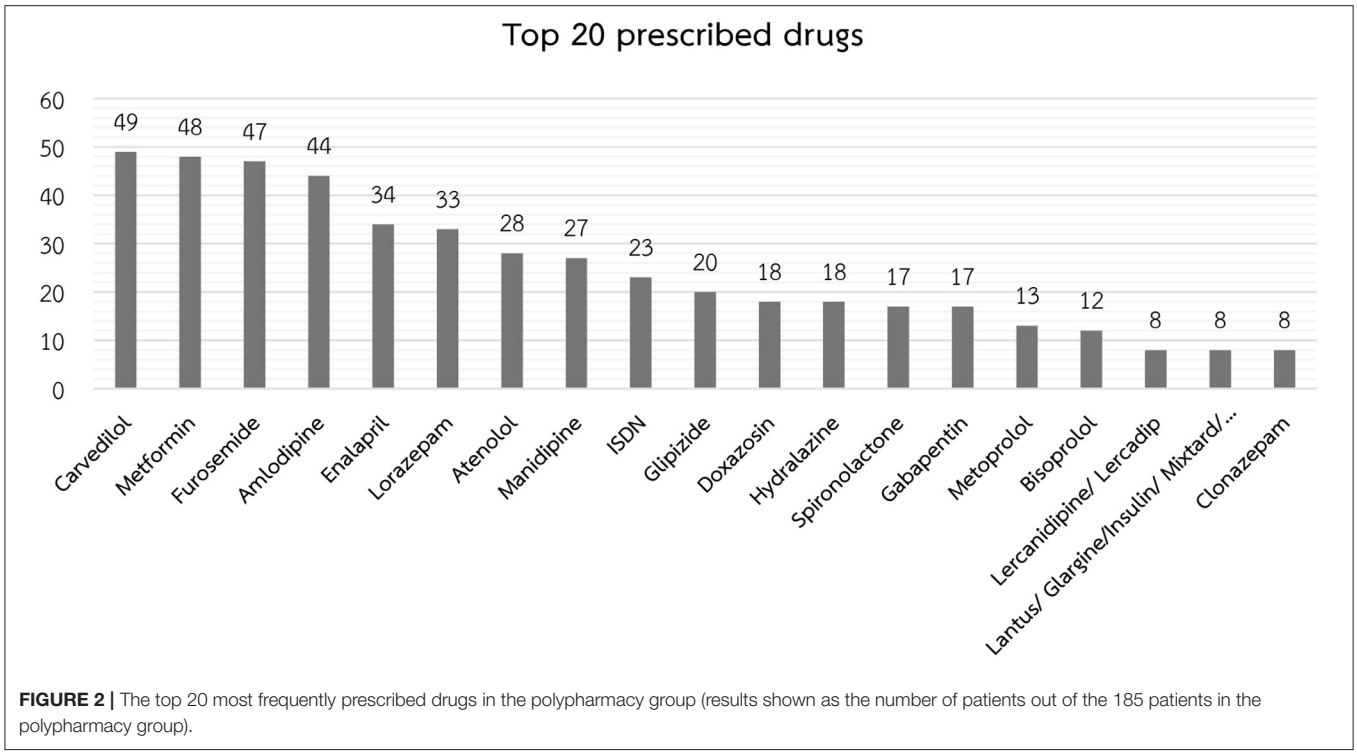


**FIGURE 2 |** The top 20 most frequently prescribed drugs in the polypharmacy group (results shown as the number of patients out of the 185 patients in the polypharmacy group).

that require a larger number of drugs to manage their illnesses (25, 26). However, the prevalence of polypharmacy is under-studied and under-reported in the surgical setting. Recent studies have reported a prevalence of polypharmacy of 40–55% (9, 10, 27, 28). The negative consequences of polypharmacy in a postopearative setting have been variously reported (9, 10, 27, 28). Some studies found polypharmacy to be associated with poor postoperative outcomes, such as postoperative complications, functional decline, hospitalization, and increased mortality (10, 27); however, other studies did not report those results (9, 28). A prospective multicenter observational study (28) in older adults undergoing elective surgery found polypharmacy to be unrelated to the development of POCD. The present study found a very high 74% prevalence of polypharmacy, which is substantially higher than the rates reported in previous studies. Even though we found only a trend ($p = 0.08$) toward significant association between polypharmacy and POCD among older adult patients who underwent elective major surgery, careful monitoring of older adults with polypharmacy should still be recommended in this setting.

POCD is a postoperative phenomenon that has been increasingly studied during the last decade. Although the exact pathology of POCD is unknown, it is thought to be caused by an inflammatory process in the brain. The systemic responses induced by anesthesia and surgery may trigger neuroinflammation and subsequent POCD. Many risk factors are thought to influence POCD onset, including increasing age, poor education, a history of cerebrovascular disease with no residual impairment, the duration and type of surgery, preexisting cognitive impairment, poor functional status, multiple comorbidities and severity of illness, and postoperative respiratory complications (29, 30). In line with the finding from other studies, older adults in the polypharmacy group have a greater percentage of comorbidities. However, consider from the chalrlson's comorbiditity index between the POCD and non-POCD group, it appears that the complexity of comorbid diseases were not influence the occurence of POCD in the present study.

Our multivariate analyses showed POCD to be independently associated with intraoperative benzodiazepine and isoflurane, which are anesthetic drugs, but not with other baseline risk factors. The association between anesthetics and cognitive impairment is of both interest and concern. Many medications used during anesthesia have systemic effects, with particular effects on cognitive abilities after surgery. However, the mechanism of anesthetics in POCD remains unknown, but several mechanisms have been proposed. Factors that may contribute to POCD include anesthetic approach, monitoring modality, and intraoperative complications. These factors influence modification of the tau protein, inflammation process, calcium dysregulation, and mitochondrial dysfunction, which have all been proposed to influence postoperative cognitive impairment (13).

Benzodiazepines are categorized as delirium-inducing medications (DIMs) (31, 32). This group of drugs is contraindicated in patients at high-risk for developing delirium (33). However, the studies in the relationship between benzodiazepine and POCD are few, and the results are conflicting. Li et al. (34) conducted a prospective randomized controlled trial of 164 older adult patients who underwent hip

**TABLE 3 |** Intraoperative data compared between the non-polypharmacy and polypharmacy groups.

| Data | Non-polypharmacy ($n = 65$) | Polypharmacy ($n = 185$) | *p*-value |
|---|---|---|---|
| Choice of anesthesia | | | <0.001* |
| GA and RA | 17 (26.1%) | 21 (11.4%) | |
| GA | 36 (55.4%) | 153 (82.7%) | |
| RA | 12 (18.5%) | 11 (5.9%) | |
| **Special monitoring** | | | |
| BIS | 2 (3.1%) | 5 (2.7%) | 1.000 |
| NIRS | 4 (6.2%) | 11 (5.9%) | 1.000 |
| Benzodiazepine use | 27 (41.5%) | 102 (55.1%) | 0.059 |
| Dexmedetomidine use | 9 (13.8%) | 19 (10.3%) | 0.440 |
| Intraoperative adverse events | 33 (51.6%) | 75 (42.6%) | 0.218 |
| **Induction agents** | | | |
| Thiopental | 2 (3.1%) | 3 (1.6%) | 0.607 |
| Propofol | 43 (66.2%) | 141 (76.2%) | 0.113 |
| Etomidate | 1 (1.5%) | 3 (1.6%) | 1.000 |
| Propofol TCI | 7 (10.8%) | 14 (7.6%) | 0.423 |
| Inhalation use | | | 0.004* |
| No | 19 (29.2%) | 25 (13.5%) | |
| Yes | 46 (70.8%) | 160 (86.5%) | |
| **Inhalation type** | | | |
| Desflurane | 13 (20.0%) | 29 (15.8%) | 0.422 |
| Sevoflurane | 25 (38.5%) | 66 (35.9%) | 0.688 |
| Isoflurane | 8 (12.3%) | 65 (35.1%) | <0.001* |
| **Muscle relaxant for intubation** | | | |
| Pancuronium | 0 (0.0%) | 0 (0.0%) | NA |
| Atracurium | 13 (20.0%) | 29 (15.7%) | 0.422 |
| Cis-atracurium | 25 (38.5%) | 63 (34.1%) | 0.522 |
| Succinylcholine | 2 (3.1%) | 1 (0.5%) | 0.167 |
| Rocuronium | 13 (20.0%) | 78 (42.2%) | <0.001* |
| **Analgesia used** | | | |
| Morphine | 18 (27.7%) | 102 (55.1%) | <0.001* |
| Pethidine | 1 (1.5%) | 2 (1.1%) | 1.000 |
| Fentanyl | 59 (90.8%) | 179 (96.8%) | 0.084 |
| Ketamine | 0 (0.0%) | 3 (1.6%) | 0.570 |
| COX-2 inhibitor | 1 (1.5%) | 2 (1.1%) | 1.000 |
| Reversal agents used[a] | 30 (46.2%) | 48 (26.4%) | 0.003* |
| Blood product used | 30 (46.2%) | 121 (66.9%) | 0.003* |
| **Other complications** | | | |
| Hypertension | 1 (1.6%) | 4 (2.2%) | 1.000 |
| Hypotension | 29 (44.6%) | 67 (37.0%) | 0.302 |
| Hypotension[b] | 3 (4.7%) | 4 (2.2%) | 0.382 |
| Severe arrhythmia | 9 (13.8%) | 29 (16.0%) | 0.677 |
| Anesthetic time (min) | 289 (40, 610) | 282 (53, 775) | 0.715 |

*Data presented as number and percentage or median and range (minimum, maximum).*
*A $p < 0.05$ indicates statistical significance.*
[a]*Reversal of muscle relaxant at the end of surgery.*
[b]*Hypotension requiring continuous intravenous inotropic/vasopressor support.*
*GA, general anesthesia; RA, regional anesthesia; BIS, bispectral index; NIRS, near-infrared spectroscopy monitoring; Propofol TCI, target-controlled infusion of propofol; COX, cyclooxygenase.*
*$p < 0.05$.

**TABLE 4 |** Variables potentially associated with postoperative cognitive dysfunction (POCD) compared between the non-POCD and POCD groups.

| Variables | Non-POCD ($n = 124$) | POCD ($n = 51$) | *p*-value |
|---|---|---|---|
| **Preoperative data** | | | |
| Polypharmacy | 89 (71.8%) | 43 (84.3%) | 0.080 |
| PIMs | 35 (22.7%) | 14 (27.5%) | 0.917 |
| Age (years) | 73.26 ± 6.46 | 71.10 ± 7.24 | 0.074 |
| Male gender | 69 (55.6%) | 34 (66.7%) | 0.178 |
| ASA Status | | | 0.061 |
| II | 34 (27.4%) | 5 (9.8%) | |
| III | 85 (68.5%) | 41 (80.4%) | |
| IV | 5 (4.0%) | 5 (9.8%) | |
| **Comorbidity** | | | |
| CCI | 5.78 ± 1.74 | 5.76 ± 2.04 | 0.954 |
| CCI < 6 | 61 (49.2) | 26 (51.0) | |
| CCI ≥ 6 | 63 (50.8) | 25 (49.0) | |
| Congestive heart failure | 10 (8.1%) | 8 (15.7%) | 0.131 |
| Ischemic heart disease/myocardial infarction | 51 (41.1%) | 31 (60.8%) | 0.018* |
| Valvular heart disease | 25 (20.2%) | 17 (33.3%) | 0.064 |
| **Preoperative medication** | | | |
| Antiemetic drug | 2 (1.6%) | 3 (5.9%) | 0.151 |
| Antihypertensive drug | 99 (79.8%) | 47 (92.2%) | 0.046* |
| Pre-benzodiazepine | 23 (18.5%) | 10 (19.6%) | 0.889 |
| Site of surgery | | | <0.001* |
| Non-CVT | 63 (50.8%) | 12 (23.5%) | |
| CVT | 61 (49.2%) | 39 (76.5%) | |
| **Intraoperative data** | | | |
| Choice of anesthesia | | | 0.022* |
| GA and RA | 22 (17.7%) | 5 (9.8%) | |
| GA | 86 (69.4%) | 45 (88.2%) | |
| RA | 16 (12.9%) | 1 (2.0%) | |
| Benzodiazepine | 56 (45.2%) | 36 (70.6%) | 0.002* |
| Inhalation use | | | 0.442 |
| No | 23 (18.5%) | 7 (13.7%) | |
| Yes | 101 (81.5%) | 44 (86.3%) | |
| **Inhalation type** | | | |
| Desflurane | 24 (19.4%) | 2 (3.9%) | 0.009* |
| Isoflurane | 29 (23.4%) | 27 (52.9%) | <0.001* |
| **Muscle relaxant for intubation** | | | |
| Rocuronium | 40 (32.3%) | 27 (52.9%) | 0.011* |
| Reversal agents used | 41 (33.6%) | 9 (17.6%) | 0.035* |
| Received blood product | 70 (57.4%) | 41 (80.4%) | 0.004* |
| **Postoperative data** | | | |
| Post-benzodiazepine | 48 (39.6%) | 26 (50.9%) | 0.178 |

*Data presented as number and percentage or mean ± standard deviation.*
*A $p < 0.05$ indicates statistical significance.*
*POCD, postoperative cognitive dysfunction; PIMs, potentially inappropriate medications; ASA, American Society of Anesthesiologists; CVT, cardiovascular thoracic; GA, general anesthesia; RA, regional anesthesia; Pre-benzodiazepine, The patient was given benzodiazepine before the operation; Post-benzodiazepine, The patient was given benzodiazepine after the operation; CCI, charlson comorbidity index.*
*$p < 0.05$.

**TABLE 5 |** Univariate analysis to identify factors that are potentially independently associated with postoperative cognitive dysfunction (POCD) in older adult patients after major surgery.

| Factors | Non-POCD ($n = 124$) | POCD ($n = 51$) | Univariate analysis | |
|---|---|---|---|---|
| | | | Crude OR (95% CI) | *p* |
| Polypharmacy | 89 (71.8%) | 43 (84.3%) | 2.11 (0.90–4.94) | 0.084 |
| Age | 73.26 ± 6.46 | 71.10 ± 7.24 | 0.95 (0.90–1.00) | 0.056 |
| IHD/MI | 51 (41.1%) | 31 (60.8%) | 2.21 (1.14–4.32) | 0.019* |
| **Site of surgery** | | | | |
| Non-CVT | 63 (50.8%) | 12 (23.5%) | Reference | |
| CVT | 61 (49.2%) | 39 (76.5%) | 3.35 (1.60–7.01) | <0.001* |
| Benzodiazepine | 56 (45.2%) | 36 (70.6%) | 2.91 (1.44–5.86) | 0.003* |
| Desflurane | 24 (19.4%) | 2 (3.9%) | 0.17 (0.03–0.74) | 0.019* |
| Isoflurane | 29 (23.4%) | 27 (52.9%) | 3.68 (1.85–7.34) | <0.001* |
| Rocuronium | 40 (32.3%) | 27 (52.9%) | 2.36 (1.21–4.60) | 0.011* |
| Reversal agents used | 41 (33.6%) | 9 (17.6%) | 0.42 (0.18–0.95) | 0.038* |
| Received blood product | 70 (57.4%) | 41 (80.4%) | 3.04 (1.39–6.63) | 0.005* |

*Factors with a $p < 0.10$ in univariate analysis will be entered into multivariate analyses. POCD, postoperative cognitive dysfunction; OR, odds ratio; CI, confidence interval; IHD/MI, ischemic heart disease/myocardial infarction; CVT, cardiovascular thoracic surgery.*
*$p < 0.05$.*

**TABLE 6 |** Multivariate analyses (models A and B) to identify independent predictors of postoperative cognitive dysfunction (POCD) in older adult patients after major surgery.

| Predictors | Model A (Included desflurane) | | Model B (Included isoflurane) | |
|---|---|---|---|---|
| | Adjusted OR (95% CI) | *p*-value | Adjusted OR (95% CI) | *p*-value |
| Polypharmacy | 1.91 (0.92–4.62) | 0.149 | 1.44 (0.58–3.55) | 0.426 |
| Benzodiazepine | 2.27 (1.10–4.68) | 0.026* | 2.11 (1.00–4.43) | 0.048* |
| Desflurane | 0.21 (0.47–0.95) | 0.066 | – | – |
| Isoflurane | – | – | 2.80 (1.35–5.81) | 0.006* |

*A $p < 0.05$ indicates statistical significance.*
*Multivariate backward stepwise logistic regression was analyzed separately for multicollinearity variables.*
*POCD, postoperative cognitive dysfunction; OR, odds ratio; CI, confidence interval.*
*$p < 0.05$.*

or knee replacement under spinal-epidural anesthesia (CSE) to assess the effects of dexmedetomidine, propofol, or midazolam sedation on POCD. They found that the group given midazolam had the highest prevalence of POCD. However, another study that was conducted by Mansouri et al. (35) in 150 candidates aged over 65 years who underwent cataract surgery under general anesthesia found that patients who received midazolam had a lower incidence of POCD than those who received a placebo. A systematic review by Kok et al. (36) investigated the function of benzodiazepine as a sedative agent during ICU admission. Their results revealed benzodiazepine to be a significant risk factor for cognitive impairment during and after admission. Several pathogeneses via the GABAergic neurotransmitter system have been proposed. The independent associations identified in the present study add to the existing body of evidence regarding the short-term detrimental effect of benzodiazepine in older adult patients.

Isoflurane, sevoflurane, and desflurane are inhalation anesthetics that are essential agents for maintaining general anesthesia for longer periods. However, they can effectuate neuroinflammation, which can adversely affect the cognitive function of older adults. Nevertheless, limited studies have been conducted in human that addressed the association between specific inhalation agent and the occurrence of POCD (13). Several studies in animal models demonstrated derangement in neurotransmitters and cytokines during isoflurane administration. Acharya et al. reported that isoflurane increased blood-brain barrier (BBB) permeability by destroying brain vascular endothelial cells in aging mice, which resulted in the secretion of various cytokines and proinflammatory mediators into the brain. This combined release caused abnormal brain function, and contributed to postoperative cognitive dysfunction (37). According to the findings of Cao et al., isoflurane induces inflammatory processes by secreting a significant quantity of proinflammatory cytokines, including tumor necrosis factor (TNF), interleukin 6 (IL-6), and interleukin 1 beta (IL-1β), which resulted in the deposition of a large amount of hypoxia-inducible factor 1-alpha (HIF-1α) protein at the hippocampus. They reported these findings to be associated with cognitive impairment (38). In the laboratory, Xie et al. (39) investigated H4 human neuroglioma cells that had been exposed to isoflurane. Their results showed increased amounts of β-amyloid protein and amyloid precursor protein (APP), which are both known to be associated with Alzheimer's disease (AD). Another study in animals that was conducted by Liu et al. (40) found that isoflurane enhanced the development of Alzheimer's disease (AD) (specific to spatial memory impairment) by increasing amyloid-beta (A-beta) levels and tau phosphorylation in the hippocampus of older rats. The accumulating evidence from animal studies suggests that isoflurane exposure in older adult population may be, in part, responsible for pathogenesis of POCD observed in the present study.

Desflurane is a volatile anesthetic drug with a low blood-gas partition coefficient that provides faster recovery following general anesthesia, which is thought to protect against cognitive

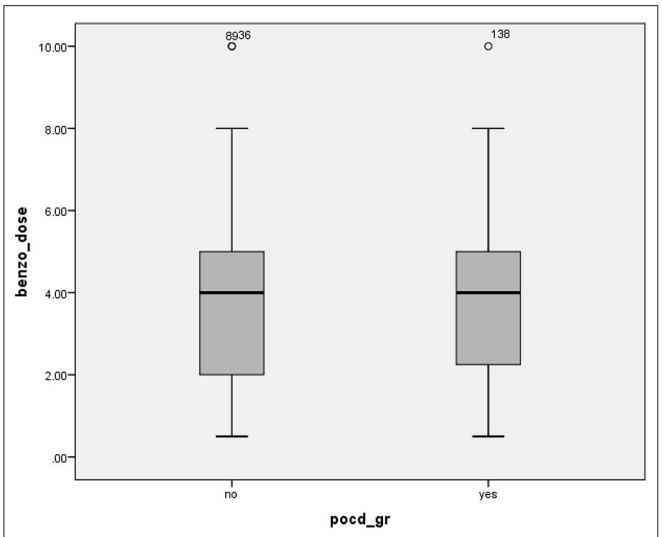


**FIGURE 3 |** Median (IQR) dosage of intraoperative benzodiazepine compared between the non-POCD and POCD groups ($p > 0.05$). IQR, interquartile range; POCD, postoperative cognitive disorder.

**TABLE 7 |** Prevalence of the independent factors that predict postoperative cognitive dysfunction (POCD) in older adult patients after major surgery compared between the non-polypharmacy and polypharmacy groups.

| Factors | Non-polypharmacy ($n = 8$) | Polypharmacy ($n = 43$) | *p*-value |
|---|---|---|---|
| Intraoperative benzodiazepine | 3 (37.5%) | 33 (76.7%) | 0.039* |
| Isoflurane | 1 (12.5%) | 26 (60.5%) | 0.019* |

*A $p < 0.05$ indicates statistical significance.*
*$p < 0.05$.

decline after anesthesia. The present study demonstrated a significant association effect of desflurane to reduce risk of POCD in univariate analysis; however, that significant effect was not sustained in multivariate analysis. It was reported that desflurane enhanced neurologic outcomes in patients who underwent cardiac bypass surgery, and in patients with hypoxic neuronal impairment (41). However, another study that investigated the effect of two volatile anesthetic drugs (desflurane and sevoflurane) on POCD reported no significant difference in the incidence of POCD between the two drugs (42). Given the mixed results from current and previous studies, further studies are needed to explore the effect of desflurane on POCD in older adult patients requiring anesthesia.

## Strengths and Limitations

This study has several mentionable strengths and limitations. Regarding strengths, medication reconciliation is a routine practice in our center, so our determination of the medication used could be considered reliable. Second, this study was a prospective study designed to investigate for POCD, potential confounding factors and outcome were preplanned and therefore less likely to underestimate or misclassification. Concerning limitations, the first weakness of this study is that our data were derived from a single center, the generalizability might be limited and should preferable be further explored in other setting. Second, MoCA retesting was only performed in 175 patients out of 250 subjects initially included. This might lead to bias estimation of prevalence of POCD. However, we have compared baseline characteristics of those for whom MoCA was not repeated and discovered no statistical difference with the included group. We, therefore, hypothesize that the missing data would have minimal effect on the prevalence of POCD identified in the study. However, although the sample size was sufficient to investigate the prevalence of polypharmacy, it might not be adequate for exploring the associated with POCD. Should the observed odd ratios of two from the association between polypharmacy and POCD be considered as clinically significant, it would require sample size around 400, estimated from the prevalence of polypharmacy. This study could have been underpowered to explore the association for polypharmacy and POCD. Furthermore, based on the finding of this study and a review of previous findings, it emerges that the effects of isoflurane and desflurane on POCD in humans are fascinating and could lead to changes in anesthetic practice. Further study with a larger sample size to explore the associations between those anesthetic agents, polypharmacy, and POCD is warranted.

# CONCLUSIONS

The prevalence of polypharmacy in this study was a very high 74%. There was a non-significant trend toward ($p = 0.08$) increased risk of POCD in the polypharmacy group. Intraoperative benzodiazepine and isoflurane were identified as independent predictors of POCD, whereas desflurane was found to be an independent protective factor against POCD. Since POCD can significantly adversely impact older adult patients and their families, strategies to manage these modifiable factors are needed to improve patient outcomes.

# DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

# ETHICS STATEMENT

The protocol for this study was approved by the Institutional Review Board of the Human Research Protection Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA No. Si 189/2019). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the

individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

SL, WL, NM, WW, AS, and VS contributed to the study's conception and design. SL, AS, PS, and VS were responsible for data collection and analysis. SL, WL, AS, and VS interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

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REVIEW
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# Inflammation and Cardiovascular Diseases in the Elderly: The Role of Epicardial Adipose Tissue

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Human aging is a complex phenomenon characterized by a wide spectrum of biological changes which impact on behavioral and social aspects. Age-related changes are accompanied by a decline in biological function and increased vulnerability leading to frailty, thereby advanced age is identified among the major risk factors of the main chronic human diseases. Aging is characterized by a state of chronic low-grade inflammation, also referred as inflammaging. It recognizes a multifactorial pathogenesis with a prominent role of the innate immune system activation, resulting in tissue degeneration and contributing to adverse outcomes. It is widely recognized that inflammation plays a central role in the development and progression of numerous chronic and cardiovascular diseases. In particular, low-grade inflammation, through an increased risk of atherosclerosis and insulin resistance, promote cardiovascular diseases in the elderly. Low-grade inflammation is also promoted by visceral adiposity, whose accumulation is paralleled by an increased inflammatory status. Aging is associated to increase in epicardial adipose tissue (EAT), the visceral fat depot of the heart. Structural and functional changes in EAT have been shown to be associated with several heart diseases, including coronary artery disease, aortic stenosis, atrial fibrillation, and heart failure. EAT increase is associated with a greater production and secretion of pro-inflammatory mediators and neuro-hormones, so that thickened EAT can pathologically influence, in a paracrine and vasocrine manner, the structure and function of the heart and is associated to a worse cardiovascular outcome. In this review, we will discuss the evidence underlying the interplay between inflammaging, EAT accumulation and cardiovascular diseases. We will examine and discuss the importance of EAT quantification, its characteristics and changes with age and its clinical implication.

**Keywords: elderly, inflammation, epicardial adipose tissue, cardiovascular diseases, coronary artery disease, aortic stenosis, atrial fibrillation, heart failure**

## INTRODUCTION

Life expectancy has improved and, therefore, the proportion of older individuals in the general population is increased. These demographic changes led to a considerable increase in the prevalence of chronic diseases, such as cardiovascular diseases, a major public health problem (1, 2).

Aging is characterized by a chronic low-grade proinflammatory state that results in a progressively greater susceptibility to multimorbidity, disability, and death. This pro-inflammatory status, a condition often named inflammaging, is promoted by sustained high levels of pro-inflammatory markers (3–5).

Inflammaging represents a risk factor for cardiovascular diseases and is associated to adverse outcomes in the elderly (6, 7). It has been recognized a potential role of visceral adipose tissue (VAT) in inflammaging. The accumulation of visceral fat depots is accompanied by an increased production and secretion of inflammatory mediators. Accumulation of abdominal VAT is closely associated with increased prevalence of insulin resistance and metabolic syndrome, and it is related to an increased risk of worse cardiovascular outcomes (8).

Accumulating evidence strongly support the role of structural and functional changes of epicardial adipose tissue (EAT), the visceral fat depot of the heart, in the pathogenesis of various cardiovascular diseases. Interestingly, a greater EAT amount is observed in the elderly. EAT is a metabolically active tissue that in pathological conditions assumes a pro-inflammatory phenotype. EAT-derived pro-inflammatory mediators and neuro-hormones act on the myocardium and coronary vessels in a paracrine and vasocrine manner, thus contributing to development and progression of cardiovascular diseases (9).

Different imaging methods, such as echocardiography, computed tomography (CT) and cardiac magnetic resonance (CMR), have been used for the quantification of EAT in clinical research studies. Given the association between EAT with the onset and progression of cardiovascular disease and its impact on cardiovascular outcome, EAT measurement has been proposed as useful marker for assessment of cardiovascular and metabolic risk (10). However, there is still a lack of consensus on how to measure EAT in clinical practice.

In this review, we will discuss the evidence underlying the interplay between inflammaging, EAT accumulation and cardiovascular diseases. We will examine and discuss the characteristics and changes of EAT occurring with age and its clinical implication (**Figure 1**). Finally, we will describe the different available image methods to measure EAT amount and we will emphasize the importance of EAT quantification in assessing cardiovascular and metabolic risk.

