



Assessment of risk factors associated with long-term mortality in nursing homes: result from the SENIOR cohort

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Abstract

Background Previous studies on risk factors for death in nursing homes have focused on short-term observation and limited number risk factors.

Aims This study aims to identify factors predictive of 8-year survival in nursing homes.

Methods The study used the baseline measurements from the SENIOR cohort collected in 2013–2014. Data included clinical assessments (i.e., body composition, nutritional status, physical performance, level of dependence and cognition, frailty phenotype) as well as demographic information, number of medications and medical history. Mortality data were collected annually for 8 years. Univariate analyses were initially performed to assess potential predictive factors, followed by a Cox regression model using stepwise selection.

Results Of the 662 participants enrolled in the cohort, 58 (8.8%) were not further assessed due to the withdrawal of 2 nursing homes and 71 (10.7%) had no mortality data available (i.e., relocation, refusal to continue the study). Among the 533 patients included, 111 (20.8%) were still alive in 2022. Median survival time was 4 years (1.93–6.94). Multivariate regression showed that younger age (HR = 1.04 (1.03–1.06)), higher body mass index (HR = 0.96 (0.94–0.98)), higher score on the Mini-Mental State-Examination (HR = 0.97 (0.94–0.99)) and higher score on the Short Physical Performance Battery (HR = 0.93 (0.90–0.97)) were protective factors against mortality.

Conclusions This study highlights that certain modifiable factors related to physical or mental health contribute to increased survival in nursing homes. Because of its ability to improve physical performance and partly cognitive function, promoting physical activity in nursing homes appears to be a public health priority.

Keywords Mortality · Physical performance · Long-term care facilities

Introduction

The world is witnessing a shift in the population pyramid, with the number of people over the age of 65 steadily increasing. According to the World Health Organisation (WHO), the number of people aged over 80 is expected to triple by 2050 [1]. The aging process can be accompanied by functional decline, reflected in a loss of mobility, cognitive impairment, dependency in daily living and other

contributors to old age [2]. At a certain stage, the impact of the decline on older people living in the community may lead to their placement in adapted structures such as nursing homes (NHs) [3]. The current context of an ageing population, combined with medical progress but also the development of age-related chronic pathologies and functional decline is likely to have a significant impact on the need for more beds in NHs in most developed countries in the coming years [4].

The care provided by NHs aims to meet the needs of this specific population. Indeed, they have some particularities compared to the community-dwelling older people. For example, NH residents have been shown to be older, more dependent and have more comorbidities than other older people [5, 6]. In addition, older adults living in NHs tend to have lower life expectancy and higher mortality rates than community-dwelling older people [5]. A study conducted

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in the UK showed that the risk of dying within a year was 4 times higher for NH residents than for other senior of the same age [7]. In fact, in addition to mortality risk factors such as smoking, chronic disease and physical inactivity [8], prevalent geriatric conditions in NH residents have been associated with poorer short-term survival, such as frailty, low physical performance, low cognitive status and malnutrition [9–12].

Few studies have examined long-term mortality in NHs and they have focused on a limited number of potential risk factors. In the present study, we aimed to use a wide range of data from the SENIOR (Sample of Elderly Nursing Home Individuals: An Observational Research) cohort to identify factors associated with mortality 8 years after cohort entry.

Methods

Study design

This study used baseline data from the Sample of Elderly Nursing Home Individuals: An Observational Research (SENIOR) cohort, which is a prospective longitudinal study, to examine the mortality after 8 years.

Population

A full and detailed description of the SENIOR cohort has been described previously [13]. Concisely, 662 residents were recruited from 28 different NHs in Belgium. All the participants met the inclusion criteria, namely: living in an NH, being mobile with or without walking aids, being able to sign and understand an informed consent form. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Teaching Hospital of the University of Liège (reference 2013/178) with an amendment related to this study in July 2022.

Mortality

The primary outcome of the study was 8-year mortality. All-cause mortality was recorded from the residents' medical records in each year of follow-up and retrospectively with an update in 2022. Patients who were considered lost to follow-up (i.e., relocated, withdrew from the study) were considered as censoring event in the survival analyses.

Data collection

Data were collected by trained researchers with the active participation of the residents (i.e., physical tests and

questionnaires) and from medical records, especially for socio-demographic and anthropometric data.

Frailty

Frailty was assessed according to Fried's phenotype criteria, namely: an unintentional weight loss of 5 kg or $\geq 5\%$ in the previous year by self-report, physical weakness assessed by grip strength, poor endurance and energy assessed by two items of the Centre for Epidemiological Studies Depression Scale, slowness assessed by gait speed and low physical activity level measured by self-reported time spent in physical activity in the previous 7 days based on the Minnesota scale. An individual was defined as frail if three or more criteria were met, as pre-frail if one or two criteria were met and as robust if none of the criteria were met [14].

Body composition

Appendicular lean mass (ALM) was measured using a validated multi-frequency bioelectrical impedance analyser (Biospace Co, Ltd, Korea/Model JMW140). BIA electrodes were placed at 8 points on the body for a multisegmental frequency analysis and the sum of the lean mass of the arms and legs was used to calculate ALM [15]. The ALM was then divided by the square of the height (ALM/h^2) to obtain the appendicular lean mass index (ALMI).

Nutritional status

Nutritional status was assessed using the Mini Nutritional Assessment Short-Form (MNA-SF) which consists of 6 nutritional questions (i.e., weight loss, mobility, acute illness or psychological stress, neuropsychological problem, body mass index) and provides a score between 0 and 14. The score is used to determine nutritional status: normal (12–14), at risk of malnutrition (8–11) or malnutrition (0–7) [16].

Cognitive level

Cognition was assessed using the Mini Mental State Examination (MMSE). This questionnaire consisted of questions to measure cognitive impairment (i.e., repetition of word lists, language use and comprehension, basic motor skills, orientation, concentration, short-term memory, language skills, visuospatial skills and ability to understand and follow instructions). Out of a maximum score of 30 points, a score of 27 or more indicates a normal cognitive status. Below this threshold, mild, moderate and severe cognitive impairment are indicated by scores between 19 and 24, between 10 and 18 and below or equal to 9 [17].

Muscle strength

Muscle performance was assessed by grip strength measured using a hydraulic dynamometer (Seahan Corporation, MSD Europe Bvba, Belgium). Specifically, the participants had to squeeze the dynamometer as hard as possible 3 times in each hand. The highest value was taken as the reference.

Physical performance

Physical performance was first assessed using the Short Physical Performance Battery test (SPPB), a geriatric-validated tool consisting of 3 different tests: balance, gait speed and chair stand. Each of the test is scored separately and then summed to give an overall score. Secondly, physical performance was assessed using the Tinetti test or Performance-Oriented Mobility Assessment (POMA) which is designed to assess body balance and gait abnormalities in older people. The test is made up 16 items: 9 assessing balance and 7 assessing gait. Each item is scored on a scale of 0 to 2, with a maximum score of 28 and lower scores indicating impairment [18].

Level of autonomy and dependence

The ability to perform activities of daily living (ADLs) was assessed using the Katz scale which is based on 6 categories of assessment: bathing, dressing, toileting, transferring, continence and feeding. Scores between 1 and 4 are assigned for each activity category, with higher scores reflecting higher dependence in ADLs [19].

Health related quality of life

The EuroQol-5-Dimension (EQ-5D) questionnaire was used to assess self-reported health state. The dimensions covered by this test are mobility, self-care, usual activities, pain or discomfort and anxiety or depression. Each dimension