## AGING AND INFLAMMATION

Chronic inflammation is a typical feature of aging. Therefore, the term inflammaging has been introduced to describe the

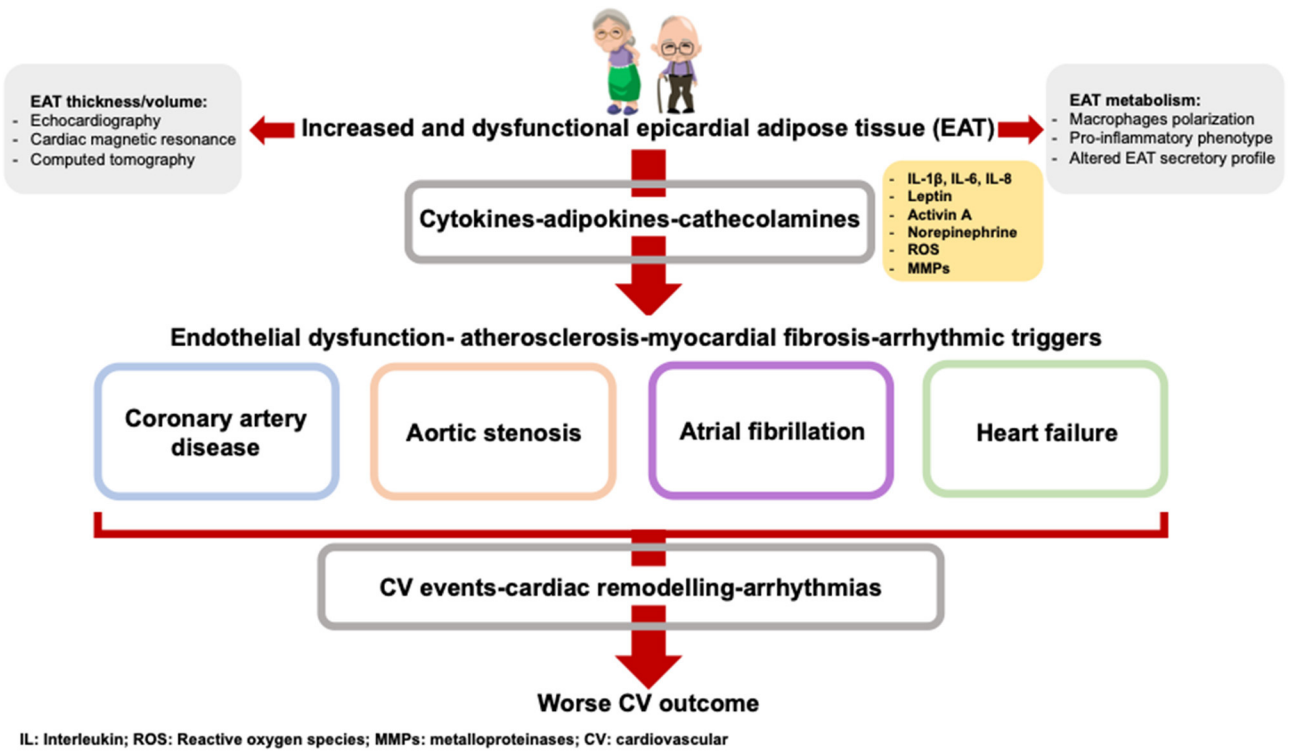


**FIGURE 1 |** The age-related EAT accumulation is paralleled by an EAT altered metabolism. The adipokines produced by EAT contribute to the onset and the worse prognosis of cardiovascular diseases.

low-grade, chronic, systemic, and sterile inflammation that occurs without overt infection, and results in progressive tissue damage and degeneration. This proinflammatory state is characterized by high circulating levels of proinflammatory mediators, including interleukin (IL)-1, IL-6, IL-8, IL-18, C-reactive protein (CRP), interferon (IFN)-α and IFN-β, growth factor transformant-β (TGF-β), tumor necrosis factor (TNF) and serum amyloid A, even in the absence of risk factors and clinically active diseases (11).

Inflammation, which represents an important defense mechanism against infections or foreign molecules, becomes particularly harmful when it is sustained and prolonged. Thus, inflammaging is recognized as a determinant of adverse health outcomes, leading to a higher risk of morbidity and mortality in older people.

The etiology of inflammaging is very complex and heterogeneous, including genetic factors, cellular senescence, immunosenescence, age-related changes in coagulation and gut microbiota (12).

Genetic polymorphisms can promote a pro-inflammatory state with aging, leading to the upregulation of immune and inflammatory pathways and influencing the circulating levels of various inflammatory markers, such as IL-6 (13).

Several evidence suggest that age-related changes in microRNAs (miRNAs) could contribute to inflammaging. miRNAs are non-coding, single-stranded RNAs, that play important roles in regulating gene expression. Significant differences have been described in the specific miRNAs levels in elderly circulating cells, plasma and whole blood compared to younger subjects, with over-representation of miR-21–5p and miR-126–3p and under-representation of miR-25–3p, miR-92a-3p, miR-93–5p, miR-101–3p, miR-106b-5p, miR-142–5p, miR-151a-3p, and miR-181a-5p. Age-related changes in miRNAs can affect cellular senescence and modulate immune responses, thus promoting the low-grade inflammation state (12, 14, 15).

Among the identified major sources of inflammation in the elderly, there is the imbalance between production and elimination of host-derived endogenous cell debris. The accumulation of biological debris with age support chronic inflammation, by miming bacterial products and activating innate immunity. In these conditions, cellular and organelle components, free radicals and metabolites are recognized as harmful by a network of sensors, including the NOD-, LRR- and pyrin domain-containing protein 3 (Nlrp3) inflammasome, and activate the immune reactions for physiological repair. However, over time, such responses can become chronic and therefore maladaptive (16).

The multiprotein complex of Nlrp3 inflammasome acts in response to cellular danger through the activation of procaspase-1, with consequent production and secretion of proinflammatory cytokines IL-1β and IL-18. Among the activators of the Nlrp3 inflammasome, there are both pathogen-associated molecular patterns and endogenous host-derived molecules indicative of cellular damage, that act mainly through the production of mitochondrial reactive oxygen species (ROS). Furthermore, the mitochondrial cardiolipin, in the case of mitochondrial dysfunction, can act as endogenous pathogen-associated molecular pattern and as binding site for Nlrp3, capable of activating the Nlrp3 inflammasome proinflammatory pathway (17).

Cellular senescence, the normal cellular response to damage and stress, is another important determinant of inflammaging. On the one hand, it suppresses the proliferation of genotypically damaged cells, preventing cancer, and contributes to the wound healing process. On the other hand, senescent cells accumulate with age in many tissues and through their secretory profile (secretory phenotype associated with senescence or SASP), they promote age-related disease by altering the structure and function of different organs. SASP consists in a wide range of soluble molecules, including IL-1α, IL-1β, and IL-6, chemokines (IL-8 and growth-regulated-α protein), growth factors (fibroblast growth factor 2 and hepatocyte growth factor), metalloproteinases (MMP1, MMP3, and MMP13), and other insoluble proteins and extracellular matrix components. These secretory mediators act in a paracrine manner, influencing neighboring cells, but can be also released in systemic circulation, thereby strongly contributing to inflammaging. Interestingly, a marked accumulation of senescent cells has been described in the visceral fat of obese individuals, that is an important source of inflammatory cytokines (18, 19).

The VAT, usually defined as abdominal accumulation of adiposity, mainly localized at the omental and mesenteric level, is composed not only of adipocytes, but also of other cells, such as the stromal vascular fraction of fibroblasts, endothelial cells, macrophages and preadipocytes (20).

Accumulation of abdominal VAT is closely associated with increased prevalence of insulin resistance and metabolic syndrome, and it is related to an increased risk of cardiovascular outcomes (8, 21).

The persistent positive caloric balance, as it occurs in obesity, induces an enlargement and consequent metabolic and immune dysfunction of the adipocytes. The increase in visceral fat correlates to a proatherogenic alteration of the lipid profile, with a reduction of high-density lipoproteins and an increase in small low-density lipoprotein particles (LDLs) (22). Pathogenetic mechanisms underlying the complications related to visceral obesity include the activation of lipolysis and production of free fatty acids (FFAs), increased oxidative stress, adipocyte hypoxia and apoptosis (23). Furthermore, the monocyte infiltrate turns toward an inflammatory sense with generation of M1 macrophages, that lead to increased secretion of proinflammatory cytokines, which contribute to a chronic inflammatory state (24).

The age-related changes of the immune system, also named immunosenescence, also contributes to inflammaging. With aging, in fact, an immune dysregulation with a reduction in adaptive immunity and a hyperactivity of innate immunity has been described. In the elderly, CD4 + lymphocytes show greater intrinsic activation of the nuclear factor-κB (NF-κB) pathways than in younger individuals. These age-related changes are promoted by genetic factors, by intrinsic cellular changes of the immune system and by permanent exposure to antigens and pathogens, and can be accelerated and aggravated by persistent

infections, sustained by some viruses, such as Epstein–Barr virus and Cytomegalovirus (25).

Another source of inflammation seems to be linked to age-related changes in the gut microbiota, capable of inducing an inflammatory response through the production of harmful products and metabolites. This process seems to be favored by the age-related reduced intestinal ability to sequester pathogenic microbes and their products. Age-related gut microbiota changes consist, on the one hand, in a decrease in beneficial commensal microorganisms, such as Coprococcus, Faecalibacterium, and Lactobacillus, which counteract the development of microbial pathogens and maintain the integrity of the intestinal barrier; on the other hand an increase in anaerobic pathogenic bacteria—such as Fusobacterium and Staphylococcus—occur, associated with an increase in the mucosal barrier permeability, thereby allowing bacteria and their products into the circulatory system. These factors contribute to increased plasma levels of inflammatory cytokines, thus sustaining the chronic pro-inflammatory state (26).

Overall, the possible sources of inflammation are various and very heterogeneous. Probably, these different mechanisms are interconnected and act synergistically with different relevance and combinations in selected individuals.

## INFLAMMATION AND CARDIOVASCULAR DISEASES

The role of inflammation in the pathophysiology of several cardiovascular diseases is widely recognized and its involvement in the pathogenesis and progression of atherosclerotic processes has already been hypothesized many years ago, based on autopsy studies which showed the presence of abundant inflammatory infiltrates in the adventitia of the coronary arteries of patients who died for acute coronary syndrome (27).

The close association between inflammation, immune response and cardiovascular diseases has been then supported by several experimental data, that identified immune cells and inflammatory mediators involved in atherogenesis. The binding and penetration of lymphocytes into the vascular endothelium, the proliferation and migration of smooth muscle cells toward the intima of the vessel wall, the transendothelial recruitment of macrophages have been described as key events in the formation of atherosclerotic plaque (28).

Subendothelial accumulation of LDLs, the increased generation of ROS, and the consequent LDLs oxidation by ROS are associated with endothelial injury and dysfunction, thereby promoting the onset and progression of atherosclerotic process. Oxidized LDL induce endothelial cell expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), that bind monocytes and T lymphocytes, allowing them to penetrate the intima. In addition, chemoattractant mediators such as complement factors and monocyte chemoattractant protein-1 (MCP-1), are responsible for recruitment into the vascular wall of mononuclear cells, that differentiate into macrophages, ingest oxidized LDLs, and become foam cells. Interestingly, activated T cells and macrophages can release a variety of proinflammatory and fibrogenic mediators, such as IL-1, IL-3, IL-8, IL- 18, and TNF-α, able to attract circulating inflammatory cells and trigger and perpetuate the local inflammatory response. Inflammation is involved not only in the onset and progression of atherosclerotic process, but also in the complication of plaque rupture, probably due to the ability of activated macrophages and T cells to release hydrolytic enzymes, chemokines, cytokines, and growth factors. These factors lead to focal necrosis of the fibrous cap, which becomes highly susceptible to rupture, whereas the macrophage production of procoagulant mediators, such as tissue factor, triggers plaques thrombosis (29, 30).

Furthermore, endogenous products from damaged cells, such as cholesterol crystals, may trigger and amplify inflammatory response, through the activation of the Nlrp3 inflammasome, a complex of proteins involved in the proteolytic cleavage, maturation and secretion of IL-1 beta (31). IL-1 beta promotes smooth muscle cell proliferation, the recruitment of inflammatory cells and the increased production of other cytokines, such as IL-6, thus playing a crucial role in the inflammatory cascade (32).

Numerous inflammatory mediators have been extensively studied in the physiopathological processes of atherogenesis and plaque vulnerability. Elevated IL-6 levels have been associated with an increased risk of future myocardial infarction in apparently healthy men, thus supporting the role of inflammation in the early stages of atherogenesis. Furthermore, clinical studies have demonstrated the prognostic role of CRP and serum amyloid A levels in patients with coronary artery disease (CAD). The increase in these sensitive inflammation markers predicted an unfavorable outcome in patients with unstable angina (33–35).

Moreover, the large randomized controlled Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) emphasized the role of IL-1b in cardiovascular risk and outcome, demonstrating that its inhibition with canakinumab significantly reduces the recurrence of new cardiovascular events in subjects with stable CAD and persistent increase in CRP (36). The use of Canakinumab has been also associated with a reduction in IL-6, CRP and fibrinogen circulating levels in patients with high vascular risk (37).

Inflammation plays a crucial role, not only in the pathophysiology of CAD, but also in the pathogenesis of aortic stenosis (AS). Interestingly, age, smoking, hypertension, hypercholesterolemia, obesity and diabetes, which are traditional risk factors for atherosclerosis, are also associated with development and progression of AS. Indeed, AS is a progressive and active process that shares many physio-pathological aspects with vascular atherosclerosis, such as endothelial dysfunction, lipid infiltration, oxidative stress, and activation and penetration of inflammatory cells into the endothelium of the aortic valve leaflets.

Secretion of the proinflammatory cytokines, IL-1β, TNF-a, IL-6, IL-8, insulin-like growth factor 1, TGF-β, promotes osteogenic differentiation of resident interstitial valve cells, leading to bone deposition and calcification (38).

More and more evidences are also accumulating on the role of inflammation in the pathogenesis of atrial fibrillation (AF). Histological analysis of the atria of AF patients revealed infiltration of lymphomononuclear cells and necrosis of adjacent myocytes (39).

Several inflammatory pathways may contribute to structural remodeling of the atria by modulating calcium homeostasis, cardiomyocyte apoptosis, and fibrosis. Furthermore, AF itself can induce inflammation that further improves atrial remodeling, activating a vicious cycle that perpetuates and increases the severity of the arrhythmia (40). Indeed, higher levels of inflammatory markers, such as IL-6, IL-8, and TNF, have been described in AF patients compared with subjects in sinus rhythm (41). The association between elevated CRP levels and the presence of AF has also been reported; furthermore, an increase in baseline CRP levels seems to predict the development of new onset AF. Overall, these data confirm the central role of persistent inflammatory state in the development of AF (42, 43).

Many lines of evidence demonstrate the involvement of inflammation also in development and progression of heart failure (HF). Elevated circulating levels of pro-inflammatory cytokines in HF patients compared with healthy individuals, have been reported, thereby revealing a potentially important role for the innate and adaptive immune system in the pathogenesis of the disease (44, 45).

Immune systems activation in HF comprises non-cellular and cellular components, including macrophages, mast cells, B cells, and T cells.

The hyperactivity of the sympathetic nervous system (SNS) and of the renin-angiotensin-aldosterone system (RAAS), that occur in HF, promote the activation of inflammatory cells, such as macrophages, T cells and B cells, as well as the loss of cardiomyocytes. In turn, myocardial damage acts as a trigger to support and amplify the inflammatory response in the heart (46).

The key role of inflammation in the pathogenesis of HF is also supported by histological studies of specimens of heart from HF patients, that revealed infiltrates of activated T lymphocytes, monocyte, macrophages and natural killer cells (47).

HF with preserved ejection fraction (HFpEF) also recognizes an inflammatory pathogenesis. HFpEF is a very challenging syndrome, very common in elderly patients, especially in the presence of multiple comorbidities, such as chronic kidney disease, chronic obstructive pulmonary disease, sleep disordered breathing, diabetes mellitus, sarcopenia, that can significantly contribute to a systemic proinflammatory state. All these chronic diseases are associated with high plasma levels of cytokines and inflammatory mediators, such as IL-6, TNF-a, soluble ST2 (sST2) and pentraxin, which promote endothelial dysfunction and the expression of VCAM and E-selectin, which in turn result in the migration and activation of monocytes in the subendothelium and the release of transforming growth factor (48). The conversion of fibroblasts into myofibroblasts determines the deposition of collagen in the interstitial space and the remodeling of the extracellular matrix. Furthermore, oxidative stress and the production of ROS result in reduced bioavailability of nitric oxide in cardiomyocytes with consequent lower activity of soluble guanylate cyclase (sGC) and consequently lower concentration of cyclic guanosine monophosphate and reduced activity of protein kinase G. The result is the induction of cardiomyocyte hypertrophy. Both hypertrophic cardiomyocytes and interstitial fibrosis, driven by inflammation, contribute to high diastolic stiffness of the left ventricle (LV) and to onset of HF (48).

## EPICARDIAL ADIPOSE TISSUE IN ELDERLY

Accumulating evidence strongly support the role of structural and functional changes of EAT in the pathogenesis of various cardiovascular diseases.

EAT is the visceral fat depot of the heart. It covers 80% of the heart's surface, thereby contributing for the 20% to the total heart weight. It is located between the visceral pericardium and the myocardium, lodges in the atrioventricular and interventricular furrows and along the major branches of the coronary arteries. It is a distinct tissue from the pericardial fat, while shares embryological origins and morphological aspects with visceral fat, both originating from mesodermal cells. EAT is mainly composed of small adipocytes, but also stromo-vascular and immune cells, and histological analysis also demonstrated the presence of ganglia and intercommunicating nerves, and variable degrees of leukocyte accumulation, with T lymphocytes, macrophages and mast cells (49).

Human EAT shows the molecular signature of a beige fat depot in middle-aged patients. These special features might portray stages in the trans-differentiation of the EAT from brown adipose tissue to white adipose tissue (50).

The anatomical closeness to the myocardium is one of the most interesting features of EAT; the two tissues are anatomically and functionally contiguous, without dividing boundaries, and share the same microcirculation. These aspects underlie the strong interaction between EAT and myocardium.

EAT is a metabolically very active tissue and performs numerous functions, through mechanical, vasocrine and paracrine actions. Epicardial fat, by surrounding coronary arteries, provides mechanical support and restricts their movement, thus protecting them from the tensions and torsion induced by the arterial pulse wave and cardiac contraction (51).

In physiologic conditions, EAT acts like brown adipose tissue, thereby having thermogenic function, and represents a local storage for FFAs. Thus, on the one hand, it ensures protection of cardiomyocytes and coronary arteries from toxic exposure to high circulating levels of FFAs; on the other hand, this deposit represents a direct source of FFAs, readily available for cardiomyocytes to generate energy under conditions of increased metabolic demand. Furthermore, EAT is a tissue with relevant endocrine properties, capable of producing and secreting antiatherogenic and anti-inflammatory cytokines. The anatomical contiguity to the myocardium and the sharing of the same microcirculation allow EAT to perform not only endocrine actions but also paracrine effects, directly disseminating cytokines and FAAs into the myocardium and coronary lumen. Among the EAT-released adipocytokines, there are adiponectin, adrenomedullin and omentin, which allow the

control of vascular tension, by promoting vasodilatation, and hinder vascular remodeling (52).

Adrenomedullin also has antioxidant and antiapoptotic properties, inhibits the migration and proliferation of vascular smooth muscle cells and contributes to cardiac output, by increasing the availability of calcium for cardiomyocytes. Adiponectin, instead, is known to be an anti-inflammatory cytokine involved in the regulation of glucose and lipid metabolism: the increase in adiponectin levels is associated with an increase in insulin sensitivity, a reduction in the hepatic and muscular triglyceride content and in circulating FFAs levels (49).

In case of changes in the local microenvironment, the positive and beneficial effects of EAT can turn into harmful effects. This switch occurs in various pathological conditions, such as obesity, diabetes, and vascular diseases, which favor the shift of the EAT phenotype and secretome toward a pro-inflammatory, profibrotic and pro-atherosclerotic profile. In these conditions, an increase in the EAT thickness occurs, accompanied by a greater inflammatory and immune infiltrate, consisting of dendritic cells, T and B lymphocytes, macrophages and eosinophils and by an increase in production and secretion of cytokines and pro-inflammatory mediators, such as IL-1β, IL-6, TNFα, MCP-1, resistin and visfatin. Furthermore, in addition to an increase in the number of infiltrating macrophages, a cellular polarization is observed in the thicker and dysfunctional EAT. It consists in a phenotype shift of anti-inflammatory macrophages M2 toward the pro-inflammatory macrophages M1 and seems to correlate to the levels of EAT-derivated inflammatory cytokines (53).

Aging is one of the determining factors of the EAT amount. In subjects over 65 years of age, a mean total EAT amount of 22% greater compared to young subjects has been reported, thus suggesting an increase in epicardial fat with age (54). In patients without metabolic syndrome the presence of EAT $\geq$ 5 mm significantly increased with age (55). Similary, Silaghi et al. (56) described age as one of the main covariates associated with EAT extent, together with waist circumference and myocardial hypertrophy. Interestingly, Guglielmini et al. (57) reported that EAT depots appear to be more strongly associated with age than waist circumference or body mass index (BMI).

Human and animal studies demonstrated that aging is accompanied by increasing changes in body mass and fat redistribution and has a profound impact on EAT phenotype. Epicardial fat of old rats exhibited significantly lower levels of adiponectin and an increase in IL-6, compared to young rats (58). A population of 120 patients has been enrolled by Karadag et al. to examine the correlation between anthropometric values and EAT in young and old subjects. Simple regression analysis revealed statistically significant positive correlation between EAT and age, waist circumference and thigh circumference. Importantly, the effect of age and waist circumference on EAT continued to be statistically significant also on multivariate regression analysis. Interestingly, a statistically significant correlation between fasting insulin, insulin resistance and EAT was observed only in the geriatric group. This data, probably influenced by the higher incidence of insulin resistance with advancing age, highlights the importance of epicardial fat assessment in estimating the cardiometabolic risk of elderly patients (59).

The recognition of EAT as a marker of visceral adiposity and cardiometabolic risk is also confirmed by a study conducted on geriatric population, that identified obesity, HOMA index, fasting plasma glucose, weight, total and LDL cholesterol levels as determinants of EAT amount (60).

Other authors evaluated the relationship between visceral fat, hepatic steatosis, and metabolic syndrome occurrence in the elderly. Abdominal fat thickness and EAT thickness resulted to be greater in old patients with metabolic syndrome compared to non-metabolic syndrome patients. It has been observed an association between both increased abdominal fat thickness and EAT thickness with hepatic steatosis but only EAT resulted to be strongly correlated to metabolic syndrome (61).

In elderly patients with isolated severe calcific AS the increased EAT thickness, measured by echocardiography, correlates with levels of EAT secreted inflammatory and pro-atherogenic mediators, such as IL1β, IL-6, MCP-1, and TNF-α. These results support the thesis of an involvement of cardiac visceral fat in inflammatory and atherogenic phenomena that promote the development of cardiovascular diseases in the elderly and underlie the potential clinical utility of routinely EAT assessment (62).

Overall, the cytokines produced by EAT may contribute to the systemic inflammatory state, which in turn can promote the accumulation of EAT, inducing local and systemic inflammation and organ dysfunction. Thus, a continuous interlink is established between EAT, systemic inflammation and cardiovascular disease (9).

## EAT AND CORONARY ARTERY DISEASE

The central role of inflammation in endothelial dysfunction and cardiovascular diseases has been widely described (63, 64). Obesity promotes systemic inflammation and is recognized as a major cardiovascular risk factor (65, 66). Of note, the cardiovascular risk in obese subjects is mainly associated with visceral rather than subcutaneous adiposity (67). A dysfunctional perivascular adipose tissue, also through the down- and up-regulation of adiponectin and leptin, favors many features of atherosclerosis (68). A greater deposit of VAT has been observed in non-obese patients with CAD (69). Interestingly, in CAD patients, VAT has been associated with the presence of multivessel disease (70) and the risk of coronary plaque progression (71).

Importantly, obesity and the consequent chronic systemic inflammation result to be associated to a significant increase of the EAT amount (72). Therefore, several researchers have investigated the role of EAT in the development and progression of CAD. Ahn et al. (73) conducted a study on 527 patients undergoing their first coronary angiography, to explore the relationship between EAT, CAD risk factors and the extent of coronary atherosclerosis. EAT thickness was correlated with age and abdominal VAT and, above all, a greater EAT thickness was observed in CAD subjects compared to controls. Accumulation

of EAT also correlated with the extent and activity of CAD, being thicker in patients with unstable angina than those with stable angina or atypical chest pain.

These data agree with the results of another study, that showed higher EAT thickness in patients with angiographic CAD. The authors also reported the correlation between EAT increase and number of vessels with >50% diameter stenosis (74).

The positive association between EAT volume and CAD burden is supported by studies conducted on pig models of CAD. In these animal studies, surgical resection of EAT depot decreased the progression of CAD, thus suggesting that EAT may contribute locally to CAD and exacerbate coronary atherosclerosis (75, 76).

In vulnerable plaques 18-fluorodeoxyglucose ($^{18}$F-FDG) imaging detect higher macrophage infiltrates, evidenced by an increased standardized uptake value (SUV) (77). Positron emission tomography (PET)-CT was performed in a prospective study conducted on patients with low or intermediate risk of acute coronary syndrome to investigate whether the inflammatory activity of pericoronary adipose tissue could be associated to plaque composition. Interestingly, the maximum SUV of $^{18}$F-FDG was measured in the fat surrounding the coronary arteries and correlated with plaque burden and necrotic core component of coronary plaque (78).

The prominent impact of EAT inflammation on CAD risk, has been confirmed by studies that compared expression of inflammatory mediators in epicardial and subcutaneous adipose stores in patients undergoing coronary artery bypass graft surgery (CABG). A greater inflammatory cell infiltrates has been shown in EAT with respect to the subcutaneous adipose tissue, accompanied by a marked increase of EAT-derived inflammatory mediators, such as IL-1β, IL-6, MCP-1, and TNF-α, and their messenger RNA (79).

The macrophage polarization observed in EAT, characterized by a relative increase of inflammatory M1 macrophages and a relative decrease of anti-inflammatory M2 macrophages, is considered one of the underlying mechanisms of increased pro-inflammatory mediators in EAT of CAD patients. Importantly, it has been demonstrated a positive correlation between the M1/M2 macrophages ratio and the severity of CAD, thereby supporting the crucial pathological role of this phenomena in the coronary atherosclerotic process (53, 80).

Increased macrophage infiltrate and macrophage polarization are associated with increased production and secretion of inflammatory mediators (81) and may also explain the increased expression and secretion of resistin in EAT of patients with acute coronary syndrome, compared to patients with stable CAD or subjects without CAD (82). It has also been recently shown that plasma resistin levels are predictive of mortality in patients with acute myocardial infarction (83). Resistin seems to promote inflammation and atherogenesis by counteracting vasodilation and increasing the expression of adhesion molecules on endothelial cells (84, 85).

Similarly, animal studies on pig models of metabolic syndrome have indicated perivascular adipose tissue-derived leptin as a potential contributor to coronary atherogenesis. The increased leptin in EAT surrounding coronary arteries exacerbates endothelial dysfunction by reduction of nitric oxide production via protein kinase C-beta phosphorylation of endothelial nitric oxide synthase (86).

The prospective EPICHEART study evaluated proteomic EAT profile in 574 patients with severe AS and referred to cardiac surgery. EAT volume quantified by CT was associated with higher CT-derived Agatston coronary calcium score. Furthermore, EAT exhibited a pro-calcifying proteomic profile in CAD patients, consisting in upregulation of annexin-A2 and downregulation of fetuin-A. Annexin-A2 protein levels in EAT samples were also positively correlated with agatston coronary calcium score, suggesting that EAT might orchestrate pro-calcifying conditions in the late phases of CAD (87).

These results are consistent with other clinical studies documenting an increase in the volume of perivascular EAT as a risk factor for coronary atherosclerosis (88) and attributing a crucial role to the local EAT release of potentially atherogenic adipokines in the development and progression of CAD (89, 90).

The pro-atherogenic properties of EAT in CAD are determined not only by an increase in pro-inflammatory cytokines but also by a reduction in anti-inflammatory mediators. In detail, lower levels of EAT-derived adiponectin were found in CAD patients. Adiponectin is known for its vasodilatory, antiaterogenic, anti-inflammatory, antioxidant properties which it carries out through the inhibition of the expression of IL-8 by endothelial cells, the stimulation of the anti-inflammatory cytokine IL-10 and the inhibitor tissue of MMP-1 in macrophages. Thus, the imbalance between pro and anti-inflammatory cytokines confers EAT a pro-inflammatory and pro-atherosclerotic phenotype (90). Accordingly, acute coronary syndromes are characterized by an imbalance between EAT secreted IL-1ß and its receptor antagonist (IL-1ra) (91).

## EAT AND AORTIC STENOSIS

Calcific AS is a progressive and active process with multifactorial pathogenesis that includes biological mechanisms promoting the differentiation of resident valvular interstitial cells in osteoblast-like cells, thus leading to leaflets calcification. These events are mediated by proinflammatory cytokines such as IL-1β, IL-6, IL-8, insulin- like growth factor 1, TNF-a, TGF-β, mainly secreted by macrophages and activated T lymphocytes penetrating the endothelium of aortic valve leaftlets (92, 93).

In the last decades, several evidence recognized obesity and metabolic syndrome as relevant risk factors for AS, as well as for atherosclerosis, and highlighted the pathogenetic role of VAT in the development and progression of AS, thus identifying VAT as an independent risk factor for this valve disease (94).

Obesity and metabolic syndrome have been associated with the risk for both development and progression of aortic valve calcification, in the MESA (Multi-Ethnic Study of Atherosclerosis) and ASTRONOMER (Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin) studies, respectively (95, 96).

VAT production of inflammatory cytokines and ROS support its role in the pathogenesis of AS (97).

The hypothesis of a pro-inflammatory activation of EAT in AS patients is supported by several evidence which show that EAT amount is increased in these patients and its secretome is particularly rich in inflammatory mediators. It has been shown that EAT thickness correlates significantly with the levels of numerous secreted proinflammatory and pro-atherogenic cytokines, such as IL-6, TNF-α, MCP-1, IL-1β. Interestingly, this correlation was not found with plasma levels of the same mediators, which were similar in patients with and without AS, further reinforcing the hypothesis of the role of local inflammation (62).

The observation of increased EAT thickness in patients with severe AS was also confirmed by a retrospective study including a cohort of 200 consecutive patients with severe AS and 200 matched patients without AS. The logistic regression analysis showed that an increase in EAT by one standard deviation was associated with a two-fold increased occurrence of AS. Of note, EAT thickness was significantly associated with severe AS, independently from age, gender and cardiovascular risk factors (98).

The potential action of EAT in promoting inflammatory and atherosclerotic changes in the aortic valve appears to occur in the early stages of the disease. In this regard, a clinical study evaluated the correlation between EAT thickness and aortic valve sclerosis, by involving 225 patients who were admitted for coronary angiography due to new-onset angina. Transthoracic echocardiography was performed to assess both EAT thickness and the average aortic valve sclerosis score index. The authors reported that patients with an EAT thickness $\geq$7 mm were older, with more frequent hypertension and hyperlipidemia, and showed greater LV hypertrophy and a higher average aortic valve sclerosis score index, thus suggesting an association between EAT and aortic leaflets calcification already in the early stages of the disease (99).

Starting from the assumption of anti-inflammatory and anti-atherosclerotic properties of statins, several studies have suggested EAT as a potential new therapeutic target for statin therapy. In a population of elderly patients with calcific AS, statin therapy was significantly associated to a reduction of echocardiographic EAT thickness, that was paralleled by an attenuation of EAT inflammatory profile. *In vitro*, atorvastatin showed a direct anti-inflammatory effect on EAT, thereby indicating that statin may directly modulated EAT secretory profile (100).

However, there are conflicting data on the potential beneficial effect of statins in slowing the progression of AS. Rajamannan et al. developed an experimental hypercholesterolemic rabbit model and showed a proliferative atherosclerosis-like process associated with the transformation to an osteoblast-like phenotype in the aortic valve. Of note, they demonstrated that atorvastatin could inhibit these processes, by counteracting lipids deposition and oxidative stress observed in the early stages of degenerative calcified aortic disease (101).

In humans, the first prospective study showing a positive effect of statin therapy for AS progression, has been conducted by Moura et al., by evaluating 121 consecutive AS patients treated with and without rosuvastatin. Statin therapy was associated with a slowing of the hemodynamic progression of the disease (102).

However, three randomized controlled trials disagreed with these results. In fact, in patients with mild to moderate AS, the use of atorvastatin, rosuvastatin and simvastatin plus ezetimibe significantly reduced serum LDL cholesterol levels but was not associated with a beneficial effect on the progression of AS. Too late initiation of treatment could be the possible explanation for the statins failure to influence AS progression (103–105).

Long-term controlled studies enrolling patients with less advanced AS are needed to establish the true effect of statins in influencing AS progression and to better understand the possible influence of statin therapy on EAT activity.

## EAT AND ATRIAL FIBRILLATION

Numerous studies (106, 107) have reported the association between obesity and AF, and more recently, growing evidence explored the link between EAT and onset, severity and recurrence of AF. This relationship is the result of complex crosstalk between EAT and the neighboring atrial myocardium (108).

In a CT analysis of 3,217 individuals from the Framingham Heart Study, EAT volume was associated with prevalent AF, indipendently by traditional AF risk factors, including BMI (109).

EAT amount correlates not only with the presence of AF but also with AF severity and progression. Tsao and colleagues used CT images to quantify epicardial fat surrounding the atrium and demonstrated significantly increased volume of EAT surrounding the left atrium in AF patients and a tendency for persistent AF patients to have larger volumes than those with paroxysmal AF. There was no association between BMI and AF severity (110).

A recent meta-analysis reinforced the association between EAT amount and atrial arrhythmias, showing that EAT volume is higher in patients with persistent and paroxysmal AF than in healthy subjects. Interestingly, a significant increase in EAT was also found in patients with persistent AF compared to patients with paroxysmal AF, thus supporting the association between EAT amount and AF severity (111).

Several pathogenetic mechanisms have been proposed to explain the implication of EAT in AF onset and progression. EAT has been proposed as a potentially important factor involved in structural and electric atrial remodeling. Given the proximity of EAT to the underlying myocardium, it can directly influence atrial reeling by penetrating the myocardium and generating atrial fatty infiltrates, that may alter the atrial electrophysiological properties by determining the loss of side-to-side cells connection. The result is the creation of circuits that compromise the propagation of the depolarizing wave and generate the return phenomena (112, 113).

EAT can also indirectly promote atrial remodeling and alter atrial electrophysiological properties, by acting as a source of paracrine modulators of myocardial inflammation and oxidative stress.

EAT releases pro-fibrotic factors, such as TGF-β1, the adipokine activin A, a member of the TGF-β superfamily and

MMP2, MMP7, key regulators of extra-cellular matrix activity, that are up-regulated during AF, thereby contributing to atrial collagen deposition, fibrosis and remodeling (114–116).

EAT is also a relevant source of inflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNFα, that may have local pro-inflammatory effects on the adjacent atrial myocardium that facilitate arrhythmogenesis.

Several studies have associated the increase in inflammation markers, such as CRP, IL-6, IL-8, IL-1β, and TNF-α, with the presence and severity of AF (42, 117), and EAT seems to be a major source of inflammation in patients with AF. Mazurek et al. examined EAT inflammatory activity using FDG-PET/TC in patients with AF compared to non-AF controls subjects and showed that EAT inflammatory activity was increased in AF patients. Interestingly, EAT tracer uptake was greater than subcutaneous or other visceral adipose tissue depots, suggesting a marked inflammatory activity of EAT that could contribute to the development of AF (118).

EAT can contribute to the pathogenesis of AF also through the induction of oxidative stress. EAT is a source of ROS, that exert detrimental local effects on atrial myocardium. The role of oxidative stress from EAT in the genesis of AF is confirmed by experimental data showing that ROS inhibition by antioxidants attenuates atrial remodeling in animal models (119).

EAT could promote AF also through the production of an enzymatic protein, known as aromatase, that acts converting androgens into estrogens, and which seems to play an important role in modulating electromechanical properties, with consequent susceptibility to atrial arrhythmias. Aromatase is abundantly expressed in adipose tissue as well as in the myocardium and in EAT and its expression is remarkably upregulated with aging. In experimental models, EAT aromatase levels were higher in aged than in young animals and its estrogen conversion capacity resulted to be significantly enhanced with the increase in EAT amount, suggesting an association between EAT, aromatase estrogenic capacity and atrial arrhythmogenicity (120).

In addition to these pathogenetic mechanisms, it has been hypothesized that EAT could function as a trigger at the pulmonary veins level and/or other sites. An increased EAT volume may alter the function of ganglionated plexi, located near the pulmonary veins, leading to spontaneous, rapid and repetitive electrical activity that can promote AF (121).

Ganglionated plexi have been identified also in EAT, and their activation can cause autonomic nervous system stimulation resulting in shortening of action potential duration and increasing in calcium transient in the atrial myocardium, thereby contributing to arrhythmogenesis. These hypotheses are supported by the efficacy of botulinum toxin injection into EAT in suppressing atrial tachyarrhythmia, potentially through the inhibition of ganglionated plexi (122).

EAT, through the local production and secretion of catecholamines, may directly contribute to an increased sympathetic tone and to a sympatho-vagal imbalance, thus promoting atrial arrhythmias. Interestingly, EAT has a higher adrenergic activity compared to subcutaneous adipose tissue, demonstrated by the increased catecholamine levels and expression of catecholamine biosynthetic enzymes, thus supporting a potential role of EAT in arrhythmogenesis (123).

## EAT AND HEART FAILURE

The role of EAT in the pathogenesis and progression of HF has been described by several studies and includes complex pathophysiological mechanisms. EAT can sustain the processes underlying HF by regulating myocardial remodeling, insulin resistance, and RAAS. Adipokines and inflammatory cytokines secreted by EAT could mediate these actions. In fact, proinflammatory polarization of EAT is common in patients with HF and consequent changes in the EAT secretome promote myocardial dysfunction, by exacerbating inflammation and cardiac fibrosis, thus favoring arrhythmogenesis and HF progression (124).

EAT obtained from obese HFpEF patients showed a marked increase in inflammatory infiltrate, consisting in T lymphocytes and M1 macrophages, supporting the potential role of local inflammation in the pathogenesis of disease (125).

It has been shown that in obese individuals the increase in EAT volume was significantly associated with impaired myocardial microcirculation, abnormal cardiac diastolic properties, increased vascular stiffness and left atrium dilatation, all typical features of HFpEF (126, 127).

The leptin-aldosterone-neprilysin axis appears to be the main pathway linking EAT dysfunction to the development and progression of HF (128). EAT contributes to increased circulating levels of leptin, promoting systemic inflammation, thereby negatively affecting the heart and other visceral organs (128–130).

Leptin can stimulate adrenergic- and angiotensin-dependent mechanisms and represents the major stimulus to the production of aldosterone, thereby being responsible for the excessive mineralocorticoid receptor signaling observed in HF (131, 132). Leptin is linked to the accumulation and dysfunctional biology of EAT which may directly lead to inflammation, microcirculatory abnormalities, and fibrosis of the underlying myocardium (133, 134). Moreover, the activation of leptin-aldosterone-neprilysin axis, which results in sustained increased levels of aldosterone and neprilysin, further promotes EAT accumulation and inflammation (135, 136).

The local activation of the RAAS promotes lipotoxicity, reduced mitochondrial respiration and insulin resistance, thus contributing to HF. The detrimental effects of RAAS activation on heart function have been observed both in humans and in experimental models (125).

Protracted exposure to inappropriately elevated aldosterone levels causes significant changes in LV structure and function. Of note, hyperaldosteronism promotes sodium retention, increases cardiac filling pressures, induces worse remodeling, and accelerates HF progression (137).

Angiotensin II stimulates the secretion of complement-C1q and TNF-related protein 1 (CTRP1), that is an adipokine associated with metabolic syndrome, adiponectin deficiency, platelet aggregation, athero-inflammation and hypertension. CTRP1 acts as a stimulating factor for endogenous aldosterone

(138). In animal studies, proinflammatory cytokines including TNF-α and IL-1β caused elevations of CTRP1 levels in adipose tissue, indicating that CTRP1 expression may be associated with a low-grade chronic inflammation status in fat depots (139). Interestingly, Yang et al., showed that CTRP1 levels were increased both in the plasma and EAT of HF patients and proposed the CTRP1 involvement in the pathogenesis of HF by modulating IL-6 levels and aldosterone release (140).

EAT-derived miRNAs can also be involved in the pathophysiology of HF. In HF patients, increased secretion of several miRNAs was observed by EAT-resident macrophages, such as miR-21, which was upregulated in response to cardiac overload, and correlated with the TGF-β pathway causing hypertrophy and myocardial fibrosis (141).

Moreover, EAT-secreted MiR-17-5p is upregulated in obese subjects, increases adipogenesis, and has been described as a biomarker for HF (142). Similarly, miR-27 suppresses adipocyte differentiation and contributes to HF (143).

Cardiac SNS hyperactivity is associated to HF and results in sympathetic denervation of the heart. Several evidence indicate a possible contribute of EAT to SNS activation in HF (144). EAT may promote HF-related cardiac adrenergic derangement by stimulating central SNS activity through dysregulated adipokines production and secretion (145).

In patients with systolic HF, it has been demonstrated that EAT is a local source of catecholamine, and its increased thickness is associated to myocardial impaired autonomic function. Therefore, EAT seems to play an additive role in determining cardiac sympathetic denervation, in the context of HF-SNS hyperactivity (123). SNS activation in HF patients has been associated also with the prevalence of central sleep disordered breathing. Interestingly, EAT thickness resulted to be increased in these patients and correlated with increased circulating levels of norepinephrine and with the presence and the severity of sleep apneas (146, 147).

Overall, the evidence on the possible contribution of EAT to SNS activation in HF reinforces the potential negative role of EAT in the pathogenesis and progression of HF.

## EAT AND CARDIOVASCULAR OUTCOME

Given the role of EAT in the pathophysiology of cardiovascular diseases, numerous studies have been conducted to explore the potential impact of EAT on cardiovascular outcome (**Table 1**).

Tanindi et al. showed that CAD patients with EAT thickness more than 7 mm are at higher risk of myocardial infarction and cardiovascular death (148).

EAT thickness proved to be also useful for predicting in-intensive care unit complications after CABG surgery, such as AF onset, prolonged inotrope use, and fever (149).

Eberhard et al. evaluated the prognostic value of CT-EAT volume in 503 patients with severe AS undergoing transcatheter aortic valve implantation (TAVI). In this population, EAT volume was independently associated with all-cause mortality at 1, 2, and 3 years after TAVI. Therefore, these authors proposed the pre-TAVI assessment of EAT volume as a relevant prognostic factor for risk stratification of AS patients (151).

Similarly, Davin et al. investigated the contribution of EAT and late gadolinium-enhancement, quantified by CMR, on AS patients outcome. They showed that EAT volume predicts adverse outcome in AS asymptomatic patients, thus EAT volume assessment may improve the risk-stratification of asymptomatic AS patients (152).

The association between EAT and worse prognosis in AS patients probably lie in the potential EAT-related unfavorable LV-remodeling in response to the chronic pressure overload of LV.

Initially, LV hypertrophy is an adaptive phenomenon allowing the heart to maintain adequate cardiac output, by overcoming the obstacle of valve stenosis. However, over time it becomes a maladaptive phenomenon and evolves toward diastolic dysfunction, leading to HF and poor prognosis (156).

Numerous evidence support the role of EAT in promoting myocardial hypertrophy. Coisne et al. showed an independent and significative association between EAT echocardiographic thickness and pathological LV remodeling in AS patients. The intense metabolic and pro-inflammatory activity of EAT could account for this association (157). These data confirm the previous hypothesis of Iacobellis et al. (158) who found for the first time that an increase in epicardial fat, assessed by echocardiography, is significantly related to an increase in LV mass.

EAT echocardiographic thickness has been also identified as a marker of cardiac adrenergic derangement that is strongly correlated with adverse prognosis in HF patients. EAT thickness proved to be an independent predictor of cardiac sympathetic innervation evaluated by $^{123}$I-metaiodobenzylguanidinescintigraphy ($^{123}$I-MIBG) (123), and, in high-risk patients, is associated to both clinical and arrhythmic outcome (155). Furthermore, in HF patients, echocardiographic EAT thickness was found higher in presence of sleep-disordered breathing and progressively and significantly increased with the severity of the sleep apneas (147), that are associated with a greater SNS activation (159) and a worse outcome in patients with HF (160). This association supports the hypothesis that EAT abnormalities might represent a novel pathophysiological link between sleep-disordered breathing, SNS hyperactivation and prognosis in HF patients.

The correlation between EAT and cardiovascular outcome has been described also in AF patients. Tsao et al. described an independent association between increased EAT around the left atrium and stroke in AF patients. EAT amount was correlated with the contractile dysfunction of the left atrium and the circulatory stasis of the atrial appendage, two important risk factors for AF-related stroke (161). Similarly, Chu et. Al reported the negative prognostic role of EAT thickness in AF patients. EAT was associated with adverse cardiovascular outcome, being correlated to cardiovascular mortality, hospitalization for HF, myocardial infarction, and stroke. Moreover, adding EAT thickness to a predictive model containing CHA2DS2-VASc score, left atrial volume, and LV systolic and diastolic

**TABLE 1 |** Associations between EAT and cardiovascular diseases.

| References | Patients source | EAT measure | Outcome | Association/Distribution |
|---|---|---|---|---|
| Ahn et al. (73) | 527 patients undergoing PCA for suspected CAD | EAT thickness ≥ 3 mm in diastole at echocardiography | CAD (stenosis ≥50%) | OR: 3.36 (95%CI: 2.2 - 5.2) |
| Picard et al. (74) | 970 patients undergoing PCA for suspected CAD | EAT thickness EAT ≥2.8 mm at CT | CAD (stenosis ≥50%) | OR: 1.67 (95%CI: 1.23 - 2.26) |
| Tanindi et al. (148) | 200 CAD patients (stable angina pectoris or acute coronary syndrome) | EAT thickness ≥7 mm in end-systole at echocardiography | - Cardiovascular death<br>- AMI | HR: 1.9 (95%CI: 0.4-8.3)<br>HR: 2.4 (95%CI: 0.6-10.0) |
| Mirdamadi et al. (149) | 78 CAD patients referred to CABG | Intraoperative EAT thickness measure ≥6.5 mm | In-intensive care unit complications after CABG | OR: 1.33 (95%CI: 1.04–1.71) |
| Nelson et al. (150) | 356 subjects referred to cardiovascular risk assessment | EAT thickness ≥ 5 mm in end-diastole at echocardiography | Coronary calcium score | HR: 2.26 (95%CI: 1.44–3.53) |
| Jeong et al. (89) | 203 CAD patients undergoing PCA | EAT thickness ≥7.6 mm in end-diastole at echocardiography | CAD (stenosis ≥ 50%) | OR: 10.53 (95%CI: 2.2–51.2) |
| Parisi et al. (62) | 95 severe AS patients referred to AVR vs. 44 healthy subjects | EAT thickness in end-systole at echocardiography | Association to AS | 9.85 ± 2.78 mm (AS) vs. 4.91 ± 1.27 mm (controls); $p < 0.0001$) |
| Mahabadi et al. (98) | 200 severe AS patients vs. 200 matched non-AS patients | EAT thickness increase of 1 SD at echocardiography | Occurrence of AS | OR: 2.10 (95%CI: 1.65-2.68) |
| Eberhard et al. (151) | 503 AS patients referred to TAVR | EAT volume > 125 $mm^3$ at multi-detector CT | All cause 3-year mortality after TAVR | HR: 2.27 (95%CI: 1.44–3.57) |
| Davin et al. (152) | 118 patients with moderate or severe AS | Indexed EAT volume >60 $ml/m^2$ at CMR | Adverse cardiovascular outcome | Indexed EAT volume > 60 $ml/m^2$ vs. ≤ 60 $ml/m^2$; $p = 0.0088$ |
| Thanassoulis et al. (109) | 3217 individuals from the Framingham Heart Study | EAT Volume increase of 1 SD at multidetector CT | Prevalence of AF | OR: 1.28 (95%CI: 1.03-1.58) |
| Tsao et al. (110) | 68 AF patients vs. 34 non-AF controls | EAT Volume at multidetector CT | - AF occurrence | EAT Vol: 29.9 ± 12.1 (AF) vs. 20.2 ± 6.5 $cm^3$ (non-AF); $p < 0.001$ |
| | | | - AF recurrence after ablation | EAT Vol: 26.8 ± 11.1 (AF) vs. 35.2±12.5 (non-AF): $p = 0.007$ |
| Chu et al. (153) | 190 persistent AF patients | EAT thickness ≥ 6 mm in end-diastole at echocardiography | Adverse cardiovascular events | OR: 1.224 (95%CI: 1.096-1.368) |
| Maeda et al. (154) | 218 AF patients undergoing AF ablation | EAT Volune Index cut off ≥116 $mL/m^2$ at multidetector CT | post-ablation recurrence of AF | HR: 1.02 (95%CI: 1.00-1.03) |
| Parisi et al. (155) | 69 sistolic HF patients referred to ICD | Echocardiographic EAT thickness increase of 1 SD | Composite clinical and arrhythmic outcome | HR: 1.16 (95%C.I: 1.08–1.24) |
| Wu et al. (124) | 58 systolic HF patients, 63 HFpEF patients, 59 non-HF patients | EAT volume at CMR | - HF patients vs. HFpEF patients vs. non-HF patients | Indexed EAT vol: 27.0 (22.7-31.6) vs. 25.6 (21.4-31.2) or 24.2 (21.0-27.6) $mL/m^2$; $p < 0.05$ |
| | | | - Cardiac fibrosis | r: 0.49; 95% CI: 0.12-0.86, $p < 0.01$ |
| Nakanishi et al. (126) | 372 patients undergoing CFR examination | Increase EAT volume of 10 ml at multi-detector CT | Deterioration of LV diastolic function | OR: 1.11 (95%CI: 1.02-1.21) |

*EAT, epicardial adipose tissue; PCA, percutaneous coronary angiography; CAD, coronary artery disease; AS, aortic stenosis; AF, atrial fibrillation; HF, heart failure; AVR, aortic valve replacement; TAVR, transcatheter aortic valve replacement; ICD, implantable cardioverter defibrillator; CABG, coronary artery bypass graft surgery; CFR, coronary flow reserve; LV, left ventricle; AMI, acute myocardial infarction; CT, computed tomography; CMR, cardiac magnetic resonance; SD, standard deviation; 95%CI, 95% confidence interval; OR, odds ratio; HR, hazard ratio; r, correlation (Pearson or Spearman).*

function, EAT significantly improved the risk stratification (153). Interestingly, EAT thickness is positively correlated with CHA2DS2-VASc risk score, thus confirming the association between EAT, stroke and adverse cardiovascular outcomes in AF patients (162). Maeda et al. showed a close correlation between EAT and recurrence of post ablation AF, that was more frequent in patients with higher EAT amount, measured using CT. These authors demonstrated that an EAT volume index cutoff $\geq$116 mL/m$^2$ may be useful for prediction of recurrent AF after catheter ablation (154). Another study showed that the EAT pro-arrhythmic influence is greater in the early post-ablation phase. EAT amount independently predicted early recurrence after AF ablation whereas it did not have an impact on late recurrence (163). According to these findings, a systematic review and meta-analysis that compared EAT amount between patients with and without AF recurrence showed that total and left atrial-EAT volumes, as well as EAT thickness, were higher in patients with AF recurrence (164).

Overall, these evidence indicate that EAT quantification can be used along with traditional predictors, as a new imaging marker to predict cardiovascular outcome.

## EAT QUANTIFICATION

Several imaging methods have been used to provide a reliable quantification of EAT, such as echocardiography, CT and CMR (**Table 1**).

CMR imaging is considered the gold standard for the detection and quantification of EAT, providing an accurate and volumetric EAT measurement (165), especially once new CMR imaging method using the three-dimensional summation of slices approach has been developed (166).

The measure of absolute amount of EAT is obtained volumetrically in consecutive short-axis views by means of the modified Simpson's rule. In addition, maximum EAT thickness at the right ventricular free wall is measured in a transversal 4-chamber view and averaged in consecutive short-axis views covering the whole ventricle; the mean EAT thickness is calculated by averaging results from the long-axis and short-axis measurements. The results of these measurements appear to be consistent with the autopsy measurement of EAT thickness obtained in 200 human hearts at the ventrolateral edge of the right ventricle (167).

Despite the great diagnostic potential, CMR imaging is an expensive and time-consuming procedure, so it is hardly available routinely in clinical practice.

CT allows the measurement of the EAT with the higher spatial resolution as compared to echocardiography and CMR imaging, thus providing the best sensitive and accurate assessment of EAT thickness, volume and total area (168). The three-dimensional image reconstruction with multidetector-row CT further improves spatial resolution and the specificity and sensitivity of measurements (169). In the populations of the Framingham Heart Study and the Multi-Ethnic Study of Atherosclerosis, variable values of mean EAT volume detected by CT, ranging from 68 $\pm$ 34 mL to 124 $\pm$ 50 mL, have been reported (170, 171). In a cohort of 226 subjects with a low Framingham Risk Score referred to non-contrast cardiac CT for coronary calcium scoring, the value of 68.1 mL/m$^2$ at CT has been identified as the 95th percentile of EAT volume indexed to body surface area. EAT volume exceeding this value seems to predict major adverse cardiovascular events (172). The reproducibility of volumetric quantification of EAT by cardiac CT appears to be superior to that of thickness and area measurements. However, volumetric assessment is time consuming, requires an advanced cardiac imaging workstation and qualified and experienced observers (168). Furthermore, the high cost and the exposure to ionizing radiation limit the use of this procedure in routine clinical practice.

Echocardiography allows the assessment of the EAT thickness in a safe, inexpensive and easily repeatable way, without the risk of exposure of the patient to ionizing radiations. Despite these intuitive advantages, it has some important limitations: it is an operator-dependent procedure and allows an accurate estimation of EAT thickness but not of EAT volume. In addition, a poor acoustic window, such as in obese or very thin subjects, can prevent optimal visualization of the EAT thickness or make it difficult.

Over the years, various authors have proposed different echocardiographic approaches for measuring epicardial fat, which differ both in the measurement site and in the phase of the cardiac cycle chosen for the EAT thickness assessment. Some authors have proposed the EAT measurement at end-diastole (73), to conform with other imaging techniques, such as CT and CMR; conversely, other authors recommend measuring EAT at end-systole (173), thus avoiding any EAT compression during diastole.

Iacobellis et al. described EAT as an echo-free or a hyperechoic space, if it is massive, visible from both parasternal longitudinal and transverse views, and suggested to measure the maximum EAT thickness in systole, between the right ventricular free wall and the parietal pericardium, as the average of three consecutive beats. In healthy people with body max index $>$ 22 kg/m$^2$, they showed a variability of EAT thickness values, between 1.8 and 16.5 mm (174). By using the same echocardiographic method, Malvazos et al. observed an EAT thickness of 6.5 $\pm$ 0.8 mm in obese patients, and of 1.3 $\pm$ 0.2 mm in healthy subjects, from both parasternal long- and short-axis views (175).

On the 2-dimensional echocardiography, EAT was described by Jeong et al. as an echo-free space in the pericardial layers (89) and its thickness was measured in more than 200 subjects referred to coronary angiography, at end-diastole, from the parasternal long-axis views, perpendicularly on the free wall of the right ventricle, for 3 cardiac cycles. The authors reported an average EAT thickness value of 6.4 mm (1.1–16.6 mm). Nelson et al. (150) also measured EAT thickness in asymptomatic subjects presenting for cardiovascular preventive care. The measurement was obtained on the free wall of the right ventricle in parasternal long-axis view, at end-diastole, perpendicular to the aortic annulus. They found a mean value of 4.7 $\pm$ 1.5 and suggested a cut-off EAT thickness value of 5 mm as marker of cardiovascular risk.

However, as known, EAT is localized between the myocardium and the visceral layer of the pericardium; therefore, some of these approaches are not faithful to its true anatomical site and can cause confusion between epicardial fat and pericardial fluid. Our group validated the measurement of EAT at the level of the Rindfleisch fold, between the free wall of the right ventricle and the anterior surface of the ascending aorta (173). In this pericardial recess, the space between the two pericardial layers is greater, so that EAT, not compressed by parietal pericardium, can expand and EAT can be visualized in its maximum thickness and directly measured. This approach is faithful to the true anatomical site of the EAT, located between the myocardium and the visceral layer of the pericardium, allows for direct visualization of the fat depot and its simple measurement. Furthermore, unlike other echocardiographic methods (176), this measurement seems to reflect the total amount of EAT, and well correlates with EAT thickness and volume at CMR, the current gold standard for EAT quantification.

Further studies are required on comparing and improving fat measurement techniques and methods, to reach a consensus on how to measure EAT, define normal reference values and introduce this measurement into clinical practice.

## CONCLUSIONS

Human aging is characterized by a state of chronic low-grade inflammation that significantly contributes to cardiovascular diseases and adverse outcomes in the elderly. The accumulation of visceral fat depots is accompanied by an increased inflammatory status and promotes the low-grade inflammation. A greater EAT amount, the visceral fat depot of the heart, is observed in the elderly. The anatomical features and biological function of EAT raised a growing interest on the potential role of EAT in cardiovascular diseases. Increased EAT is paralleled by an increased inflammatory status and a greater EAT secretion of inflammatory mediators and neuro-hormones, which may penetrate the myocardium and coronary vessels in a paracrine and vasocrine manner and express their toxicity in the neighboring tissues, thus contributing to development and progression of cardiovascular diseases. Accumulating evidence strongly support the correlation between EAT accumulation and a worse cardiovascular outcome.

Different imaging methods, such as echocardiography, CT and CMR, are available for EAT quantification in clinical practice, and EAT measurement has been proposed as useful marker for assessment of cardiovascular and metabolic risk. Further studies are needed to validate standardized measurement methods and to better define normal reference values of EAT, thereby leading to a routinely use of EAT volume/thickness in cardiovascular risk stratification.

## AUTHOR CONTRIBUTIONS

MC and VP conceived the manuscript structure and wrote the manuscript with support from LP, PP, and VV. SC contributed to the writing and revision of the Aging and inflammation and Epicardial adipose tissue in elderly sections. PC and GC contributed to the writing and revision of the coronary artery disease and aortic stenosis sections. EA and VR contributed to the writing and revision of the atrial fibrillation and heart failure sections. EP and PF contributed to the writing and revision of the cardiovascular outcome section. VP and DL supervised other authors and contributed to the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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# Long-Term Medical Resource Consumption of Radical Prostatectomy vs. Intensity-Modulated Radiotherapy for Old Patients With Prostate Cancer: A Nationwide Population-Based Cohort Study

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**Purpose:** Few studies have compared the long-term medical resource consumption between radical prostatectomy (RP) and intensity-modulated radiation therapy (IMRT) among old (≥80 years) patients with localized prostate cancer (LPC), particularly in those at high risk of prostate adenocarcinoma.

**Patients and Methods:** The propensity score matching was conducted to investigate the medical expenditure of two therapeutic modalities (RP and IMRT) in elderly patients with high-risk LPC (HR-LPC). The generalized linear mixed and logistic regression models were employed to evaluate the number of postdischarge visits and medical reimbursement for urinary diseases or complications and the number of hospitalizations for treatment-related complications over 5 years after treatment, respectively.

**Results:** Significant differences were observed in the median or mean urology clinic visit numbers across the two therapeutic modalities from the first until fifth year post treatment ($p < 0.0001$). After adjustment for covariates, the mean difference [95% confidence interval (CI)] of urology clinic visit numbers between RP and IMRT was 13.07 (10.45–15.49, $P < 0.0001$), 7.47 (8.01–14.92, $P < 0.0001$), 8.24 (4.59–9.90, $P < 0.0001$), 6.63 (3.55–11.70, $P < 0.0001$), and 5.02 (1.12–8.73, $P < 0.0001$)

for the first, second, third, fourth, and fifth years, respectively. In the logistic regression multivariate model with adjustment for covariates [therapy type, age, diagnosis year, income, hospital area, hospital level (academic or nonacademic), clinical and pathological T-stage, grade (Gleason score), pretreatment PSA level (ng/ml), and D'Amico risk classification], the adjusted odds ratio (95% CI) of IMRT was 2.10 (1.37–2.56, $P = 0.0013$), 1.55 (1.08–2.21, $P = 0.0151$), 1.35 (1.08–2.21, $P = 0.0084$), 1.24 (1.07–2.21, $P = 0.0071$), and 1.09 (1.02–1.81, $P = 0.0379$) for the first, second, third, fourth, and fifth years, respectively, compared with those of RP. The mean difference (95% CI) of total medical claims amounts of RP and IMRT between the RP and IMRT + ADT groups was 2,69,823 New Taiwan Dollars (NTD) (247,676–291,970, $P < 0.0001$), 40,803 NTD (17,379–54,228, $P < 0.0001$), 36,202 NTD (24,375–68,029, $P < 0.0001$), 26,708 NTD (11,179–54,595, $P = 0.0321$), and 12,173 NTD (17,140–41,487, $P = 0.0187$) for the first, second, third, fourth, and fifth years, respectively.

**Conclusion:** The long-term medical resource consumption was higher in old men with HR-LPC undergoing IMRT than in those undergoing RP.

**Keywords: medical resource consumption, radical prostatectomy, intensity-modulated radiation therapy, old-age, localized prostate cancer**

## INTRODUCTION

Localized prostate cancer (LPC) mean prostate cancer is still confined within prostate glands without extension to other sites in the patients. LPC is commonly asymptomatic if it has been diagnosed in the early stage, because slowly progression of disease (1, 2). Consequently, fewer older men receive curative-intent therapy, namely radiotherapy (RT) or radical prostatectomy (RP), compared with younger men, because elderly patients with LPC might receive conservative treatments (1). Active surveillance is generally the treatment strategy applied in older men (2). Between the aforementioned two curative-intent therapies, RT is preferable for older men, who are typically aged more than 70 years, (1, 3, 4) whereas another therapy, such as watchful waiting or androgen deprivation therapy (ADT) with luteinizing hormone-releasing hormone agonist, are preferable for men older than 80 years (1). In Taiwan, the most common risk classification used is the National Comprehensive Cancer Network (NCCN) risk classification depending on the clinical tumor (T) stage; Gleason scores and Pretreatment Prostate-Specific Antigen (PSA) are applied for further decision-making based on NCCN guidelines (5). Even for old (≥80 years) men with NCCN high-risk LPC (NCCN-HR-LPC) with a life expectancy of >5 years, more aggressive treatments such as RT or RP are suggested as per NCCN guidelines (5). understanding the medical resource consumption of the two curative treatments is valuable for establishing health policies, and the results can be used as a reference for implementing relevant national health services.

The treatment of patients with PC is expensive (3). Studies have provided inconsistent results regarding the cost of RP and RT (3, 6, 7). Some studies have shown that the expenditure incurred in RP is higher than that incurred in RT (3), which was most likely caused by the emergence of the advanced RP techniques, namely laparoscopic RP and robot-assisted radical prostatectomy (RARP) (8). In addition, the hospitalization cost of RP is significantly higher than that of RT, as the major proportion of RT patients are outpatients (3). A study evaluated the value of RP based on the morbidity and mortality rates and found that overall adjusted in-hospital mortality after radical prostatectomy was relatively low (0.25%), with a decreased length of hospitalization (6). Intensity-modulated radiation therapy (IMRT) is the contemporary RT technique; it is more suitable for HR-LPC, with a higher radiation dosage, higher dose conformity to cancer, and less radiation to normal tissues (9–13). RT with IMRT technique is more costly than the RT techniques applied in the studies that identified RP as more expensive than RT (7, 14). The medical resource consumption of RP and IMRT for men with NCCN-HR-LPC is unclear (3, 7, 14), especially in elderly patients. However, no long-term evaluation with a follow-up duration of >5 years has been conducted for the medical resource consumption of RP and high-dose IMRT plus long-term ADT in old men with NCCN-HR-LPC.

Geriatric medicine has gained increasing importance for cancer treatment because the average life span is increasing (15). The two curative-intent modalities of RP and IMRT are effective

**Abbreviations:** PC, Prostate cancer; HR-LPC, high-risk localized prostate cancer; RP, radical prostatectomy; RT, radiotherapy; IMRT, intensity-modulated radiotherapy; aOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval; NCCN, National Comprehensive Cancer Network; PSA, prostate-specific antigen; NCCN-HR-LPC, National Comprehensive Cancer Network high-risk localized prostate cancer; PSM, propensity score matching; NHIRD, National Health Insurance Research Database; TCRD, Taiwan Cancer Registry Database; AJCC, American Joint Committee on Cancer; ADT, androgen deprivation therapy; ICD-9-CM, International Classification of Diseases, Ninth Revision, Clinical Modification; CCI, Charlson comorbidity index; T, tumor; SD, standard deviation; RARP, robot-assisted radical prostatectomy; NHI, National Health Insurance; NTD, New Taiwan Dollars; US, United States; USD, United States Dollars.

for improving the survival of old men with NCCN-HR-LPC (16–22), but no comparative study has been conducted for the medical resource consumption of the two treatments. This research gap leads to difficulty in shared decision-making between old patients and physicians. Therefore, we conducted this comparative study of the medical resource consumption of RP and high-dose IMRT with long-term ADT using propensity score matching (PSM) among old (≥80 years) men with NCCN-HR-LPC. The results would provide a valuable reference for shared decision-making between old patients and physicians in the future. Selection of the same clinical outcomes therapeutic option with less financial toxicity on patients and Taiwan's healthcare financing system would be important to establish the more cost-effective health policy in the near future, because Taiwan's health care system on the verge of collapse (23).

# PATIENTS AND METHODS

## Study Cohort

The data were collected retrospectively from the Taiwan Cancer Registry Database (TCRD) and the Taiwan National Health Insurance (NHI) Research Database (NHIRD). All medical costs have been paid by NHI and data recorded in the NHIRD. The index date was the date of PC diagnosis. The cohort included patients aged ≥80 years who had been diagnosed with LPC and who had received RP or high-dose IMRT and long-term ADT between January 1, 2011, and December 31, 2016.

In the inclusion criteria, (1) RP was defined as surgical procedures to remove the entire prostate gland and its surrounding lymph nodes for men with LPC (24). (2) High-dose IMRT was defined for RT administered a 54 Gy to the seminal vesicles as well as cone-down boosts of 72–81 Gy to cover the prostate in 1.8 Gy per fraction. (3) Patients were confirmed through a review of following information: pathological data, magnetic resonance imaging for PC stratification (cT1-T3a), pretreatment PSA levels (>20 ng/ml), and grade based on GS ≥8. (4) According to the aforementioned criteria, patients were included in our cohort and were defined as having NCCN-HR-LPC (5). RP and high-dose (≥72 Gy) IMRT with long-term (≥18 months) adjuvant ADT were included as the curative-intent therapies for men with NCCN-HR-LPC and a life expectancy of >5 years.

In the exclusion criteria, (1) patients who had other cancers, clinical lymph node metastasis, or distant metastasis [based on the staging system of the American Joint Committee on Cancer (AJCC), 7$^{th}$ edition] were excluded from this study. (2) Inadequate doses of IMRT (<72 Gy) were excluded from this study. (3) Patients with unidentified clinical or pathological stage, unidentified D'Amico risk classification, unidentified Gleason score, unidentified postoperative Gleason grade, missing data on pretreatment PSA levels, and nonadenocarcinoma histology were excluded from this study.

Furthermore, the comparison of the two procedures, were specified into the RP and high-dose IMRT plus long-term ADT groups, respectively. The follow-up duration was 5 years after the index date; the medical resource consumption of the two curative-intent therapies was calculated over these 5 years. The study protocols were reviewed and approved by the Institutional Review Board of Tzu-Chi Medical Foundation (IRB109-015-B).

## Prospensity Score Matching

To improve analysis precision, we employed head-to-head PSM between the RP and high-dose IMRT plus long-term ADT groups (25). Most of the independent variables were matched at a ratio of 1:2; the other variables were matched at a ratio of 1:2 or 1:1. To reduce the effects of potential confounders when comparing all-cause death between the RP and high-dose IMRT+ ADT groups, the participants were matched based on propensity scores. The matching variables used were age, year of diagnosis, CCI scores, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, diabetes, hypertension, income levels, hospital areas, hospital levels (academic or non-academic hospitals), clinical T-stage, Gleason score, Grade (max of Gleason grade), preoperative PSA (ng/ml), and D'Amico classification. Comorbidities were determined according to ICD-9-CM codes in the main diagnosis of inpatient records or if the number of outpatient visits was ≥2 within 1 year. Continuous variables are presented as means ± standard deviations or medians (first and third quartiles), as appropriate. We matched the participants at a ratio of 1:1 or 1:2 by using the greedy method, matched with a propensity score within a caliper of 0.2 (26). Matching is a common technique for selecting controls with identical background covariates as study participants, and it is done to minimize differences among study participants (that the investigator deems necessary to be controlled for).

## Covariates and Endpoints

The primary independent variables in this study were RP and IMRT. The covariates were therapy type, age, diagnosis year, income, hospital area, hospital level (academic or nonacademic), clinical and pathological T-stage, grade (Gleason score), pretreatment PSA level (ng/ml), and D'Amico risk classification, which might be correlated with all-cause mortality. Comorbidities were evaluated using the Charlson comorbidity index (CCI) (27, 28). Comorbidities that were correlated with all-cause death and which occurred 6 months prior the index date were examined in this study. Comorbidities were identified based on International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnostic codes; comorbidities were defined as those with more than two repetitive primary diagnostic codes for visits to the outpatient department or the first admission. The dependent variables were as follows: (1) the number of urology outpatient clinic visits, (2) the proportion of patients being hospitalized for urinary diseases or treatment-related complications, and (3) medical reimbursement for urinary diseases or treatment-related complications.

## Statistical Analysis

In this nationwide population-based cohort study, the generalized linear mixed model with multivariate analysis, with adjustment for covariates including age, clinical and pathological T-stage, Gleason score, preoperative PSA, D'Amico

**TABLE 1 |** Generalized linear mixed model of numbers of urology outpatient clinic visits stratified by RP and IMRT.

| Numbers of outpatient clinic visits | | RP (Ref) $N = 277$ $N$, % | IMRT $N = 382$ $N$, % | Mean difference (95% CI)* | $p$-value |
|---|---|---|---|---|---|
| First year after treatment | Mean (SD) | 31.7 (12.9) | 44.8 (16.8) | 13.07 (10.45, 15.49) | <0.0001 |
| | Median (IQR, Q1–Q3) | 31 (22–39) | 42 (32–53) | | |
| Second year after treatment | Mean (SD) | 28.2 (14.6) | 35.7 (18.8) | 7.47 (8.01, 14.92) | <0.0001 |
| | Median (IQR, Q1–Q3) | 25 (18–35) | 33 (23–46) | | |
| Third year after treatment | Mean (SD) | 27.8 (15.3) | 35.0 (18.7) | 8.24 (4.59, 9.90) | <0.0001 |
| | Median (IQR, Q1–Q3) | 26 (17–35) | 32 (21–44) | | |
| Fourth year after treatment | Mean (SD) | 24.5 (14.3) | 30.7 (20.8) | 6.63 (3.55, 11.70) | <0.0001 |
| | Median (IQR, Q1–Q3) | 23 (12–33) | 24.5 (15–41) | | |
| Fifth year after treatment | Mean (SD) | 20.8 (10.5) | 25.1 (17.4) | 5.02 (1.12, 8.73) | <0.0001 |
| | Median (IQR, Q1–Q3) | 20 (11–31) | 22 (13–39) | | |

**Multivariate model with adjustment for covariates: Age, year of diagnosis, CCI score, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, diabetes, hypertension, income, hospital area, hospital level, clinical T stage, Gleason score, grade, preoperative PSA, and Damico risk classification. Least square mean difference for continuous variables and odds ratio for binary variables through fitting the generalized linear model with stratification of matched pairs.*
*RP, radical prostatectomy; SD, standard deviation; IQR, interquartile range; IMRT, intensity-modulated radiation therapy; Ref, reference group; CI, confidence interval.*

risk classification, hospital level, and therapeutic modality, was applied to compare the RP and high-dose IMRT+ ADT groups. The generalized linear mixed model fitted with the random intercept was used for grouping patients by the hospital level, and Type III tests of fixed effects were conducted. As a result, the $p$-value was the only indicator that could be observed. Descriptive statistics were used to describe patient characteristics based on the therapeutic modality. The descriptive statistics were the mean and standard deviation for normal continuous data, median and interquartile range for nonnormal continuous data, and number and proportion for categorical data. Student's $t$ test, analysis of variance, and nonparametric counterpart tests were applied, as appropriate. Two types of multivariate mixed models stratified by the hospital level were fitted to ensure the effect of therapeutic modalities on the outcomes: (1) a linear model for continuous outcomes, number of urology outpatient clinic visits, and medical costs for therapeutic complications and (2) a logistic regression model for the number of hospitalizations for therapeutic complications, with adjustment for covariates. The significance level was set at 5%.

# RESULTS

## Clinicopathological Characteristics

Totally, 659 patients were included in this study, comprising 277 and 382 who underwent RP and IMRT + ADT, respectively. Patients who underwent RP and IMRT + ADT were followed up for a mean period of 61.7 [standard deviation (SD) = 18.9] months and 58.4 (SD = 18.4) months, respectively. No statistically significant differences were observed in age, diagnosis year, CCI score, clinical T-stage, T-stage, postoperative Gleason score, Gleason grade, pretreatment PSA level, D'Amico risk classification, hospital level and area, follow-up duration, and income (**Supplementary Table 1**, online only).

## Number of Urology Outpatient Clinic Visits Stratified by RP and IMRT

**Table 1** presents the number of urology outpatient clinic visits per patient classified by treatment approaches (RP and IMRT). Significant differences were observed in the median or mean urology clinic visit numbers across the two therapeutic modalities from the first until fifth year post treatment ($p < 0.0001$). The numbers of urology outpatient clinic visits per patient were significantly more in the IMRT group than in the RP group (**Table 1**). In the generalized linear mixed model with adjustment for covariates (**Supplementary Table 1**, online only), the mean difference [95% confidence interval (CI)] of RP and IMRT + ADT was 13.07 (10.45–15.49), 7.47 (8.01–14.92), 8.24 (4.59–9.90), 6.63 (3.55–11.70), and 5.02 (1.12–8.73) for the first, second, third, fourth, and fifth years, respectively, with all $p$-values of <0.0001. The median and mean clinic visit numbers significantly reduced from the initial year post treatment (median of 31 and 42 visits for RP and IMRT, respectively, $p < 0.0001$) to the latest follow-up in the fifth year (median of 20 and 22 visits for RP and IMRT, respectively, $p < 0.0001$).

## Hospitalization for Urinary Diseases or Treatment-Related Complications Stratified by RP and IMRT

A significant decrease was observed in the rate of hospitalization for urinary diseases or treatment-related complications for both modalities (**Table 2**, $p < 0.05$) from the first year (hospitalization rates of 29.96% and 51.83% for RP and IMRT + ADT, respectively, $p = 0.0013$) onward after treatment until the last follow-up (hospitalization rates of 9.80% and 15.97% for RP and IMRT + ADT, respectively, $p = 0.0379$). In the logistic regression multivariate model with adjustment for covariates, the adjusted odds ratio (aOR) (95% CI) of IMRT was 2.10 (1.37–2.56), 1.55 (1.08–2.21), 1.35 (1.08–2.21), 1.24 (1.07–2.21), and

**TABLE 2 |** Logistic regression model of hospitalization for urinary diseases or treatment-related complications stratified by RP and IMRT.

| Hospitalization (%) | RP (Ref), $N = 277$ | | IMRT, $N = 382$ | | aOR (95% CI)* | | *p*-value |
|---|---|---|---|---|---|---|---|
| | *N*, % | | *N*, % | | | | |
| First year after treatment | 83 | 29.96 | 198 | 51.83 | 2.10 | (1.37, 2.56) | 0.0013 |
| Second year after treatment | 67 | 24.19 | 119 | 31.15 | 1.55 | (1.08, 2.21) | 0.0151 |
| Third year after RP | 53 | 19.13 | 101 | 26.44 | 1.35 | (1.08, 2.21) | 0.0084 |
| Fourth year after treatment | 39 | 14.08 | 81 | 21.20 | 1.24 | (1.07, 2.21) | 0.0071 |
| Fifth year after treatment | 27 | 9.80 | 61 | 15.97 | 1.09 | (1.02, 1.81) | 0.0379 |

**Multivariate model with adjustment for covariates: Age, year of diagnosis, CCI score, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, diabetes, hypertension, income, hospital area, hospital level, clinical T stage, Gleason score, grade, preoperative PSA, and Damico risk classification. Least square mean difference for continuous variables and odds ratio for binary variables through the fitting generalized linear model with stratification of matched pairs.*
*RP, radical prostatectomy; IMRT, intensity-modulated radiation therapy; Ref, reference group; CI, confidence interval.*

1.09 (1.02–1.81) for the first, second, third, fourth, and fifth years, respectively, compared with that of RP.

## Medical Reimbursement for Urinary Diseases or Treatment-Related Complications Stratified by RP and IMRT

Treatment costs were lower for RP because treatment-related complications were fewer after RP than after IMRT, with approximately 55% reduction in the first year ($p < 0.0001$) and ∼30% reduction in the second to fourth years (**Table 3**). The total medical claims amounts of RP and IMRT + ADT over 5 years were 5,65,313 New Taiwan Dollars (NTD) and 9,60,692 NTD in terms of the mean value (**Table 3**) and 3,51,598 NTD and 7,24,702 NTD in terms of the median value, respectively. RP was associated with a saving of 395,709 NTD, which was approximately 80% of the medical cost of IMRT at that time (**Table 3**). In the generalized linear mixed model with adjustment for covariates (**Supplementary Table 1**, online only), the mean difference (95% CI) between RT and IMRT + ADT was 2,69,823 (2,47,676–2,91,970, $p < 0.0001$), 40,803 (17,379–54,228, $p < 0.0001$), 36,202 (24,375–68,029, $p < 0.0001$), 26,708 (11,179–54,595, $p = 0.0321$), and 12,173 (17,140–41,487, $p < 0.0187$) for the first, second, third, fourth, and fifth years, respectively. The bar plots of medical costs trends by time were presented in **Figure 1**.

## DISCUSSION

The incidence of LPC is likely to increase in the future due to population aging (the number of older persons is projected to double) and increased life expectancy worldwide (15). In the United States, cancer medical costs showed a 27% increase within 2010–2020, with the largest proportion (42%) of cost accounting for PC (29). In addition, the global cost of PC has increased considerably, from USD11.85 billion in 2010 to USD18.53 billion in 2020 (30). Gaining a comprehensive understanding of therapy cost requires comprehensive knowledge; thus, measuring health care costs is a great challenge faced by health care providers. The society and the national government health departments have prevented such unnecessary expenditure by encouraging bundled payments provided by insurance reimbursement. Such action can systematically reduce the cost throughout PC treatment (30). Nevertheless, no comparative study has evaluated the long-term medical resource consumption of the curative-intended therapies of RP and IMRT for men with HR-LPC until now. To the best of our knowledge, our study is the first population-based study of the long-term medical resource consumption of the RP and IMRT modalities according to the number of urological clinical visits, hospitalization rate, and medical costs for treatment-related complications (**Tables 2**, **3**). According to our findings, RP significantly decreased the number of urology outpatient clinic visits required postoperatively compared with IMRT + ADT and effectively reduced the hospitalization rate for urinary diseases or treatment-related complications as well as succeeding medical reimbursement arise for urinary diseases or treatment-related complications compared with IMRT (**Tables 1**–**3**).

From the first year post treatment onward, the number of urology outpatient clinic visits showed a significant difference between the RP and IMRT plus long-term ADT groups (**Table 1**). The higher number of outpatient clinic visits in the IMRT group indicated the significant medical resource consumption for old men with NCCN-HR-LPC compared with the RP group (median visit numbers of 12.9% and 16.8% for RP and IMRT, respectively, $p < 0.0001$). In each follow-up year (first, second, third, fourth, and fifth years), a higher disparity was observed in the medical resource consumption of the modalities. This finding indicated lower medical resource consumption for old patients with NCCN-HR-LPC who underwent RP than for those who underwent IMRT plus ADT. The number of outpatient clinic visits after 5 years was similar between both modalities, as affected by the slowly regressing medical resource consumption between the RP and IMRT groups. This medical resource consumption (**Tables 1**–**3**) might be attributed to treatment-related complications. Side effects after IMRT + ADT include urinary incontinence (31), gastrointestinal toxicity, and soreness and swelling, as after-effects of radiation exposure.

The trend of hospitalization for urinary diseases or treatment-related complications was similar to that of the number of urology outpatient clinic visits (**Tables 1**, **2**). The medical resource consumption of the RP group was superior to that of the IMRT group in the long-term 5-year follow-up due to the fewer hospitalizations (**Table 2**). With time, the difference in the medical resource consumption between the two treatments

**TABLE 3 |** Generalized linear mixed model of medical reimbursement for urinary diseases or treatment-related complications stratified by RP and IMRT.

| Medical cost (NTD) | | RP (Ref), $N = 277$ | | IMRT, $N = 382$ | | Mean difference (95% CI)* | $p$-value |
|---|---|---|---|---|---|---|---|
| First year after treatment | Mean (SD) | 2,17,606.6 | (1,32,341.8) | 4,87,430.0 | (1,64,201.9) | 2,69,823 (2,47,676, 2,91,970) | <0.0001 |
| | Median (IQR, Q1–Q3) | 1,76,838 | (1,44,113–2,21,615) | 4,70,451 | (4,25,597–5,23,395) | | |
| Second year after treatment | Mean (SD) | 90,479.5 | (1,53,852.6) | 1,31,282.9 | (1,59,277.0) | 40,803 (17,379, 54,228) | <0.0001 |
| | Median (IQR, Q1–Q3) | 45,563 | (2,57,55–77,938) | 70,793 | (37,366–1,32,908) | | |
| Third year after treatment | Mean (SD) | 86,160.1 | (1,12,929.6) | 1,22,362.0 | (1,69,854.7) | 36,202 (24,375, 68,029) | <0.0001 |
| | Median (IQR, Q1–Q3) | 42,384 | (23,572–84,132) | 70,088 | (34,006–1,36,271) | | |
| Fourth year after treatment | Mean (SD) | 85,983.9 | (1,74,618.0) | 1,12,692.2 | (1,75,758.5) | 26,708 (11,179, 54,595) | 0.0321 |
| | Median (IQR, Q1–Q3) | 37,628 | (17,125–67,590) | 52,330 | (23,338–1,26,386) | | |
| Fifth year after treatment | Mean (SD) | 85,083.3 | (1,30,088.1) | 97,256 | (1,45,400.2) | 12,173 (17,140, 41,487) | 0.0187 |
| | Median (IQR, Q1–Q3) | 49,185 | (28,120–91,347) | 61,040 | (31,871–1,00,628) | | |

**Multivariate model with adjustment for covariates: Age, year of diagnosis, CCI score, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, diabetes, hypertension, income, hospital area, hospital level, clinical T stage, Gleason score, grade, preoperative PSA, and Damico risk classification. Least square mean difference for continuous variables and odds ratio for binary variables through fitting generalized linear model with stratification of matched pairs.*
*RP, radical prostatectomy; SD, standard deviation; IQR, interquartile range; IMRT, intensity-modulated radiation therapy; Ref, reference group; NTD, New Taiwan Dollars; N, number; CI, confidence interval.*

narrowed. The hospitalization rates for urinary diseases or treatment-related complications of RP and IMRT in the first and fifth years were 29.96% and 51.83% ($p = 0.0013$) and 9.80% and 15.97% ($p = 0.0379$); thus, in terms of hospitalization, the medical resource consumption of the RP group was half of that of the IMRT group. These findings support the findings of Cooperberg et al. (7) that IMRT is actually less effective and more expensive than RP. The NHI universal program is a compulsory enrollment system for all citizens and foreign residents of Taiwan and thus covers almost the entire population. Up to now, it includes up to 99.8% of the 23.57 million inhabitants of Taiwan. This insurance system ensures that everyone has the same accessibility and affordability to medical care, with the extensive coverage of emergency care, inpatient and outpatient care, imaging and laboratory tests, prescription drugs, traditional Chinese medicine, dental services, and home nursing care (32–35). Therefore, our study represents a comprehensive comparative study of the long-term medical resource consumption of RP and IMRT. Our findings indicate that RP is associated with less medical resource consumption in old men with NCCN-HR-LPC compared with IMRT; this is prevalent even in HR-LPC with more aggressive cancer behavior and advanced tumor stages.

In this study, the generalized linear mixed model was used to evaluate medical reimbursement for urinary diseases or treatment-related complications for RP or IMRT (**Table 3**). Based on the analysis results, RP was more cost-effective than IMRT in each year or overall from the beginning until the end of the follow-up period. Therefore, this hints that RP has more favorable outcomes with potential fewer complications and side effects, and it has less medical resource consumption. RP is correlated with positive margin rates of up to 50% (36). In addition, the RP approach for NCCN-HR-LPC requires adherence to several principles (37), as follows: (1) complete removal of the gland, (2) confirmed negative surgical margins intraoperatively on the frozen section, and (3) great performance of the extended pelvic lymph node dissection. In general, RP might be more complicated and difficult to perform for advanced tumor stages in men with NCCN-HR-LPC; post-RP complications might be more in men with HR-LPC, especially in old men (38, 39). Our results contradict the hypothesis that old men might be more suitable for IMRT rather than for RP (1, 3, 4). In our results, higher medical resource consumption was found for IMRT than for RP in old men with NCCN-HR-LPC (**Tables 1**–**3**). In addition, the mean follow-up time for the two treatments was similar (**Supplementary Table 1**, online only); therefore, there was no competing risk of mortality in the endpoint of the medical resource consumption between RP and IMRT (40).

RT has several disadvantages, namely time and resource consuming (9, 41). IMRT is the advanced RT technique that enables higher conformal therapy for differentiating the adjacent normal tissue from the targeted tumor, allowing better dose distribution and delivering an escalated dose to the targeted area (9–12). In terms of advantages of IMRT, the cost of IMRT likely depends on radiation conformity, which can decrease the area of tissue exposed to high-dose radiation (9–12). Moreover, the radiation costs are mostly influenced by the total number of treatments and the fixed costs of the equipment (30). With the progression of contemporary RT techniques, the cost of IMRT might be higher in the near future (42, 43). Our study showed a higher medical resource consumption in IMRT than in RP. We believe that the medical resource consumption might be different in the next generation of proton therapy with fewer RT-related complications and toxicities, although the proton therapy is very expensive (42–44).

This study has many strengths. First, the entire dataset of old men with NCCN-HR-LPC undergoing RP and IMRT

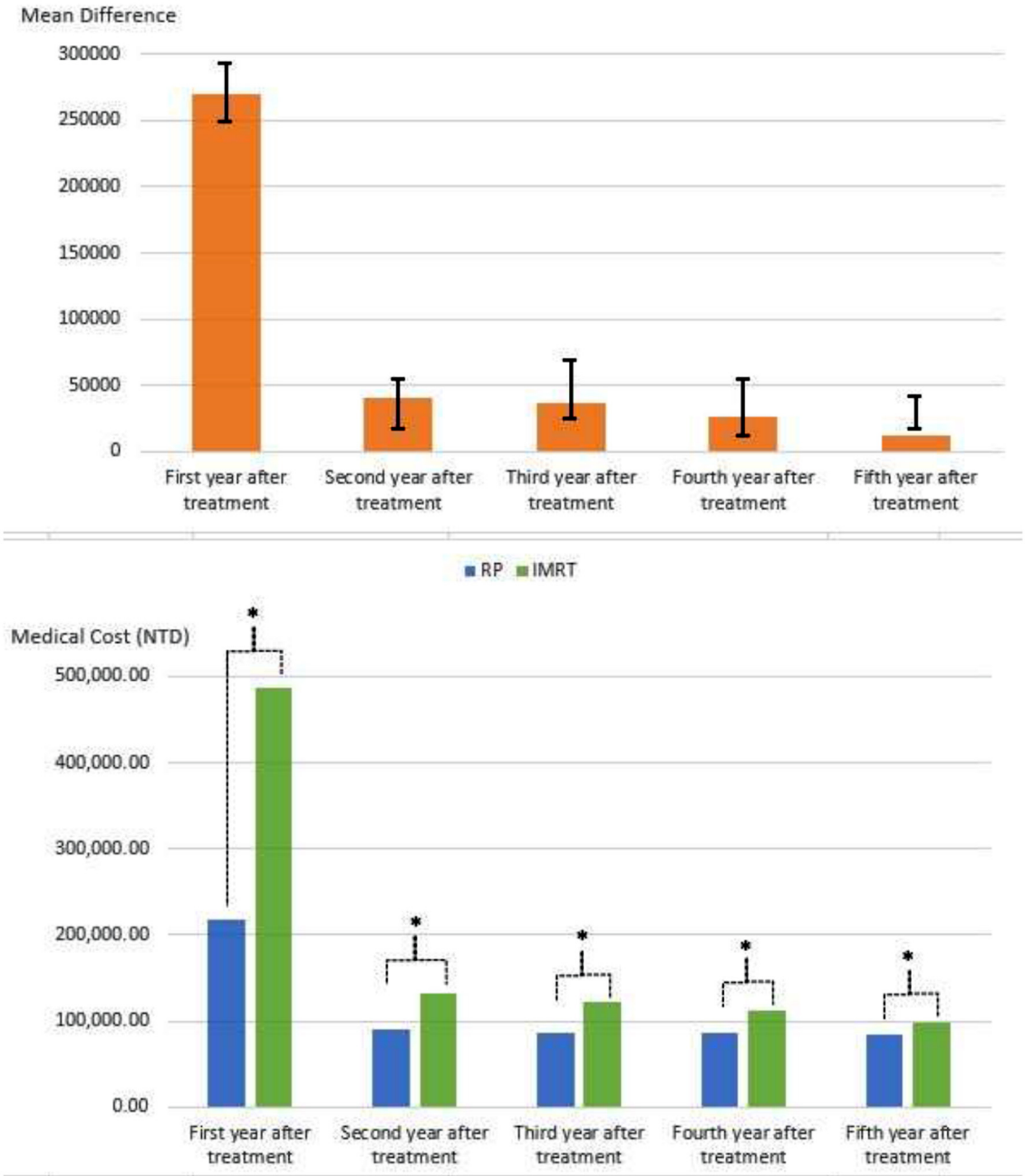


**FIGURE 1 |** The bar plots of medical costs trends by time stratified by RP and IMRT. RP, radical prostatectomy; IMRT, intensity-modulated radiation therapy; NTD, new Taiwan dollars. *$P < 0.05$.

was retrieved from the TCRD in Taiwan, which represented almost the entire population. Second, the data were collected periodically, and the study population was followed up successively for 5 years. Third, covariates were balanced between the RP and IMRT groups, which decreased bias probability. Additionally, all medical costs have been paid by NHI and data recorded in the NHIRD. Therefore, there is no non-direct costs of care that may confound and/or influence interpretation of these direct costs findings. The findings of this study can assist physicians and patients in choosing the most effective and optimal therapy for old patients with NCCN-HR-LPC considering the medical cost, quality of life, and treatment-related complications. Our findings provide a valuable reference for shared decision-making by old patients and physicians and for establishing health policies for providing national health services. Quality of life and empirical clinical outcomes should be considered when selecting curative-intent treatments in old men with HR-LPC, which are expected to have a higher economic burden in the future, and the most cost-effective treatment option should be determined, especially for HR-LPC. This study

provided the first complete nation-wide empirical population-based evidence that RP could be the preferred treatment option for old men with NCCN-HR-LPC considering both clinical and economical endpoints.

However, this study has several limitations. First, it only considered patients with treatment covered by Taiwan's NHI system and did not consider treatment with out-of-pocket payment. However, such old men with NCCN-HR-LPC were most likely to be few. Furthermore, the cost might vary between countries. Therefore, the findings may not be generalized to other countries. Despite these limitations, this is the first population-based cohort study with current updated information and long-term follow-up for the medical resource consumption of RP and IMRT. The results can help in formulating health care policies, particularly for the medical reimbursement of the treatment modalities for the old men with NCCN-HR-LPC.

## CONCLUSIONS

The total medical resource consumption in the RP group of old men with NCCN-HR-LPC was less in terms of the number of urology outpatient clinic visits, the number of hospitalizations for urinary diseases or treatment-related complications, and medical reimbursement for urinary diseases or surgical complications compared with the high-dose IMRT plus long-term ADT group.

## DATA AVAILABILITY STATEMENT

The data used in this study is from the National Health Insurance Research Database and Taiwan Cancer Registry Database. The data cannot be made available due to the Personal Information Protection Act executed by Taiwan's government, starting in 2012. Requests for data can be sent as a formal proposal to obtain approval from the Ethics Review committee of the appropriate governmental department in Taiwan. Specifically, links regarding contact info for which data requests may be sent to are as follows: http://nhird.nhri.org.tw/en/Data_Subsets.html#S3 and http://nhis.nhri.org.tw/point.html. Requests to access the datasets should be directed to szuyuanwu5399@gmail.com.

## ETHICS STATEMENT

The study protocols were reviewed and approved by the Institutional Review Board of Tzu-Chi Medical Foundation (IRB109-015-B). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

S-YW, FE, JP, and C-CH: conception, design, and manuscript writing. S-YW and C-CH: collection and assembly of data. S-YW: data analysis, interpretation, and administrative support. All authors: final approval of manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.843709/full#supplementary-material

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# Evaluation of Bedtime vs. Morning Levothyroxine Intake to Control Hypothyroidism in Older Patients: A Pragmatic Crossover Randomized Clinical Trial

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**Introduction:** Drug scheduling in older adults can be a challenge, especially considering polypharmacy, physical dependency, and possible drug interactions. Properly testing alternative treatment regimens could therefore help to overcome treatment barriers. Hypothyroidism is a prevalent condition in older adults, however, studies evaluating L-thyroxine treatment effectiveness in this specific age group are still lacking. Most studies testing an evening administration of levothyroxine were mainly composed of younger adults. Therefore, this trial is aimed to assess if evening levothyroxine (LT4) administration can effectively control hypothyroidism in older patients.

**Materials and Methods:** A randomized crossover clinical trial was conducted between June 2018 and March 2020 at the Hospital de Clínicas de Porto Alegre, a teaching hospital in Brazil, to compare the efficacy of morning and evening administration of LT4 for hypothyroidism control in older patients. The study protocol is published elsewhere. A total of 201 participants, ≥60 years old, with primary hypothyroidism treated with LT4 for at least 6 months and on stable doses for at least 3 months were included. Participants were randomly assigned to a starting group of morning LT4 intake (60 min before breakfast) or bedtime LT4 intake (60 min after the last meal). After ≥12 weeks of follow-up, a crossover between strategies was performed. The primary outcome was the change in serum thyrotropin (Thyroid-Stimulating Hormone; TSH) levels after 12 weeks of each LT4 administration regimen.

**Results:** A total of 201 participants with mean age of 72.4 ± 7.2 years were included, out of which 84.1% were women; baseline characteristics and frequency of controlled

hypothyroidism were similar between groups. Mean baseline TSH was 3.43 ± 0.25 mUI/L. In total, 118 participants attended three meetings, allowing 135 comparisons by crossover analytic strategy. Mean TSH levels after follow-up were 2.95 ± 2.86 in the morning group and 3.64 ± 2.86 in the bedtime group, $p = 0.107$.

**Discussion:** Thyroid-Stimulating Hormone levels and frequency of controlled hypothyroidism were similar during the follow-up period regardless of the treatment regimen (morning or bedtime).

**Keywords: older adults, thyroxine, hypothyroidism, clinical trial, treatment, evening**

## INTRODUCTION

The reduced production of thyroid hormones results in a clinical state named hypothyroidism; the thyroid gland (primary) is affected in 99% of the cases and autoimmunity is the major etiology (90% of the cases) in sufficient iodine areas (1). Robust epidemiologic data on population thyroid function levels come from large studies from Europe and the United States (2–4). The mean annual incidence of spontaneous hypothyroidism in the Whickham Survey Cohort (1972–1993) was 35 cases per 10,000 women and 6 cases per 10,000 men (3). The National Health and Nutrition Examination Survey–NHANES–(1988–1994) reported a 4.6% prevalence of hypothyroidism (0.3% clinical hypothyroidism and 4.3% subclinical hypothyroidism) and a 97.5% increase in Thyroid-Stimulating Hormone (TSH) levels with aging (4). Hypothyroidism treatment seeks to resolve the signals and symptoms of the condition and to re-establish serum TSH levels to reference limits (5).

When thyroxine sodium salt was first introduced in 1949, levothyroxine (LT4) became a treatment option for hypothyroidism and, eventually, monotherapy was established as the standard choice of therapy (6). Tablets need the physiologic acid gastric environment to dissolve, and the small intestine (duodenum and jejuno-ileum) is the main location of absorption (7, 8). Healthy volunteers can reach 60–80% of levothyroxine bioavailability and require about 2–3 h from the oral intake to reach maximum serum concentration (9–11). Changes in pharmacokinetics can impair levothyroxine replacement and are caused by (1) gastrointestinal disorders, (2) drug-food interactions, (3) drug-drug interactions, and (4) old age (12, 13). In a study with euthyroid participants over 70 years old, thyroxine absorption was 9.4% lower (62.8 ± 13.5% SD vs. 69.3 ± 11.9% SD; $p < 0.001$) than for younger counterparts (13). A complex drug regimen is prevalent in older ages and can result in negative clinical effects, such as lower adherence (14). In cross-sectional surveys, the non-compliance of levothyroxine, defined as proportion of days covered (PDC) as <80%, reaches 50% or more of individuals with hypothyroid (15, 16) and is associated with the presence of other comorbidities (17). Furthermore, a Brazilian study showed that 40.6% of the samples used levothyroxine together with other medication, such as proton-pump inhibitors (PPIs) and calcium and iron supplements (18).

Other levothyroxine intake regimens have thus been proposed to improve treatment effectiveness. As an example, Bevenga et al. (19) showed that preponing levothyroxine intake from 15–20 to 60 min before breakfast resulted in higher TSH suppression during a follow-up of 4–15 months. In a crossover study based on their pilot trial (20), Bolk et al. assessed the effects of bedtime levothyroxine administration vs. administration 30 min before breakfast. At the end of 24 weeks, LT4 bedtime intake was as effective as morning intake regarding thyroid hormone levels (mean age 48 years) (21). Recently, a meta-analysis that grouped six studies ($n = 527$) on LT4 timing; altogether, LT4 administration for 30 min between the administration and before breakfast and at bedtime had no statistically significant differences in TSH levels [standard mean differences (SEM) = −0.19, 95% CI: −0.53, 0.15; $p = 0.28$] (22).

Drug scheduling in older adults can be a challenge, especially considering polypharmacy, dependency, and possible pharmacological interactions. Proper tests of alternative drug regimens can thus help to overcome treatment barriers. Hypothyroidism is a prevalent condition in older adults, however, studies evaluating L-thyroxine treatment effectiveness in this specific age group are still lacking. Most studies, which tested a bedtime administration of levothyroxine, were mainly composed of younger adults (19–21). Therefore, this trial seeks to assess if bedtime levothyroxine (LT4) administration can effectively control hypothyroidism in older patients.

## MATERIALS AND METHODS

The detailed trial protocol was published elsewhere (23). This research was approved by the Hospital de Clínicas de Porto Alegre Research Ethics Committee, in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines (24). All participants provided written informed consent. *The Fundo de Incentivo à Pesquisa e Eventos* (FIPE) from the Hospital de Clínicas provided primary financial support for the trial. The funder had no role in the design, analysis, or reporting of the trial. National Clinical Trial Identifier number is NCT03614988.

## STUDY DESIGN AND SETTING

Study design and setting: Pragmatic, randomized, crossover clinical trial was conducted at the Endocrinology and Internal Medicine Outpatient clinics at the Hospital de Clínicas de Porto Alegre, Brazil.

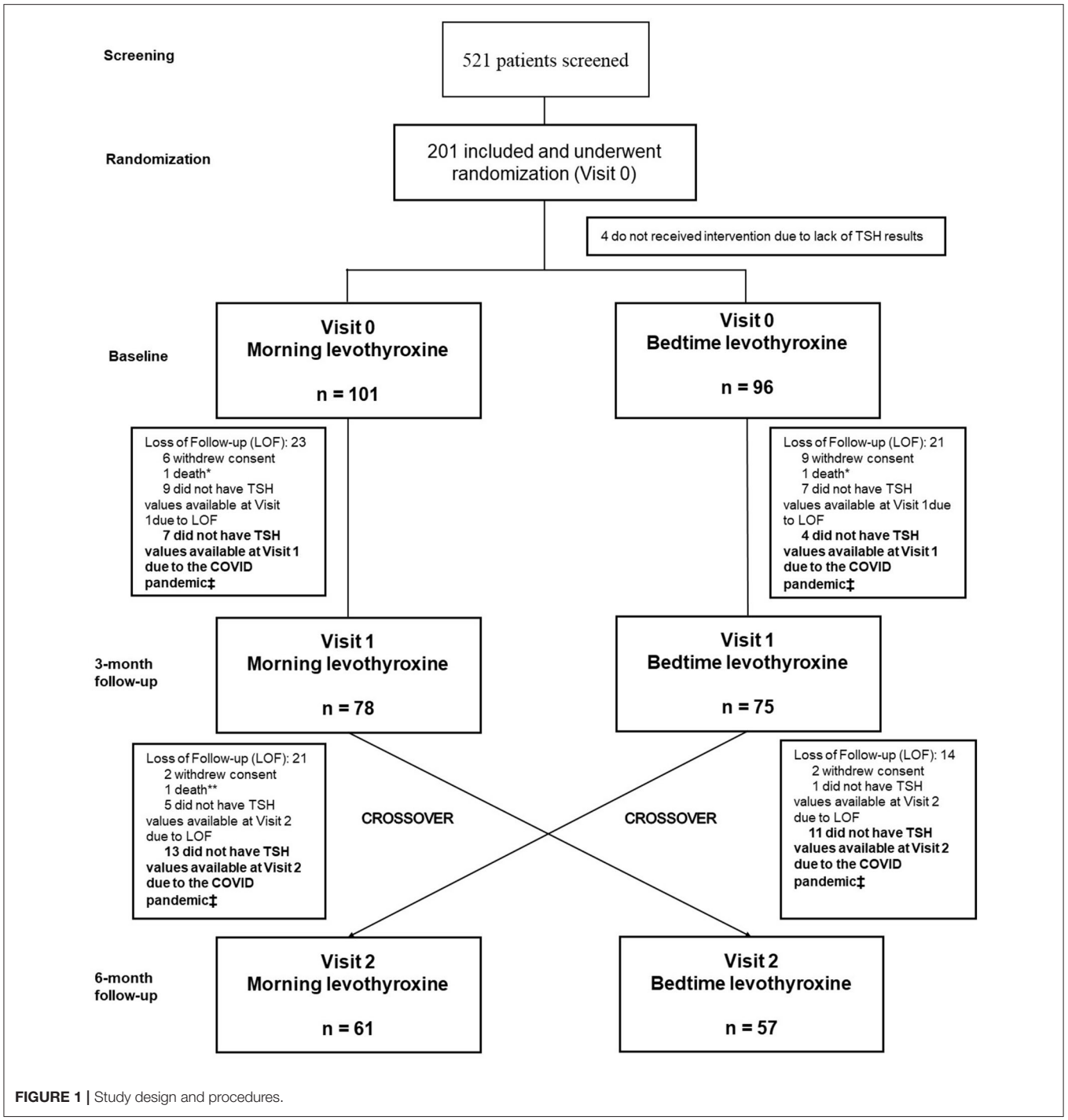


**FIGURE 1 |** Study design and procedures.

## Eligibility Criteria

Participants were identified from the Endocrinology and Internal Medicine Clinics at the Hospital de Clínicas de Porto Alegre, a teaching hospital from Universidade Federal do Rio Grande do Sul, a top-ranked university in Brazil. Inclusion criteria were outpatients ≥60 years old with primary hypothyroidism who had been using LT4 for at least 6 months and were on stable doses for the last 3 months. Exclusion criteria were severe chronic diseases, such as end-stage renal failure, severe chronic obstructive pulmonary disease (COPD), severe hepatic failure, advanced cancer, dementia, thyroid cancer, heart failure (functional class IV), patients under palliative care, three or more hospital admissions during the last year due to heart failure decompensation, and refusal to participate.

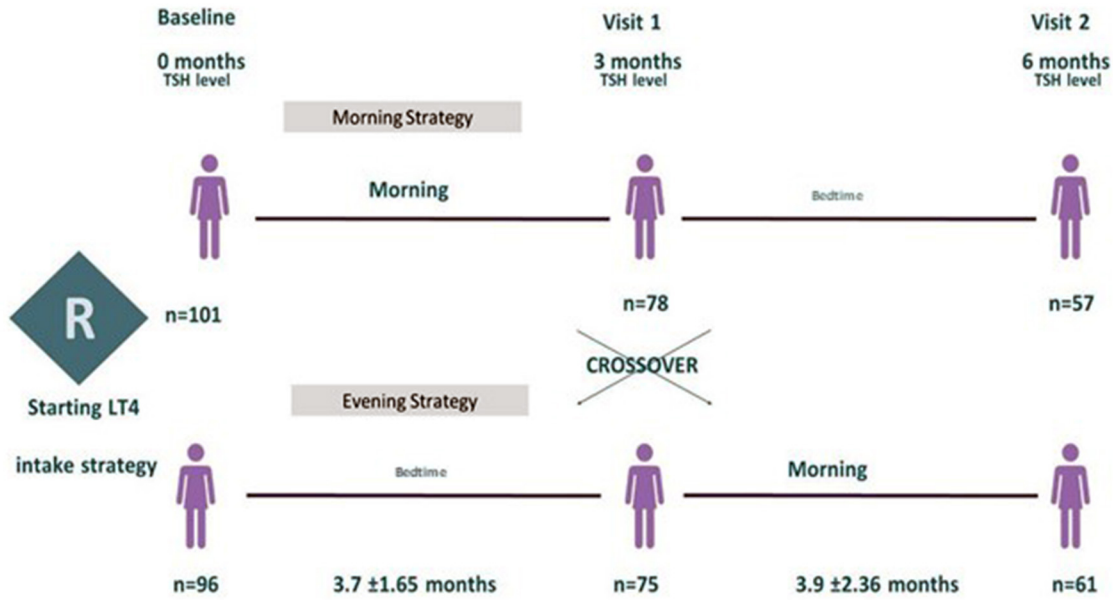


**FIGURE 2 |** Flowchart of the study displays participants enrollment from screening to study completion. *Unknow causes **Lung Cancer ‡ COVID pandemic imposed research limitations (The Clinical Research Center was closed for non-urgent or not COVID-related researches in February 2020).

## RANDOMIZATION

A randomization list stratified by sex and age (≥75 years) was created using a web-based program (http://www.randomization.com/). Interchangeable random blocks/variables' blocked randomization strategy was chosen to conceal allocation and to avoid intergroup disparities from the predicted sample size. An independent researcher was responsible for the randomization list and for treatment allocation.

After enrollment, participants underwent randomization in a 1:1 ratio to define the starting treatment regimen.

### Intervention

According to random treatment allocation, participants were instructed to take LT4 tablets 60 min before breakfast (morning strategy) or at bedtime, at least 60 min after the last meal (bedtime strategy). Different from other western countries, Brazilian breakfast is a light meal, being lunch the heaviest meal in a common day. In Brazil, the last meal is usually a light meal like breakfast, mainly based on fruits, bread, milk, and dairy, occurring 2 h after supper (similar but usually lighter than lunch). A crossover was planned to be implemented 12 weeks after the baseline assessment. Since the hypothyroidism-diagnosed patients were already under treatment, the study did not provide LT4 tablets, to encourage them to keep the same drug brand and doses they were used to take before enrollment. The intervention was the drug scheduling itself. Furthermore, levothyroxine intake was pragmatically instructed as in a doctor's office visit, reinforcing it should be taken at least 1 h before breakfast and at least 1 h after the last meal at bedtime. Similarly, as in a usual clinical visit, participants were instructed to take LT4 with water and to avoid taking other medications for at least 60 min. No treatment dose adjustments were conducted by researchers; however, those participants presenting altered TSH levels were instructed to seek their doctors to evaluate the need for LT4 treatment adjustments.

### Blinding

Considering the pragmatic intervention strategy, researchers and participants were not blinded. The researcher responsible for statistical analysis was blinded to the treatment allocation. Moreover, the primary outcome was an objective laboratory test result, not influenced by external perceptions.

## STUDY PROCEDURES

Follow-up, crossover, and study procedures: Randomized participants had to attend three study visits, baseline assessment, and 3- and 6-month follow-up visits. Detailed study procedures for each visit were described elsewhere (23). In short, the first follow-up visit was scheduled for 3 months (12 weeks) after baseline assessment and randomization to the starting treatment regimen to assess thyroid function tests and check for treatment adherence and adverse events during follow-up (**Figure 1**). At the end of the 3-month follow-up visits, participants were oriented to switch to the alternative treatment regimen and a 6-month follow-up visit was scheduled to reassess thyroid function tests. Although the LT4 has a late peak effect and long half-life, the late carryover effect was not expected since the main outcome would be assessed 12 weeks after the treatment strategy switch. Thus, a wash-out period between strategies was not applied.

**TABLE 1 |** Baseline characteristics of the 201 enrolled participants.

| | ***n* = 201** |
|---|---|
| **Characteristic** | **mean ± SD or median (IQR) or *n* (%)** |
| Age (years) | 72.5 ± 7.2 |
| Female | 169 (84.1) |
| Years of schooling | 5.6 ± 4.2 |
| TSH level mUI/L median (IQR) & | 2.59 (1.28–4.30) |
| Free T4 ng/dl median IQR # | 1.35 (1.17–1.58) |
| Hypothyroidism ethology | 170 (84.6) |
| Hashimoto's Thyroiditis | 22 (11.0) |
| Total Thyroidectomy | 7 (3.5) |
| Radioactive Iodine Therapy for Hyperthyroidism | 2 (1.0) |
| Lobectomy due to benign nodule | |
| Time of hypothyroidism diagnosis in months - median (IQR) | 120 (60–216) |
| Time on Levothyroxine stable doses in months- median (IQR) | 22.5 (8.2–53.7) |
| Levothyroxine dose (mcg) | 91.4 ± 45.7 |
| Usual levothyroxine intake regimen prior to the study | |
| Morning | 199 (99) |
| Bedtime | 2 (1.0) |
| Interval between levothyroxine and food | |
| ≤29 minutes | 46 (22.9) |
| 30–59 minutes | 72 (35.8) |
| ≥60 minutes | 83 (41.3) |
| Possible interfering medications ǂ | |
| Yes | 77 (38.3) |
| PPI | 66 (32.8) |
| Calcium supplement | 16 (8) |
| Multivitamin supplements | 2 (1.0) |
| Iron supplement | 3 (1.5) |
| No | 117 (58.2) |
| Number of comorbidities | 6 ± 1.9 |
| Number of medications in use | 7.6±3.1 |
| Functional Capacity (Barthel Scale 0–100 pts) | 94 ± 12.1 |
| Baseline TSH level categories on hypothyroidism control status | |
| TSH < 0.27 | 8 (4) |
| TSH 0.27–4.2 | 137 (68.2) |
| TSH > 4.2 | 52 (25.9) |

*ǂ 7 missing values; & 4 missing values; # data collected in 128 participants PPI = proton-pump inhibitors.*

During all follow-up visits, independent and dependent variables were collected using a standardized questionnaire: food-LT4 intervals (≤29 mins, 30–59 min, or ≥60 min); drug-LT4 interactions (use of PPIs and calcium, multivitamin, and/or iron supplements within 60 min of levothyroxine intake); and the Barthel Scale, which assesses ten items of activities of daily living (ADLs), ranging from 0 points (fully dependent) to 100 points (fully independent). Adverse events were assessed and recorded. Thyroid function (serum TSH and free T4 levels) was measured by electrochemiluminescence assay; concentrations between 0.27–4.2 mUI/L and 0.93–1.7 ng/dl were considered the normal laboratory range.

The primary outcome was the change in serum TSH levels after 12 and 24 weeks of follow-up from the baseline. Secondary outcomes included the identification of concomitant drugs interfering in LT4 absorption and the evaluation of levothyroxine effectiveness between the two treatment strategies (frequency of hypothyroidism control).

SAS Studio 3.7 was used to calculate the sample size in accordance with the parameters described by Bolk et al. (21). A total of 92 analytic pairs should be included (46 individuals randomized for each starting treatment strategy) to detect a difference of 1.0 mUI/L in mean TSH levels between treatment strategies, considering a standard deviation (SD) between 2.5 and 3.0, with 80% power and 5% significance level. Adding 10% for possible losses and refusals, the sample size should be 100 (50 per starting treatment regimen).

## Statistical Analysis

Quantitative variables with normal distribution were described as mean and SD and variables with non-normal distribution as median and interquartile range (IQR). Qualitative variables were described as absolute and relative frequencies. The treatment effect and carryover effect were analyzed by generalized estimating equation (GEE) models for crossover studies with gamma distribution. A sensitive analysis using General Linear Model (GLM) for repeated measures and a *t*-test for independent samples to compare visit 1 results was also performed and presented in the **Supplementary Material** section.

Because of the COVID pandemic, this study follow-up was initially restricted in February 2020 and then definitively interrupted at the end of March 2020. Therefore, researchers were unable to collect clinical or laboratory data for 35 enrolled participants, with 11 losses in the 3-month follow-up visit and 24 others in the 6-month follow-up visit.

# RESULTS

## Trial Population

We screened 521 outpatients diagnosed with primary hypothyroidism who were at least 60 years old. Of 201 participants who fulfilled the eligibility criteria and underwent randomization, 101 participants were assigned to the morning strategy as the first treatment regimen and 96 to the bedtime strategy. Four patients did not collect thyroid function at baseline and were excluded just after randomization. During follow-up, 135 participants (78 initially assigned to the morning group and 75 assigned to the bedtime group) were evaluated and had blood samples collected for thyroid function tests at visit 1, whereas 118 participants (61 mornings and 57 bedtimes) did so at visit 2 **Figure 2** shows the study's inclusion flowchart with losses and enrollment details, restrictions imposed by the COVID pandemic, and follow-up losses.

**Table 1** shows the baseline characteristics of the participants. Since the study is a crossover trial, patients have also their own control group; thus, baseline data are summarized and shown as a single group. The mean age was 72.5 ± 7.2 years, 169 participants were women (84.1%), and the main cause of primary hypothyroidism was Hashimoto's thyroiditis

(170; 84.6%). The overall median baseline TSH level was 2.59 (IQR 1.28–4.3) mUI/L. Most individuals were taking generic levothyroxine (110; 54.7%) in the morning (199; 99%) and 30–59 min before breakfast (72; 35.8%). The mean levothyroxine dosage was 91.4 ± 45.7 mcg. LT4 doses were stable for a median time of 22.5 months (IQR, 8.2–53.7) before inclusion. Despite multimorbidity, the mean number of concomitant diagnoses was 6 ± 1.9 and participants were mostly functionally independent (Barthel Scale 94 ± 12.1 points). Sensitivity analysis comparing independent groups defined by randomization and direct comparisons at each time-point is presented in a separate document (**Supplementary Material**). Baseline comparisons between groups are presented in **Supplementary Table S1**. **Supplementary Table S2** presents additional data for LT4 dose adjustments.

## Treatment Compliance During Follow-Up

At visit 1, in the morning group, 6 (7.6%) participants informed that they were taking LT4 < 29 min before breakfast, 24 (30.7%) were taking it 30–59 min before breakfast and 48 (61.5%) participants informed they were taking LT4 at least 60 min before breakfast. In the bedtime group, 43 (57.4%) participants informed usual LT4 intake at least 60 min after the last meal in accordance with the study's protocol; 27 (36%) were taking LT4 30–59 min after the last meal, and 5 (6.6%) were regularly taking LT4 < 30 min after the last meal. No significant differences were found between groups regarding treatment compliance; however, it may reflect a lack of power due to additional grouping to test this hypothesis. At visit 2, numbers were slightly different, favoring better compliance in the bedtime group (not statistically significant). Morning group: 5 (8.2%) participants were taking LT4 < 30 min before breakfast; 18 (29.5%) participants were LT4 taking 30–59 min, and 38 (62.3%) participants were taking the medication 60 min before breakfast. Bedtime group: 3 (5.2%) participants were taking LT4 < 30 min after the last meal, 10 (17.5%) participants were taking it 30–59 min, and 44 (77.2%) participants were taking LT4 60 or more min after the last meal.

## Bedtime vs. Morning LT4 Intake

According to the crossover analysis, the mean TSH levels (mUI/L) were 2.95 ± 2.86 for LT4 intake in the morning and 3.64 ± 2.86 for the bedtime, with no statistical differences between groups and $p = 0.107$ (**Table 2**). Sensitivity analysis was performed on test results of participants with stable LT4 doses during the study's follow-up period and results remained similar, however, there is a trend that morning LT4 intake has lower TSH levels when compared to the bedtime intake group (**Table 2**). A stratified comparison considering three different TSH level categories (low TSH level; normal TSH level; and high TSH level) was also performed, showing no significant differences regarding hypothyroidism control rates (**Table 3**). Further sensitivity analysis showed no significant differences between groups on both TSH and free thyroxine levels when visit 1 data were analyzed individually through an independent group *t*-test analytic strategy or when GLM for repeated measures was conducted to test mean differences between groups during both study's follow-up visits (**Supplementary Tables S3, S4**).

The carryover effect was tested and was not observed ($p = 0.504$). Interestingly, of all participants who completed the study, only 22 (10.9%) said they preferred bedtime LT4 intake over morning intake. The most common adverse event was respiratory tract infection; however, no statistical difference was found between treatment intake strategies.

## Interfering Medications

In total, 77 (38.3%) patients were using possibly interfering medications within 60 min of levothyroxine intake, mostly PPIs (66; 32.8%). Different than initially thought, the mean TSH level is lower during morning LT4 intake than during bedtime intake ($p = 0.033$) as described in **Table 4**. The mean difference of TSH levels according to interfering medication status is presented in **Supplementary Table S5**.

# DISCUSSION

This pragmatic crossover clinical trial compared the effectiveness of two different LT4 treatment regimens to control hypothyroidism in older patients already under stable LT4 doses. No significant differences were found between bedtime or morning LT4 intake regarding TSH levels and hyperthyroidism control rates. Results remained similar after a sensitivity analysis that included only participants who were receiving stable doses of LT4 during the study's follow-up. To the authors' knowledge, this is the first study to compare two different LT4 intake regimens in older patients in a pragmatic real-world clinical trial that includes multimorbid participants and those using interfering medications.

This study's results corroborate with previous publications that analyzed younger populations (25–30). Rajput et al. observed two groups (mean age: 34.30 ± 11.82 years) in a parallel clinical trial, i.e., 152 levothyroxine drug-naïve patients. After 12 weeks of levothyroxine treatment, they found no significant differences in mean TSH levels between $LT_4$ intake 2 h after dinner and 30 min before breakfast (3.27 mUI/L ± 4.19 vs. 5.13 mUI/L ± 9.36; $p = 0.31$) (25). Ahmed et al. (26) and Srivastava et al. (27) tested a similar strategy but increased the LT4 intake interval to 60 min before breakfast. A total of 82 participants were included and, after 3 months of follow-up, no statistical significance was found in TSH levels between groups. The mean difference in TSH reduction from baseline was 13.6 ± 22.2 mUI/L in the morning group and 11.3 ± 22.5 mUI/L in the bedtime group, $p = 0.63$. Srivastava et al. (27) tested the same hypothesis using a crossover model; however, results were analyzed using *t*-test for independent samples, an analytic approach that can overestimate results in crossover studies, especially if a non-parametric variable is the main study outcome. In total, 59 participants were included and the bedtime LT4 administration group had better outcomes.

Different from other studies on alternative LT4 regimens for hypothyroidism control, this crossover study design intended to bring real-world treatment data. Multimorbid

**TABLE 2 |** Effects of bedtime vs. morning levothyroxine intake in Thyroid-Stimulating Hormone (TSH) levels (crossover model).

| **Total Sample** | | **Mean (95%CI)** | |
|---|---|---|---|
| | Morning ($n = 139$) | Evening ($n = 132$) | $p$-value |
| TSH mUI/L | 2.95 (2.47 to 3.43) | 3.64 (3.16 to 4.12) | 0.107 |
| **Sample with stable LT4 doses during follow-up** | | **Mean (95%CI)** | |
| | Morning ($n = 115$) | Evening ($n = 115$) | $p$-value |
| TSH mUI/L | 2.83 (2.37 to 3.29) | 3.56 (3.07 to 4.05) | 0.062 |

**TABLE 3 |** Hypothyroidism control status according to LT4 intake regimen.

| | **Visit 1** | | | **Visit 2** | | |
|---|---|---|---|---|---|---|
| | **Morning ($n = 78$)** | **Bedtime ($n = 75$)** | ***p*-value*** | **Morning ($n = 61$)** | **Bedtime ($n = 57$)** | ***p*-value*** |
| TSH < 0.27 mUI/L | 9 (11.5) | 6 (8) | 0.84 | 4 (6.6) | 5 (8.8) | 0.17 |
| TSH 0.27–4.2 mUI/L | 50 (64.1) | 51 (68) | | 35 (57.4) | 40 (70.2) | |
| TSH > 4.2 mUI/L | 19 (24.4) | 18 (24) | | 22 (36.1) | 12 (21.1) | |

**chi-square test.*

and polypharmacy-exposed older participants were included to provide data on hypothyroidism treatment effectiveness in a pragmatic real-world clinical setting. Multimorbidity and polypharmacy are a major concern in older patients and a great barrier to an optimal levothyroxine treatment regimen, especially because multimorbidity must be addressed during pharmacologic treatment plans. Thus, patients were pragmatically instructed during a visit to the doctor's office, reinforcing that levothyroxine should be taken at least 1 h before breakfast and at least 1 h before the last meal, at bedtime. Older adults are usually excluded from clinical trials mainly because of strict exclusion criteria about interfering medications and multimorbidity. Although this can better demonstrate treatment efficacy, it can also limit effectiveness tests in real-world scenarios. This study situation is suboptimal, but it simulates clinical practice reality in a pragmatic clinical trial, which compares treatment strategies despite interfering factors. Moreover, as mentioned before, randomization and the crossover design were applied to minimize the effects of known and unknown confusion variables over the primary outcome. Crossover trials are efficient since estimated treatment effects are based either wholly or largely on within-subject variance and contrasts between groups, reducing the contribution of the between-subject component and accurately estimating means for a group of patients. However, individualized treatment plans can be considered, especially when a patient is under interfering medications.

A sensitive analysis that included participants under stable doses of LT4 during the study's follow-up corroborated with previously published findings by Appayadin et al. (28) (TSH mean: $2.83 \pm 2.51$ mUI/L in the morning group and $3.56 \pm 2.68$ mUI/L in the bedtime group; $p = 0.062$).

Individuals under interfering medications are commonly excluded from clinical trials on levothyroxine efficacy (25–28). However, Skelin et al. (29) and Bach-Huynhm (30) did not exclude participants using known LT4 interfering medications from their trials. Skeling et al. found no significant differences in TSH levels regarding alternative treatment regimens, possibly because of the lack of power from a small sample size. On the other hand, Bach-Huynhm showed that TSH levels at 24 weeks were significantly lower for fasting LT4 administration than for administration two hours after dinner[1.06 mUI/L, 95% confidence interval (CI), 0.6–1.52 vs. 2.19 mUI/L, 95% CI 1.73–2.65; $p < 0,001$] (30), though both groups achieved TSH levels categorized as controlled hypothyroidism.

Previous studies (31–33) showed worse levothyroxine absorption related to PPIs and multivitamins, calcium, and iron supplements. In this study, patients in the morning group who used the investigated interfering drugs had lower TSH levels, indicating a trend toward morning intake. However, these results must be cautiously interpreted considering measurement bias (interfering medications data were collected as a dichotomous variable; dosing and time of administration were not registered) or analytical imprecision from the small number of participants in this subgroup analysis (57 analytic pairs). **Table 4** shows that the sample size decreases from 135 participant analytic pairs to 57 analytic pairs, making results more imprecise and with larger ranges of confidence intervals. Though the $p$-value is significant, a clear superposition of mean CIs indicates a non-significant result, possibly explained by the study's imprecision to test this specific secondary outcome. Even if a difference was accepted, considering 60% of patients were not taking interfering medications, we assumed a minor impact over the primary outcome, especially because there was a minimal mean TSH level difference between groups defined by the use of intervenient medications.

We emphasize that higher bedtime TSH results are a trend in all pre-specified analyses, which represents a valid and real difference, however, it does not represent a clinically relevant difference since mean TSH levels are still inside

**TABLE 4 |** Effects of bedtime vs. morning levothyroxine intake in Thyroid-Stimulating Hormone (TSH) levels of participants using interfering drugs* 60 min within levothyroxine administration (crossover model).

| | Mean (95%CI) | | |
|---|---|---|---|
| | Morning ($n = 57$) | Bedtime ($n = 52$) | $p$-value |
| TSH mUI/L | 2.81 (2.14 to 3.48) | 4.10 (2.9 to 5.3) | 0.033 |

*Proton-pump inhibitors and calcium, multivitamin, and iron supplements.

the expected target laboratory range categorized as controlled hypothyroidism. Moreover, hypothyroidism control rates were similar between groups. The authors therefore concluded that bedtime LT4 administration can be as effective as morning administration to control hyperthyroidism affecting older adults.

Furthermore, baseline data show a significant heterogeneity regarding levothyroxine intake time, a real-world treatment compliance problem that may influence hypothyroidism control rates in clinical practice. The authors believe that this is another good reason to test alternative treatment regimens in a pragmatic clinical trial since delaying breakfast to favor the absorption of a drug is hard, especially for older adults, an age group frequently affected by multimorbidity (e.g., diabetes, hypertension, and heart failure) and polypharmacy (e.g., antihypertensive and hypoglycemic drugs), conditions to which fasting can impose at least a theoretical risk. This scenario was considered during study planning as an important issue to address in a pragmatic designed study regarding hypothyroidism control in a real-world clinical setting, as we did in this study. However, we observed that the frequency of inappropriate treatment compliance patterns did not change throughout the rest of the study despite of instructions of the investigators, being similar between groups. Maybe it represents a common patient behavior that can influence results in both clinical practice and this specific study.

The main possible limitation of this study is the loss of follow-up by restrictions from the COVID pandemic. However, the final crossover comparison sample size (135 analytical pairs) guaranteed enough power to test for differences with a good precision range, as shown by the small-ranged CIs. Moreover, considering the demonstrated difference of 0.69 mUI/L instead of 1.0 mUI/L (as used to calculate sample size) in TSH levels between groups, 105 included individuals would give 80% power to detect a treatment difference in a two-sided 0.05 significance level, with total samples smaller than the 118 individuals who completed follow-up. Although this study was not blinded, the chosen outcome is an objective laboratory result, minimizing possible external interference. Even though treatment interfering factors (such as the use of interfering drugs and LT4 dose adjustments during follow-up) were considered *a priori* during study planning and proper sensitivity analyzes were conducted, residual confounding secondary to these factors can still impact the study's results. Thus, it should be considered as a possible study limitation as well.

Finally, based on this study's findings, levothyroxine administration at the bedtime was as effective as in the morning to control hyperthyroidism in older patients. However, further pragmatic trials with bigger sample sizes are still needed to confirm these findings and to test the clinical impact of interfering medications over LT4 effectiveness on real-world clinical practice settings and, thus, on hypothyroidism control rates.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Hospital de Clínicas de Porto Alegre Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

KG and RM: analysis. KG, RM, and TC: manuscript writing. KG: data collection. RM and TC: study design, revision, and final approval of the manuscript. RM, KG, GS, MM, MS, BC, VP, and TC: data collection phase, manuscript writing process, and approved the manuscript of the International Committee of Medical Journal Editors criteria for authorship have been met. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.828762/full#supplementary-material

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ORIGINAL RESEARCH
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# Prevalence of Long COVID-19 Symptoms After Hospital Discharge in Frail and Robust Patients

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**Background:** A motley postacute symptomatology may develop after COVID-19, irrespective of the acute disease severity, age, and comorbidities. Frail individuals have reduced physiological reserves and manifested a worse COVID-19 course, during the acute setting. However, it is still unknown, whether frailty may subtend some long COVID-19 manifestations. We explored the prevalence of long COVID-19 disturbs in COVID-19 survivals.

**Methods:** This was an observational study. Patients aged 65 years or older were followed-up 1, 3, and 6 months after hospitalization for COVID-19 pneumonia.

**Results:** A total of 382 patients were enrolled. Frail patients were more malnourished (median Mini Nutritional Assessment Short Form score 8 vs. 9, $p = 0.001$), at higher risk of sarcopenia [median Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls (SARC-F) score 3 vs. 1.5, $p = 0.003$], and manifested a worse physical performance [median Short Physical Performance Battery (SPPB) score 10 vs. 11, $p = 0.0007$] than robust individuals, after hospital discharge following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia. Frailty was significantly associated with: (i) confusion, as a presenting symptom of COVID-19 [odds ratio (OR) 77.84, 95% CI 4.23–1432.49, $p = 0.003$]; (ii) malnutrition (MNA-SF: adjusted B –5.63, 95% CI –8.39 to –2.87, $p < 0.001$), risk of sarcopenia (SARC-F: adjusted B 9.11, 95% CI 3.10–15.13, $p = 0.003$), impaired muscle performance (SPPB: B –3.47, 95% CI –6.33 to –0.61, $p = 0.02$), complaints in mobility (adjusted OR 1674200.27, 95% CI 4.52–619924741831.25, $p = 0.03$), in self-care (adjusted OR 553305.56, 95% CI 376.37–813413358.35, $p < 0.001$), and in performing usual activities of daily living (OR 71.57, 95% CI 2.87–1782.53, $p = 0.009$) at 1-month follow-up; (iii) dyspnea [modified Medical Research Council (mMRC): B 4.83, 95% CI 1.32–8.33, $p = 0.007$] and risk of

sarcopenia (SARC-F: B 7.12, 95% CI 2.17–12.07, $p$ = 0.005) at 3-month follow-up; and (iv) difficulties in self-care (OR 2746.89, 95% CI 6.44–1172310.83, $p$ = 0.01) at the 6-month follow-up. In a subgroup of patients (78 individuals), the prevalence of frailty increased at the 1-month follow-up compared to baseline ($p$ = 0.009).

**Conclusion:** The precocious identification of frail COVID-19 survivors, who manifest more motor and respiratory complaints during the follow-up, could improve the long-term management of these COVID-19 sequelae.

**Keywords: frailty, COVID-19, long COVID-19 syndrome, older people, prevalence**

## BACKGROUND

Coronavirus disease 2019 (COVID-19) can have heterogeneous manifestations (1), but a more severe course is expected in older people, due to the combined effect of immune-aging and accrual of comorbidities over time (2–5). In addition, the exhaustion of physiological reserves in older people, usually known as frailty (6), augments the vulnerability to stressors and enhances the risk of developing negative health outcomes (7). Indeed, a higher lethality of COVID-19 has been demonstrated in frail patients in the acute setting (8).

Worldwide reports showed that between 57 and 76% of people infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) develop persistent COVID-19 sequelae (9, 10) irrespectively of acute COVID-19 severity and age (9, 11–13). The manifestations of the postacute SARS-CoV-2 (PASC) syndrome can be: either residual symptoms and organ dysfunctions persisting after the acute infection or *de-novo* manifestations, which can develop after the resolution of COVID-19 (13, 14). Their course can be fluctuating, increasing, persisting, or relapsing. The Italian National Institute of Health (ISS) divides the PASC syndrome into two categories, according to its length: (i) persistent symptomatic COVID-19, when symptoms and signs last between 4 and 12 weeks after the acute infection and (ii) post-COVID-19 syndrome, when the manifestations prolong more than 12 weeks after the acute disease (15).

The clinical spectrum of the so-called "long COVID-19" is motley, including fatigue, muscle weakness, dyspnea, chest pain, cognitive impairment, depression, anxiety, and insomnia (10).

The etiology of the PASC syndrome has not been completely clarified yet, but it is presumably multifactorial (i.e., dysregulated immune response, endothelial injury) (16, 17) and an organic substratum seems to be present. MRI studies in patients with post-COVID-19, who suffered from either a mild or a severe SARS-CoV-2 infection, showed a multiorgan impairment inflammation, regional scarring, and ectopic fat deposition (18, 19).

An association between comorbidities (particularly obesity and psychiatric conditions) and impairment in recovering completely after SARS-CoV-2 infection has been reported (20). Indeed, the exhaustion of physiological reserves (*i.e.,* frailty) (7) could favor the persistence of COVID-19 complaints. Many COVID-19 survivors manifest sarcopenia (21) because of the intense catabolic stimuli, bed rest, weight loss, inadequate protein supply, and steroid therapies during a hospital stay. The persistence of sarcopenia after hospital discharge (22) represents a condition closely related to frailty (23) and may subtend some long COVID-19 manifestations (i.e., fatigue, myalgias) (6).

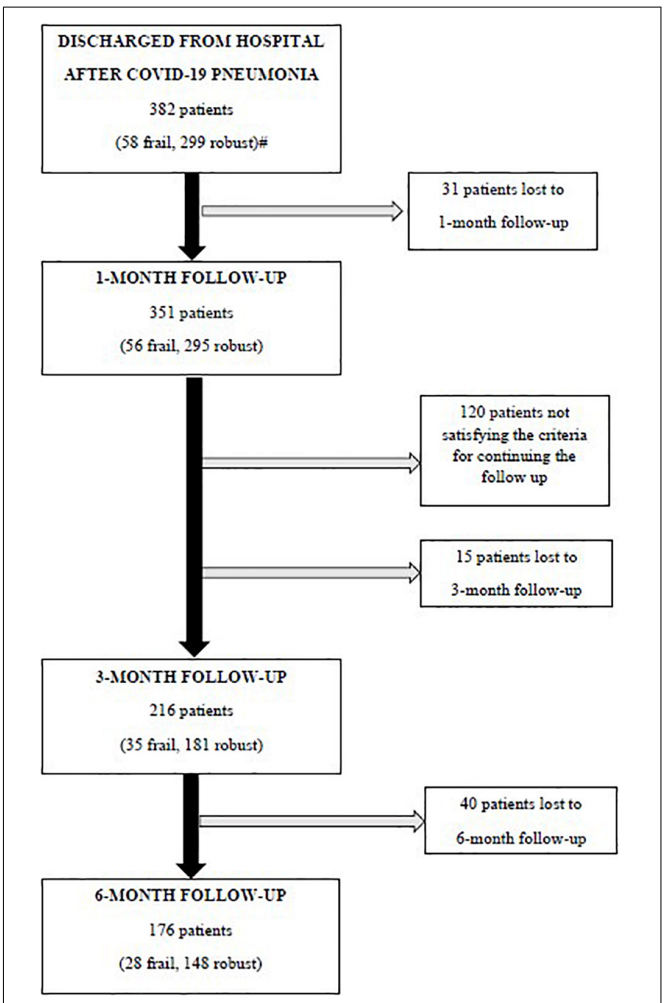


**FIGURE 1 |** Flowchart of the follow-up visits. Criteria for continuing the follow-up: (i) a respiratory rate >17 breaths/min plus, either a history of coronary heart disease, or the general Visual Analog Scale (VAS) score <86 or a C-reactive protein at hospital admission >149 mg/l or (ii) patients aged 63 years or older with a respiratory rate <17 breaths/min, but a body mass index (BMI) ≥26 kg/m$^2$, continued the follow-ups at 3 and 6 months. #Missing data for calculating the baseline frailty index in 25 patients.

No study has evaluated so far, whether long COVID-19 symptoms varied according to the frailty status in patients recovering after hospitalization for SARS-CoV-2 infection.

We analyzed whether the prevalence of the PASC symptoms was different between frail and robust patients, after hospital discharge, following COVID-19 pneumonia. Moreover, we assessed the association between frailty and the PASC manifestations at 1-, 3-, and 6-month follow-ups after hospital discharge, through regression analyses. Finally, we analyzed the variations of the frailty status over time during the follow-up visits.

## MATERIALS AND METHODS

This was a prospective observational study. We evaluated patients aged 65 years or older, who attended a dedicated post-COVID-19 outpatient clinic. These patients were previously hospitalized for SARS-CoV-2 pneumonia in the Internal Medicine Department of the San Raffaele University Hospital, Milan, Italy (24) and discharged alive. Visits took place 1, 3, and 6 months after hospital discharge from 5 November 2020 to 2 November 2021.

The present study was part of the COVID-BioB study (NCT04318366). COVID-BioB wanted to characterize patients with COVID-19 who were hospitalized for pneumonia, through the prospective collection of demographic, anthropometric, clinical, and laboratory data (25). The COVID-BioB protocol was approved by the San Raffaele University Hospital Ethics Committee (protocol no. 34/int/2020).

During the follow-up visits, the patients underwent a multidimensional evaluation consisting in anamnesis, medical examination, anthropometric measurements, screening for sarcopenia through the Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls (SARC-F) questionnaire (26), assessment of muscle strength through the hand grip strength test (27), evaluation of muscle performance with the Short Physical Performance Battery (SPPB) test (28), screening for malnutrition with the Mini Nutritional Assessment Short Form (MNA-SF) questionnaire (29), evaluation of the quality of life through the EuroQol Group Health Questionnaire 5D-3L (30), and the Visual Analog Scale (VAS) for general health (31). Moreover, the number of persistent and *de-novo* COVID-19 symptoms and manifestations were assessed at each visit. Symptoms were self-reported by the patients during the follow-up visits or written *via* email to the attending physicians of the follow-up clinic. All the patients with *de-novo* symptoms underwent a nasal swab for detecting an eventual *de-novo* SARS-CoV-2 infection.

According to a predictive algorithm (**Supplementary Material**) generated from the data of the first wave of the COVID-19 pandemic, only patients with: (i) a respiratory rate >17 breaths/min plus, either a history of coronary heart disease, or the general VAS score <86 or a C-reactive protein at hospital admission >149 mg/l or (ii) patients aged 63 years or older with a respiratory rate <17 breaths/min, but a

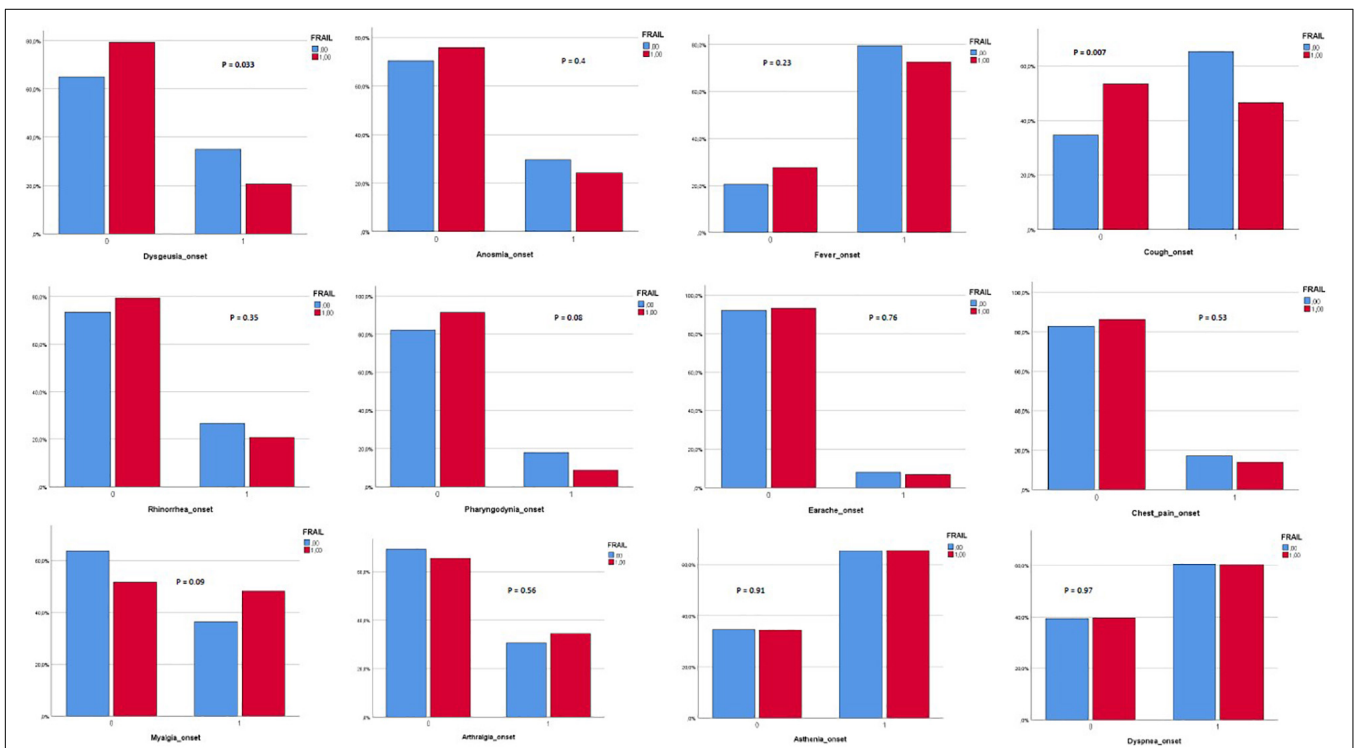


**FIGURE 2 |** Length of hospital stay in frail and robust patients. Robust patients: Median: 14 days; 25° percentile: 10 days; 75° percentile: 23 days. Frail patients: Median: 18.5 days; 25° percentile: 11 days; 75° percentile: 30 days.

**TABLE 1 |** Baseline and hospitalization characteristics of the study population[#].

| | Frail | Robust | p |
|---|---|---|---|
| | **(*N* = 58)** | **(*N* = 299)** | |
| Age | 78 (IQR 72.8–82) | 74 (IQR 69–80) | **<0.001*** |
| Males | 41 (70.7%) | 172 (57.5 %) | 0.06** |
| Ethnicity | | | 0.36** |
| *Caucasian* | 57 (98.3%) | 290 (97%) | |
| *Asiatic* | 0 (0%) | 2 (0.7%) | |
| *Latin American* | 0 (0%) | 6 (2%) | |
| *Black* | 1 (1.7%) | 1 (0.3%) | |
| BMI before COVID-19 | 27.4 (IQR 23.0–30.8) | 27.3 (IQR 24.5–30.3) | 0.76* |
| Frailty Index | 0.29 (IQR 0.26–0.32) | 0.13 (IQR 0.09–0.17) | **<0.001*** |
| Hypertension | 47 (81%) | 168 (56.2%) | **<0.001**** |
| Diabetes | 27 (46.6%) | 48 (16.1%) | **<0.001**** |
| Coronary heart disease | 25 (43.1%) | 46 (15.4%) | **<0.001**** |
| Heart failure | 24 (41.4%) | 15 (5%) | **<0.001**** |
| Arrhythmia | 31 (53.4%) | 42 (14.1%) | **<0.001**** |
| COPD/asthma/emphysema | 16 (27.6%) | 24 (8%) | **<0.001**** |
| Chronic kidney failure | 16 (27.6%) | 11 (3.7%) | **<0.001**** |
| Active neoplasia | 4 (6.9%) | 18 (6%) | 0.80** |
| Rheumatic disease | 6 (10.3%) | 17 (5.7%) | 0.19** |
| Arthrosis | 8 (13.8%) | 18 (6%) | **0.04**** |
| Osteoporosis | 8 (13.8%) | 19 (6.4%) | 0.05** |
| Psychiatric illness | 5 (8.6%) | 25 (8.4%) | 0.95** |
| Dementia | 3 (5.2%) | 7 (2.3%) | 0.23** |
| Chronic neurological disease | 8 (13.8%) | 17 (5.7%) | **0.03**** |
| Hepatic disease | 10 (17.2%) | 24 (8%) | **0.03**** |
| Peptic ulcer | 2 (3.4%) | 12 (4%) | 0.84** |
| Peripheral vascular disease | 23 (39.7%) | 35 (11.7%) | **<0.001**** |
| Cerebrovascular diseases | 15 (25.9%) | 18 (6%) | **<0.001**** |
| Length of hospital stay | 18.5 (IQR 11–30) | 14 (IQR 10–23) | **0.004*** |
| ICU stay | 3 (5.2%) | 13 (4.4%) | 0.78** |
| NIV | 18 (31%) | 71 (23.9%) | 0.25** |
| Thrombo-embolic events during hospital stay | 2 (3.4%) | 11 (3.7%) | 0.93** |
| Arrhythmic events during hospitalization | 3 (5.2%) | 9 (3%) | 0.41** |
| Days between symptoms' onset ER admission | 5 (IQR 0.25–9) | 7 (IQR 3–10) | **0.03*** |
| COVID-19 onset symptoms | | | |
| *Dysgeusia* | 12 (20.7%) | 104 (35%) | **0.033**** |
| *Anosmia* | 14 (24.1%) | 88 (29.6%) | 0.4** |
| *Fever* | 42 (72.4%) | 236 (79.5%) | 0.23** |
| *Cough* | 27 (46.6%) | 194 (65.3%) | **0.007**** |
| *Rhinorrhea* | 12 (20.7%) | 79 (26.6%) | 0.35** |
| *Pharyngodynia* | 5 (8.6%) | 53 (17.9%) | 0.08** |
| *Earache* | 4 (6.9%) | 24 (8.1%) | 0.76** |
| *Chestpain* | 8 (13.8%) | 51 (17.2%) | 0.53** |
| *Myalgia* | 28 (48.3%) | 108 (36.4%) | 0.09** |
| *Arthralgia* | 20 (34.5%) | 91 (30.6%) | 0.56** |
| *Asthenia* | 38 (65.5%) | 193 (65%) | 0.91** |
| *Dyspnea* | 35 (60.3%) | 180 (60.6%) | 0.97** |
| *Syncope* | 10 (17.2%) | 26 (8.8%) | 0.05** |
| *Headache* | 8 (13.8%) | 62 (20.9%) | 0.22** |
| *Confusion* | 24 (41.4%) | 76 (25.6%) | **0.01**** |
| *Abdominalpain* | 4 (6.9%) | 40 (13.5%) | 0.16** |
| *Nausea/vomiting* | 8 (13.8%) | 51 (17.2%) | 0.53** |
| *Diarrhea* | 16 (27.6%) | 82 (27.6%) | 0.99** |
| *Conjunctivitis* | 4 (6.9%) | 43 (14.5%) | 0.12** |
| *Skin rash* | 2 (3.4%) | 13 (4.4%) | 0.74** |
| Number of COVID-19 onset symptoms | 5 (IQR 4–7) | 6 (IQR 3–8) | 0.37* |

*BMI, Body Mass Index; ICU, Intensive Care Unit; NIV, Non Invasive Mechanical Ventilation; ER, Emergency Department.*
*[#]Missing data for calculating the baseline frailty index in 25 patients.*
**U Mann-Whitney test.*
***Chi-Square test.*
*Bold means statistically significant i.e. with p values < 0.05.*

body mass index (BMI) =26 kg/m$^2$, continued the follow-ups at 3 and 6 months.

Frailty was measured with the frailty index (FI) (2, 11) created by using the criteria proposed by Searle et al. (32, 33). The variables used to generate the FI encompass comorbidities, baseline assessment data, and blood test results. Each deficit included in the FI was scored 0 when absent and 1 when present. Thirty-one variables (**Supplementary Table 1**) were used to calculate the baseline FI and 37 variables were used to calculate the FI at 1-, 3-, and 6-month follow-up. Using more than 30 variables to calculate the FI indexes confers sufficient robustness. The FI scores above 0.25 were classified as indicative of frailty (34).

## Statistical Analyses

The baseline characteristics of the study population, such as the main aspects of the COVID-19 hospitalization, quality of life, nutritional aspects, muscle parameters, the number and type of COVID-19 onset, and persisting and *de-novo* symptoms and manifestations at 1, 3, and 6 months after hospital discharge, were described through descriptive statistics. Continuous variables were presented as mean and SD when normally distributed or with median and interquartile range (IQR), when data had a skewed distribution. Dichotomous variables were presented as number (N) and percentage (%). A comparison of the distribution of categorical and continuous variables between frail and robust patients was made through the chi-squared test for categorical variables and the Mann–Whitney *U* test for continuous variables.

The univariate linear, binary logistic, multinomial logit, and ordinal regression analyses were performed to explore the association between frailty (assessed through the FI) and the PASC manifestations at 1-, 3-, and 6-month follow-ups. Age- and sex-adjusted models were also run for the significant predictors of the univariate analyses. Regression models were also repeated considering only the subgroup of patients who attended all the three follow-up visits.

The variations of the FI over time were evaluated with the Friedman test and the changes in the frailty status during the follow-up were evaluated with the Cochran's Q test.

All the statistical analyses were performed with SPSS version 25.0 (SPSS Incorporation, Chicago, Illinois, United States).

# RESULTS

Three hundred and eighty-two patients aged 65 years or older were hospitalized at the San Raffaele Hospital for SARS-CoV-2 pneumonia between 24 August 2020 and 6 June 2021 and were discharged alive. These patients were enrolled in the present study. The compliance to 1-month follow-ups was 98.3% (351 patients). Only 60.5% of the hospitalized patients satisfied the criteria for continuing the follow-up at 3 and 6 months. However, the compliance to the 3- and 6-month follow-up visits was reduced to 56.5 (216 patients) and 49.3% (176 patients) of the initial sample, respectively (**Figure 1**). Only 15.2% (58 individuals) of hospitalized patients could be

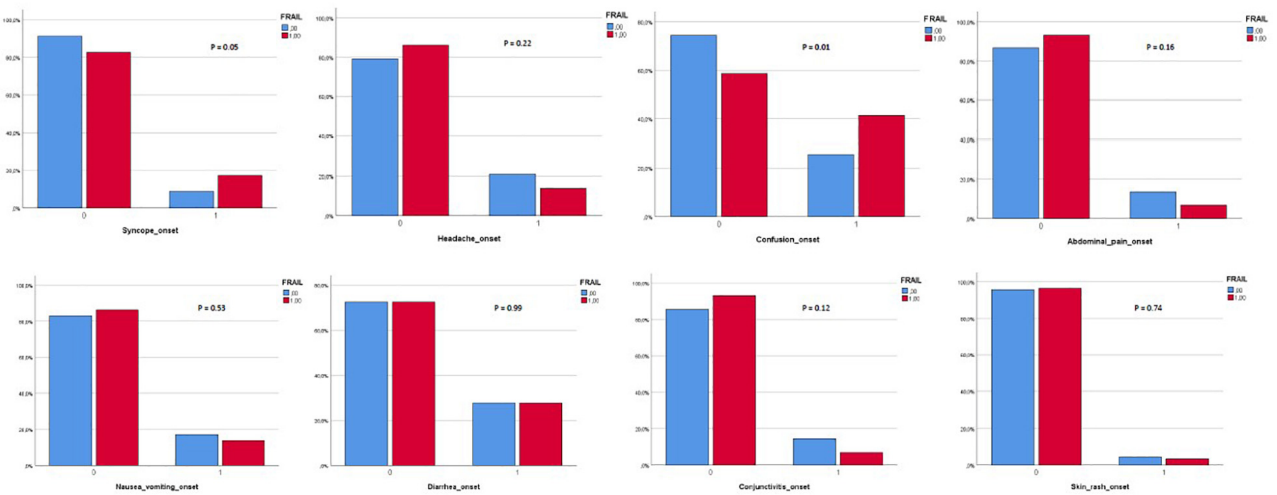


**FIGURE 3 |** Days between symptoms onset and emergency room (ER) admission in frail and robust patients. Robust patients: Median: 7 days; 25° percentile: 3 days; 75° percentile: 10 days. Frail patients: Median: 5 days; 25° percentile: 0.25 days; 75° percentile: 9 days. ER, emergency room.

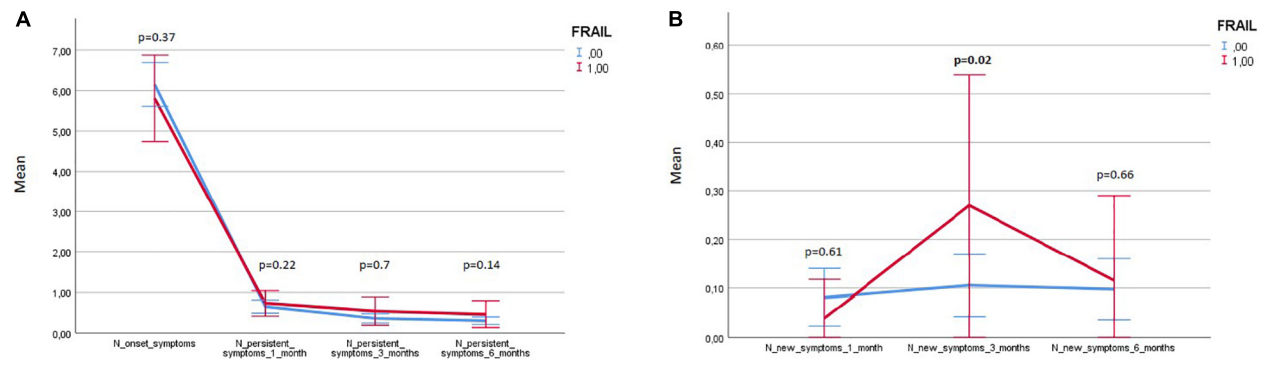


**FIGURE 4 | (A,B)** Onset of symptoms in frail and robust patients.

considered frail at hospital admission, according to their FI score above 0.25. Frail patients were significantly ($p < 0.001$) older than robust individuals (median age 78 vs. 74 years) and had longer hospitalizations (median hospital stay: 18.5 vs. 14 days, $p = 0.004$), as shown in **Figure 2**. Moreover, they suffer from more comorbidities than robust patients (**Table 1**). Even if the median number of COVID-19 onset symptoms did not differ between frail and robust patients, frail individuals had a shorter latency, between symptoms onset, and emergency department (ED) admission (5 vs. 7 days, $p = 0.03$), as shown in **Figure 3**. In addition, frail patients manifested (41.4 vs. 25.6%, $p = 0.01$) confusion more frequently, as first symptom of COVID-19. Instead, dysgeusia (35 vs. 20.7%, $p = 0.03$) and cough (65.3 vs. 46.6%, $p = 0.007$) at COVID-19 onset were more frequently observed in robust individuals (**Figures 4A,B** and **Table 1**).

1 month after hospital discharge, frail patients appeared more malnourished (median MNA-SF score 8 vs. 9, $p = 0.001$), at higher risk of being sarcopenic (median SARC-F score 3 vs. 1.5, $p = 0.003$), and displayed a worse muscle performance (median SPPB score 10 vs. 11, $p = 0.007$) than robust individuals (**Table 2**).

Moreover, frail patients complained about more problems in mobility, self-care, and in performing usual activities. Instead, no difference was observed in the prevalence of COVID-19 persisting symptoms, but frail patients manifested a *de-novo* cough (1.7 vs. 0%, $p = 0.02$) more frequently. 3 months after hospital discharge (**Table 3**), frail patients manifested more *de-novo* COVID-19 symptoms ($p = 0.02$). In particular, frail patients manifested a *de-novo* dyspnea (3.4 vs. 0%, $p = 0.001$), myalgia (5.2 vs. 1%, $p = 0.02$), and fever (1.7 vs. 0%, $p = 0.02$) more frequently. Indeed, they complained about having more dyspnea [median modified Medical Research Council (mMRC) 2 vs. 0, $p = 0.01$]. Mobility complaints ($p = 0.04$) and risk of sarcopenia (median SARC-F score 3 vs. 1, $p = 0.002$) confirmed to be higher in frail patients at the 3-month follow-up too. At the 6-month follow-up (**Table 4**), frail patients kept complaining about a *de-novo* dyspnea (1.7 vs. 0%, $p = 0.02$) and about problems in mobility ($p = 0.03$) and self-care ($p = 0.003$) more frequently. In addition, their muscle performance (median SSPB score 11 vs. 12, $p = 0.006$) and general quality of life (VAS) were worse (median VAS score 65 vs. 75, $p = 0.04$).

**TABLE 2 |** Nutritional and muscle characteristics, quality of life, and COVID-19 manifestations at the 1-month follow-up visits.

| | Frail | Robust | p |
|---|---|---|---|
| | (*N* = 56) | (*N* = 295) | |
| BMI | 26 (IQR 21.6–28.7) | 26.1 (IQR 23.8–28.7) | 0.46* |
| MNA-SF | 8 (IQR 7–10) | 9 (IQR 8–11) | **0.001*** |
| VAS | 75 (IQR 50–80) | 75 (IQR 60–90) | 0.18* |
| mMRC | 1 (IQR 0–3) | 1 (IQR 0–2) | 0.22* |
| SARC-F | 3 (IQR 1–5) | 1.5 (IQR 0–3) | **0.003*** |
| Hand Grip strength | 18.9 (14.1–22.5) | 19.2 (IQR 14.6–26.6) | 0.24* |
| SPPB | 10 (IQR 8–12) | 11 (IQR 9–12) | **0.007*** |
| **EQ 5D-3L Mobility** | | | |
| *No problems* | 25 (43.1%) | 167 (55.9%) | **0.04**** |
| *Some problems* | 23 (39.7%) | 86 (28.8%) | |
| *Extreme problems* | 2 (3.4%) | 2 (0.7%) | |
| **EQ 5D-3L Self-care** | | | |
| *No problems* | 37 (63.8%) | 221 (73.9%) | **0.03**** |
| *Some problems* | 7 (12.1%) | 29 (9.7%) | |
| *Extreme problems* | 6 (10.3%) | 9 (3%) | |
| **EQ 5D-3L Usual activities** | | | |
| *No problems* | 25 (43.1%) | 170 (56.9%) | **0.03**** |
| *Some problems* | 17 (29.3%) | 71 (23.7%) | |
| *Extreme problems* | 7 (12.1%) | 14 (4.7%) | |
| **EQ 5D-3L Pain/Discomfort** | | | |
| *No problems* | 24 (41.4%) | 163 (54.5%) | 0.12** |
| *Some problems* | 24 (41.4%) | 85 (28.4%) | |
| *Extreme problems* | 2 (3.4%) | 7 (2.3%) | |
| **EQ 5D-3L Anxiety/Depression** | | | |
| *No problems* | 30 (51.7%) | 141 (47.2%) | 0.61** |
| *Some problems* | 19 (32.8%) | 105 (35.1%) | |
| *Extreme problems* | 1 (1.7%) | 12 (4%) | |
| **COVID-19 persistent symptoms** | | | |
| *Dysgeusia* | 2 (3.4%) | 12 (4%) | 0.87** |
| *Anosmia* | 1 (1.7%) | 5 (1.7%) | 0.97** |
| *Cough* | 3 (5.2%) | 20 (6.7%) | 0.68** |
| *Rhinorrhea* | 0 (0%) | 3 (1%) | 0.45** |
| *Pharyngodynia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Chestpain* | 1 (1.7%) | 4 (1.3%) | 0.81** |
| *Myalgia* | 3 (5.2%) | 9 (3%) | 0.40** |
| *Arthralgia* | 1 (1.7%) | 3 (1%) | 0.63** |
| *Asthenia* | 6 (10.3%) | 51 (17.1%) | 0.21** |
| *Dyspnea* | 9 (15.5%) | 54 (18.1%) | 0.66** |
| *Headache* | 1 (1.7%) | 5 (1.7%) | 0.97** |
| *Confusion* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Other findings*[§] | 0 (0%) | 0 (0%) | n.a. |
| **Number of COVID-19 persistent symptoms** | | | |
| *0* | 33 (56.9%) | 181 (60.5%) | 0.22* |
| *1* | 20 (34.5%) | 68 (22.7%) | |
| *2* | 2 (3.4%) | 25 (8.4%) | |
| *3* | 1 (1.7%) | 11 (3.7%) | |
| *4* | 0 (0%) | 4 (1.3%) | |
| **COVID-19 *de novo* symptoms** | | | |
| *Cough* | 1 (1.7%) | 0 (0%) | **0.02**** |
| *Chestpain* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Myalgia* | 0 (0%) | 5 (1.7%) | 0.32** |
| *Arthralgia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Asthenia* | 1 (1.7%) | 3 (1%) | 0.63** |
| *Dyspnea* | 0 (0%) | 3 (1%) | 0.45** |
| *Confusion* | 0 (0%) | 2 (0.7%) | 0.53** |
| *Skin rash* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Deficits of short-term memory* | 2 (3.4%) | 6 (2%) | 0.49** |
| *Alopecia* | 0 (0%) | 2 (0.7%) | 0.53** |
| *Other findings#* | 0 (0%) | 0 (0%) | n.a. |
| **Number of COVID-19 *de novo* symptoms** | | | |
| *0* | 52 (89.7%) | 269 (90%) | 0.61* |
| *1* | 4 (6.9%) | 16 (5.4%) | |
| 2 | 0 (0%) | 4 (1.3%) | |

*BMI, Body Mass Index; MNA-SF, Mini Nutritional Assessment Short Form; VAS, Visual Analogue Scale; mMRC, Modified Medical Research Council Dyspnea Scale; SARC-F, Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls questionnaire; SPPB, Short Physical Performance Battery.*

[§]*Abdominal pain, nausea/vomiting, diarrhoea, conjunctivitis, skin rash, earache, syncope, fever.*

[#]*Dysgeusia, anosmia, fever, headache.*

**U Mann-Whitney test.*

***Chi-Square test.*

*Bold means statistically significant i.e. with p values < 0.05.*

**Figure 5** illustrates the variations in frail and robust patients of the number of persistent and *de-novo* long COVID-19 symptoms during the follow-up.

In spite of the presence of *de-novo* symptoms, none of the patients manifesting these complaints at any time during the follow-up had SARS-CoV-2 reinfection.

The results of the univariate and age- and sex-adjusted regression analyses, which explored the association between frailty and the PASC manifestations during the follow-up, are given in **Tables 5**, **6**, respectively. In the multivariate model, frailty was confirmed to be significantly associated with: (i) confusion, as a presenting symptom of COVID-19; (ii) malnutrition, risk of sarcopenia, impaired muscle performance, complaints in mobility, self-care, and in performing the usual activities of daily living at 1-month follow-up; (iii) dyspnea and risk of sarcopenia at 3-month follow-up; and (iv) difficulties in self-care at the 6-month follow-up.

In the subgroup analysis, including only patients who attended all the three follow-up visits, frailty was also associated with: (i) confusion, as a presenting symptom of COVID-19 [adjusted odds ratio (OR) 443.38, 95% CI 4.16–47237.39, $p = 0.01$]; (ii) malnutrition (MNA-SF: adjusted B −4.36, 95% CI −8.63 to −0.08, $p = 0.046$), risk of sarcopenia (SARC-F: adjusted B 7.1, 95% CI 1.75–12.42, $p = 0.01$), impaired muscle performance (SPPB: adjusted B −2.2, 95% CI −6.98 to −0.08, $p = 0.046$), complaints in mobility (adjusted OR 621.16, 95% CI 4.32–89415.18, $p = 0.01$), self-care (adjusted OR 1158.46, 95% CI 2.07–648668.62, $p = 0.03$), and in performing the usual activities of daily living (adjusted OR 12576.61, 95% CI 1.79–88327109.11, $p = 0.037$) at 1-month follow-up; (iii) dyspnea (mMRC: adjusted B 4.22, 95% CI 1.69–6.76, $p = 0.001$) and risk of sarcopenia (SARC-F: adjusted B 8.88, 95% CI 2.85–14.9, $p = 0.004$) at the 3-month follow-up; and (iv) difficulties in self-care (adjusted OR 2746.88, 95% CI 6.44–1172310.83, $p = 0.01$) at the 6-month follow-up.

**TABLE 3 |** Nutritional and muscle characteristics, quality of life, and COVID-19 manifestations at the 3-month follow-up visits.

| | Frail | Robust | p |
|---|---|---|---|
| | (*N* = 35) | (*N* = 181) | |
| BMI | 26.9 (IQR 23.2–30.8) | 27-3 (IQR 24.5–30) | 0.36* |
| MNA-SF | 13 (IQR 10–14) | 14 (IQR 12–14) | 0.24* |
| VAS | 75 (IQR 50–90) | 75 (IQR 70–85) | 0.68* |
| mMRC | 2 (IQR 0–3) | 0 (IQR 0–2) | **0.01*** |
| SARC-F | 3 (IQR 1–6) | 1 (IQR 0–3) | **0.002*** |
| Hand Grip strength | 19.8 (IQR 15.7–27.8) | 21.7 (IQR 14.8–29.1) | 0.71* |
| SPPB | 11 (IQR 9–12) | 12 (IQR 10–12) | 0.08* |
| **EQ 5D-3L Mobility** | | | |
| *No problems* | 19 (32.8%) | 116 (38.8%) | **0.04**** |
| *Some problems* | 14 (24.1%) | 51 (17.1%) | |
| *Extreme problems* | 1 (1.7%) | 0 (0%) | |
| **EQ 5D-3L Self-care** | | | |
| *No problems* | 23 (39.7%) | 141 (47.2%) | 0.24** |
| *Some problems* | 7 (12.1%) | 19 (6.4%) | |
| *Extreme problems* | 1 (1.7%) | 6 (3%) | |
| **EQ 5D-3L Usual activities** | | | |
| *No problems* | 18 (31%) | 120 (40.1%) | 0.14** |
| *Some problems* | 12 (20.7%) | 37 (12.4%) | |
| *Extreme problems* | 2 (3.4%) | 6 (2%) | |
| **EQ 5D-3L Pain/Discomfort** | | | |
| *No problems* | 12 (20.7%) | 94 (31.4%) | 0.06** |
| *Some problems* | 20 (34.5%) | 62 (20.7%) | |
| *Extreme problems* | 1 (1.7%) | 7 (2.3%) | |
| **EQ 5D-3L Anxiety/Depression** | | | |
| *No problems* | 16 (27.6%) | 90 (30.1%) | 0.76** |
| *Some problems* | 13 (22.4%) | 62 (20.7%) | |
| *Extreme problems* | 1 (1.7%) | 10 (3.3%) | |
| **COVID-19 persistentsymptoms** | | | |
| *Dysgeusia* | 2 (3.4%) | 4 (1.3%) | 0.25** |
| *Anosmia* | 1 (1.7%) | 2 (0.7%) | 0.42** |
| *Cough* | 1 (1.7%) | 6 (2%) | 0.89** |
| *Myalgia* | 1 (1.7%) | 6 (2%) | 0.89** |
| *Arthralgia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Asthenia* | 3 (5.2%) | 20 (6.7%) | 0.67** |
| *Dyspnea* | 7 (12.1%) | 37 (12.4%) | 0.96** |
| *Conjunctivitis* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Other findings*[§] | 0 (0%) | 0 (0%) | n.a. |
| **Number of COVID-19 persistent symptoms** | | | |
| *0* | 25 (43.1%) | 123 (41.1%) | 0.7* |
| *1* | 6 (10.3%) | 45 (15.1%) | |
| *2* | 3 (5.2%) | 8 (2.7%) | |
| *3* | 1 (1.7%) | 3 (1%) | |
| *4* | 0 (0%) | 1 (0.3%) | |
| **COVID-19 *de novo* symptoms** | | | |
| *Fever* | 1 (1.7%) | 0 (0%) | **0.02**** |
| *Cough* | 1 (1.7%) | 1 (0.3%) | 0.19** |
| *Myalgia* | 3 (5.2%) | 3 (1%) | **0.02**** |
| *Arthralgia* | 0 (0%) | 5 (1.7%) | 0.32** |
| *Dyspnea* | 2 (3.4%) | 0 (0%) | **0.001**** |
| *Deficits of short term memory* | 1 (1.7%) | 3 (1%) | 0.63** |
| *Alopecia* | 1 (1.7%) | 5 (1.7%) | 0.97** |
| *Other findings*[#] | 0 (0%) | 0 (0%) | n.a. |
| **Number of COVID-19 *de novo* symptoms** | | | |
| *0* | 25 (43.1%) | 135 (45.2%) | **0.02*** |
| *1* | 6 (10.3%) | 10 (3.3%) | |
| *2* | 0 (0%) | 3 (1%) | |
| *3* | 1 (1.7%) | 0 (0%) | |

*BMI, Body Mass Index; MNA-SF, Mini Nutritional Assessment Short Form; VAS, Visual Analogue Scale; mMRC, Modified Medical Research Council Dyspnea Scale; SARC-F, Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls questionnaire; SPPB, Short Physical Performance Battery.*

[§]*Fever, rhinorrhea, pharyngodynia, earache, chest pain, syncope, headache, confusion, abdominal pain, nausea/vomiting, diarrhea, skin rash.*

[#]*Dysgeusia, anosmia, chest pain, asthenia, headache, confusion, skin rash.*

**U Mann-Whitney test.*

***Chi-Square test.*

*Bold means statistically significant i.e. with p values < 0.05.*

Variations of the FI index and of the frailty status over time were assessed just in a subgroup of patients (78 subjects) for whom data for recalculating the FI during the follow-up visits were available. **Tables 7**, **8** illustrate the variations of the FI and the frailty status (defined as the FI >0.25) over time. Both the FI ($p < 0.001$) and the frailty status ($p = 0.009$) significantly changed during the follow-up (**Figure 5** and **Tables 7**, **8**). The highest values of the FI (median 0.2) and a number of frail individuals (23 people) were documented during the 1-month follow-up visits. **Figures 6**,**7** illustrates the length of hospital stay in frail and robust patients (**Figure 6**) and the interval between COVID-19 symptoms onset and ED admission in frail and robust patients.

## DISCUSSION

In this prospective observational study, we found that the latency between COVID-19 symptoms' onset and emergency room (ER) admission was shorter in frail patients. Frail individuals had an atypical presentation of the SARS-CoV-2 infection with confusion, more frequently. The length of COVID-19 hospitalization was longer in frail patients. 1 month after hospital discharge, frail individuals were more malnourished, at higher risk of being sarcopenic, and manifested a worse physical performance than robust people. Indeed, frail patients complained more frequently about problems with mobility, self-care, and performing the usual activities of daily living. 3 months after hospital discharge, frail patients manifested more *de-novo* COVID-19 symptoms (in particular, dyspnea and myalgia), remained at higher risk of sarcopenia, and kept complaining about difficulties in mobility. Complaints in mobility and self-care and *de-novo* dyspnea were still present 6 months after hospital discharge. Indeed, muscle performance and general quality of life were found to be worse in frail patients 6 months after hospital discharge.

The regression analyses confirmed an association between frailty, malnutrition, risk of sarcopenia, mobility, usual activity impairments, and dyspnea during the follow-up.

**TABLE 4 |** Nutritional and muscle characteristics, quality of life, and COVID-19 manifestations at the 6-month follow-up visits.

| | Frail | Robust | p |
|---|---|---|---|
| | (*N* = 28) | (*N* = 148) | |
| BMI | 25.1 (IQR 22.7–30.3) | 27.6 (IQR 24.5–30.8) | 0.09* |
| MNA-SF | 14 (IQR 11–14) | 14 (IQR 12.7–14) | 0.4* |
| VAS | 65 (IQR 50 -85) | 75 (IQR 65–85) | **0.04*** |
| mMRC | 0 (IQR 0–3) | 0 (IQR 0–1) | 0.20* |
| SARC-F | 2 (IQR 0.25–4) | 1 (IQR 0–3) | 0.07* |
| Hand Grip strength | 22.4 (IQR 17.8–27.1) | 22.2 (IQR 16.3–28.6) | 0.85* |
| SPPB | 11 (IQR 10–12) | 12 (IQR 11–12) | **0.006*** |
| **EQ 5D-3L Mobility** | | | **0.03**** |
| *No problems* | 14 (24.1%) | 99 (33.1%) | |
| *Some problems* | 13 (22.4%) | 37 (12.4%) | |
| *Extreme problems* | 0 (0%) | 0 (0%) | |
| **EQ 5D-3L Self-care** | | | **0.003**** |
| *No problems* | 16 (27.6%) | 117 (39.1%) | |
| *Some problems* | 10 (17.2%) | 16 (5.4%) | |
| *Extreme problems* | 0 (0%) | 2 (0.7%) | |
| **EQ 5D-3L Usual activities** | | | 0.26** |
| *No problems* | 16 (27.6%) | 100 (33.4%) | |
| *Some problems* | 10 (17.2%) | 30 (10%) | |
| *Extreme problems* | 1 (1.7%) | 4 (1.3%) | |
| **EQ 5D-3L Pain/Discomfort** | | | 0.91** |
| *No problems* | 13 (22.4%) | 73 (24.4%) | |
| *Some problems* | 12 (20.7%) | 56 (18.7%) | |
| *Extreme problems* | 1 (1.7%) | 6 (2%) | |
| **EQ 5D-3L Anxiety/Depression** | | | 0.65** |
| *No problems* | 17 (29.3%) | 78 (26.1%) | |
| *Some problems* | 8 (13.8%) | 50 (16.7%) | |
| *Extreme problems* | 2 (3.4%) | 7 (2.3%) | |
| **COVID-19 persistent symptoms** | | | |
| *Dysgeusia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Anosmia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Cough* | 1 (1.7%) | 3 (1%) | 0.61** |
| *Rhinorrhea* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Myalgia* | 2 (3.4%) | 2 (0.7%) | 0.06** |
| *Arthralgia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Asthenia* | 4 (6.9%) | 16 (5.4%) | 0.59** |
| *Dyspnea* | 5 (8.6%) | 18 (6%) | 0.41** |
| *Confusion* | 1 (1.7%) | 1 (0.3%) | 0.18** |
| *Conjunctivitis* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Other findings*[§] | 0 (0%) | 0 (0%) | n.a. |
| Number of COVID-19 persistent symptoms | | | 0.14* |
| 0 | 19 (32.8%) | 108 (36.1%) | |
| 1 | 6 (%) | 29 (9.7%) | |
| 2 | 2 (3.4%) | 8 (2.7%) | |
| 3 | 1 (1.7%) | 0 (0%) | |
| **COVID-19 *de novo* symptoms** | | | |
| *Fever* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Chestpain* | 0 (0%) | 2 (0.7%) | 0.54** |
| *Myalgia* | 0 (0%) | 1 (0.3%) | 0.66** |
| *Arthralgia* | 0 (0%) | 2 (0.7%) | 0.54** |
| *Asthenia* | (1.7%) | 1 (0.3%) | 0.18** |
| *Dyspnea* | (1.7%) | 0 (0%) | **0.02**** |
| *Deficits of short-term memory* | 1 (1.7%) | 1 (0.3%) | 0.18** |
| *Alopecia* | 0 (0%) | 3 (1%) | 0.45** |
| *Other findings*[#] | 0 (0%) | 0 (0%) | n.a. |
| Number of COVID-19 *de novo* symptoms | | | 0.66* |
| *0* | 26 (44.8%) | 136 (45.5%) | |
| *1* | 1 (1.7%) | 8 (2.7%) | |
| *2* | 1 (1.7%) | 2 (0.7%) | |

*BMI, Body Mass Index; MNA-SF, Mini Nutritional Assessment Short Form; VAS, Visual Analogue Scale; mMRC, Modified Medical Research Council Dyspnea Scale; SARC-F, Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls questionnaire; SPPB, Short Physical Performance Battery.*

*§*Fever, pharyngodynia, earache, chest pain, syncope, headache, abdominal pain, nausea/vomiting, diarrhoea, skin rash.*

*#Dysgeusia, anosmia, rhinorrhea, pharyngodynia, earache, syncope, cough, headache, diarrhoea, conjunctivitis, confusion, skin rash.*

**U Mann-Whitney test.*

***Chi-Square test.*

*Bold means statistically significant i.e. with p values < 0.05.*

In a subgroup analysis, the 1-month follow-up was the moment of a greater increase of the FI index over time when the highest number of frail individuals was detected.

Frail patients were older than robust individuals. This datum is consistent with the fact that the accumulation of health deficits and the decline in physiological reserve augment with aging, and as expected, the prevalence of frailty (7).

We found that the latency between symptoms' onset and ER admission was shorter in frail patients. As far as we know, this is a new finding of our study that has never been reported yet. It is plausible that COVID-19 evolves more rapidly in people with reduced physiological reserves.

Frail patients presented COVID-19 in an atypical way, with confusion more frequently. Confusion can be a symptom of delirium. It has been demonstrated that delirium can be the only presenting sign of an infection (35) in older people. Recently, an observational American study has revealed that delirium was an ED-presenting symptom of COVID-19 in 28% of older people (36).

We detected longer hospitalization in frail patients compared to robust people. This finding is in line with the results of a large multicenter European study, (37) which showed that the duration of hospitalization was significantly longer in people with high values on the Clinical Frailty Scale (CFS) ($\geq$5). Frail people need more time to recover from an acute infection and their hospitalization may more often be complicated by adverse intercurrent clinical events. Accordingly, it is reasonable to expect a more complicated postacute phase in frail COVID-19 survivors than in robust individuals. A recent study conducted by Jones et al. (38) in the primary care setting identified an association between frailty and long COVID-19 manifestations.

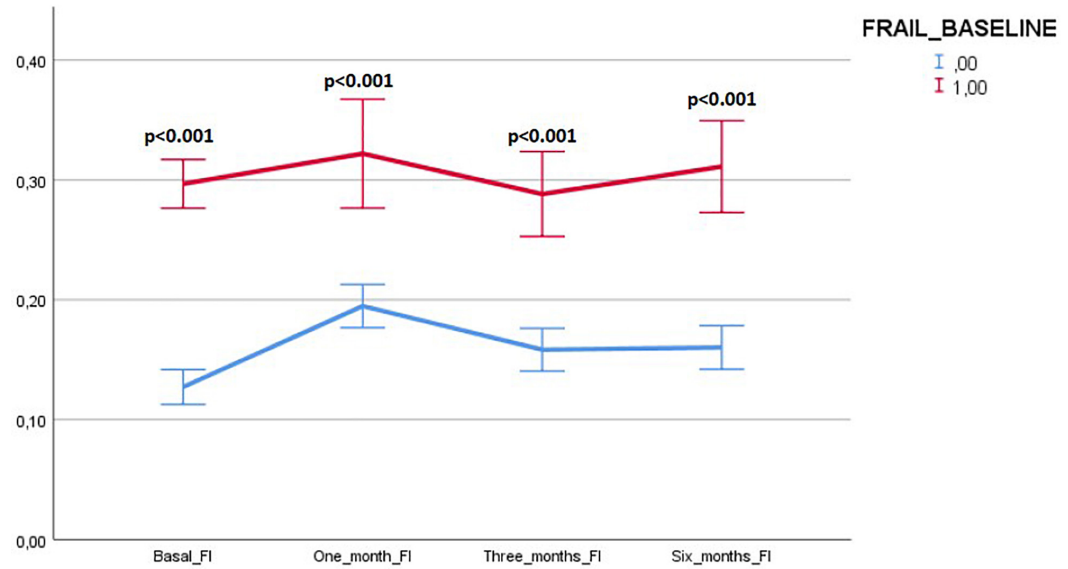


**FIGURE 5 |** Variations in frail and robust patients of the number of persisting and *de-novo* long COVID-19 symptoms during the follow-up. Results expressed as means and 95% CIs.

However, this study included a heterogeneous population, with both patients, who only believed that they had COVID-19 (but without a clinical or test diagnosis) and patients with either a clinical or a test diagnosis of SARS-CoV-2 infection. Moreover, in this study, frailty was not assessed with a validated tool, but just with an answer to a question on the level of fitness.

We found that frail COVID-19 survivors were at greater risk of malnutrition and sarcopenia and complained about great mobility impairment and difficulties in performing the activities of daily living and in usual care, 1 month after hospital discharge. More mobility complaints persisted till the 6-month follow-up and became associated with a worse muscle performance during the 6-month visits after discharge. Our findings are in line with the results of Shinohara et al. (39) in Japanese community-dwelling older adults: frail patients complained about decreased subjective leg muscle strength.

It is known that acute sarcopenia, which is common among patients with COVID-19, can evolve into chronic sarcopenia (40), especially if people do not have adequate nutritional and protein supply and do not perform enough physical activity (41). Economic difficulties during the COVID-19 pandemic could have influenced the eating habits of older people, reducing their consumption of meat and other animal proteins. In addition, COVID-19 countermeasures had a negative impact on the time spent in performing physical activities (42).

Finally, the negative skeletal muscle manifestations could have been underpinned by increased levels of angiotensin II from the classical pathway and decreased angiotensin-converting enzyme 2 (ACE2)/Ang from the nonclassical pathway, leading to muscle wasting (5). Anyway, it should be underlined that we lacked data on muscle mass and function before hospital admissions. Thus, chronic sarcopenia could have been present in some patients also before SARS-CoV-2 infection.

A more frequent *de-novo* dyspnea in frail patients emerged during our COVID-19 survivors' follow-ups. This result could be in line with the more common mobility complaints of frail patients, since both the manifestations could be underpinned by the presence of sarcopenia. Sarcopenia is particularly evident in the respiratory (43) and lower limb muscles. Sarcopenia of the respiratory muscles could impair the ability to produce appropriate tidal volumes (44) and to perform high force expulsive airway clearance maneuvers (45). Our group recently showed that reduction in respiratory muscle mass and quality was associated with extubation failure in COVID-19 critically ill patients (46).

The subgroup analyses demonstrated that frailty changed over time and COVID-19 survivors manifested a greater number of health deficits 1-month after hospital discharge.

Our findings are a bit different from the results of Lees et al. (47) who demonstrated in older people affected by influential an increased level of frailty from baseline to hospital admission, but a return to the baseline level of the frailty indexes 1 month after hospital discharge. We found instead a persistent deterioration of the frailty status in the first month after hospital discharge showing a negative impact of frailty on the recovery from the acute SARS-CoV-2 infection, which further increased the frailty of patients. However, also Lees et al. found that frailty hindered the recovery from the acute infection in flu patients (47).

Indeed, the inflammation caused by acute infections has systemic repercussions that can finally increase the frailty of the affected individuals. During infections, there is a progressive accumulation of unrepaired damages in tissues because the turnover of damaged macromolecules and organelles is inhibited (48). Moreover, both the inflammation and immobilization have catabolic effects on muscle mass contributing to physical function deterioration (49). Finally, infections can trigger other pathological conditions, which increase the level of frailty (50).

**TABLE 5 |** Results of the univariate regression models exploring the association between frailty and the PASC manifestations during the follow-up.

| | | | |
|---|---|---|---|
| **Confusion as initial symptom** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 81.86 | 5.16–1297.53 | 0.002 |
| **MNA-SF 1 month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | −5.66 | −8.31 to −3.02 | <0.001 |
| **SARCF 1 month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 10.73 | 4.52–16.95 | 0.001 |
| **SPPB 1 month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | −6.1 | −9.11 to −3.06 | <0.001 |
| **Mobility difficulties at 1 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 591832.97 | 11.01–31807224594.91 | 0.17 |
| **Difficulties in self-care at 1 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 73951,392 | 179.51–30465008,684 | <0.001 |
| **Difficulties in usual activities at 1-month follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 20891,269 | 110.48–3950397.36 | <0.001 |
| ***De novo* dyspnea at 3 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 285293790,2 | 2.43–3,3442E16 | 0.04 |
| **mMRC at 3 month-follow-up** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 4.77 | 1.46–8.10 | 0.005 |
| **Mobility difficulties at 3 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 58.34 | 1.21–2820.51 | 0.04 |
| **SARCF 3 months after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 9.28 | 4.06–14.49 | 0.001 |
| **SPPB 6 months after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | −3.24 | −6.29 to −0.18 | 0.04 |
| **Mobility difficulties at 6 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 144.43 | 1.86–11234.88 | 0.03 |
| **Difficulties in self-care at 6 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 3636.24 | 14.78–894614.89 | 0.03 |

*FI, Frailty Index; OR, Odds Ratio; C.I., Confidence Interval; MNA-SF, Mini Nutritional Assessment – Short Form; SARCF, Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls questionnaire; SPPB, Short Physical Performance Battery; mMRC, modified Medical Research Council questionnaire.*

**TABLE 6 |** Results of the age- and sex-adjusted regression models exploring the association between frailty and the PASC manifestations during the follow-up.

| | | | |
|---|---|---|---|
| **Confusion as initial symptom** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 77.84 | 4.23–1432.49 | 0.003 |
| **MNA-SF 1 month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | −5.63 | −8.39 to −2.87 | < 0.001 |
| **SARCF 1 month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 9.11 | 3.10–15.13 | 0.003 |
| **SPPB 1-month after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | −3.47 | −6.33 to −0.61 | 0.02 |
| **Mobility difficulties at 1 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 1674200.27 | 4.52–619924741831.25 | 0.03 |
| **Difficulties in self-care at 1 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 553305.56 | 376.37–813413358.35 | < 0.001 |
| **Difficulties in usual activities at 1-month follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 71.57 | 2.87–1782.53 | 0.009 |
| ***De novo* dispnea at 3 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 84066275.46 | 1.55–4,5527E15 | 0.045 |
| **mMRC at 3 month-follow-up** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 4.83 | 1.32–8.33 | 0.007 |
| **SARCF 3 months after hospital discharge** | | | |
| | **B** | **95% C.I.** | **p** |
| F.I. | 7.12 | 2.17–12.07 | 0.005 |
| **Difficulties in self-care at 6 month-follow-up** | | | |
| | **OR** | **95% C.I.** | **p** |
| F.I. | 2746.89 | 6.44–1172310.83 | 0.01 |

*FI, Frailty Index; OR, Odds Ratio; C.I., Confidence Interval; MNA-SF, Mini Nutritional Assessment – Short Form; SARCF, Strength, Assistance with walking, Rising from a chair, Climbing stairs, and Falls questionnaire; SPPB, Short Physical Performance Battery; mMRC, modified Medical Research Council questionnaire.*

The first month after hospital discharge is a critical period for older people. Previous research revealed that after hospital discharge, many older people display impaired functional levels in spite of the resolution of the acute conditions, which lead to hospital admissions. These impairments tend to persist over time and increase patients' risk of developing geriatric syndromes, novel disabilities, and hospital readmissions (51–56). In particular, the functional status 1 month after discharge was found to be associated with long-term outcomes (57). Adequate countermeasures should be introduced in the first 3 months after hospital discharge. These would favor a regain of the loss of functionality and would avoid a chronic course of the impairments (53).

Considering the symptom spectrum observed in this study in comparison to other respiratory diseases, we confirmed that frail patients more frequently manifested atypical symptoms (*i.e.,* confusion) (58). Indeed, in a previous study on the presentation of flu in a Canadian cohort, the flu diagnosis was missed in more than half of patients with laboratory-confirmed influenza, if clinicians based only on the influenza-like illness case definition (59).

**TABLE 7 |** Variations of the frailty index during the follow-up in a subgroup of 78 patients.

| | Hospital admission | 1-month follow up visit | 3-month follow up visit | 6-month follow up visit | p |
|---|---|---|---|---|---|
| **FI** | 0.14 (IQR 0.10–0.23) | 0.2 (IQR 0.15–0.27) | 0.17 (IQR 0.11–0.23) | 0.18 (IQR 0.14–0.25) | <0.001[$] |

*FI, Frailty Index; IQR, Inter Quartile Range.*
[$]*Friedman test.*

**TABLE 8 |** Variations of the frailty status during the follow-up in a subgroup of 78 patients.

| | Robust | Frail | p |
|---|---|---|---|
| Hospital admission | 61 | 17 | **0.009**[##] |
| 1-month follow up visit | 55 | 23 | |
| 3-month follow up visit | 64 | 14 | |
| 6-month follow up visit | 61 | 17 | |

*Results are presented as number of patients in each category.*
[##]*Q Cochran test.*
*Bold means statistically significant i.e. with p values < 0.05.*

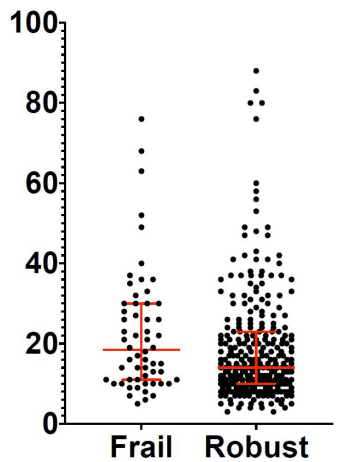


**FIGURE 6 |** Length of hospital stay in frail and robust patients.

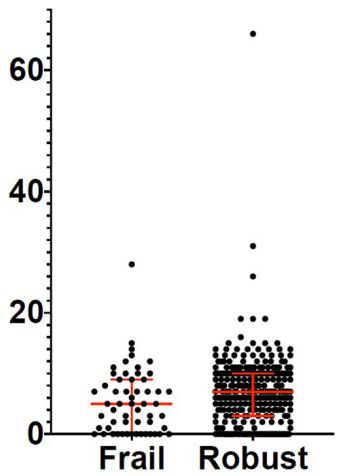


**FIGURE 7 |** Interval between symptoms onset and ED admission in frail and robust patients.

Therefore, we suggest performing a follow-up in COVID-19 survivors 1 month after the acute disease resolution. Frail patients should continue this follow-up to evaluate whether their level of frailty changes over time. For example, frailty could be reassessed 3 and 6 months after the resolution of the acute disease. Moreover, in frail patients, adequate countermeasures to revert frailty should be suggested by physicians. Otherwise, frailty would become a self-perpetuating cycle.

Our study has the merit of having described a real-world cohort of old COVID-19 survivors, followed-up over 6 months, after hospital discharge after SARS-CoV-2 pneumonia. We first depicted the prevalence of persistent and *de-novo* long COVID-19 manifestations after hospital discharge, according to the frailty status. We used the FI to describe frailty. The FI is a macroscopic indicator of biological age and has the advantage of providing integrative information, (60, 61) important to predict adverse health outcomes (62). The quantification of the negative effects of the accumulation of deficits through the FI can estimate the individual's biological age and risk profile and could be an innovative measure for assessing the risk of the PASC syndrome. This would have important therapeutic impacts. The identification of frail patients already at hospital admission would allow the prompt instauration of nutritional intervention and supervised physical exercise during a hospital stay. This could prevent and reduce the development of acute sarcopenia. Moreover, if these interventions were continued after hospital discharge, there would be also a reduction in the risk of chronicization of acute sarcopenia into chronic sarcopenia. Finally, frail individuals would benefit from a tighter follow-up that could promptly identify the PASC manifestations and ensure their adequate treatment.

However, some limits of this study are worth mentioning: the monocentric nature, the small sample, the fact that the nasal swab performed for evaluating eventual reinfections of SARS-CoV-2 was antigenic tests, the progressive reduction of the number of individuals involved in the follow-ups, and the lack of the evaluation of the frailty status over time in all the participants. Moreover, we did not have any information on patients' muscle function before hospital admission. This prevented the identification of chronic vs. acute sarcopenia as a possible cause of some long COVID-19 manifestations.

The PASC syndrome has a negative impact on the social and clinical well-being of the patients. Therefore, it would be of paramount importance to adequately address the persistence of COVID-19 symptoms. The precocious identification of frail patients, who manifest more motor and respiratory complaints during the follow-up, would allow their addressing to *ad hoc* treatment and rehabilitative services, thus improving the long-term management of COVID-19 sequelae with a cost-effective and sustainable paradigm.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by San Raffaele University Hospital Ethics Committee (protocol no. 34/int/2020). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be submitted.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.834887/full#supplementary-material

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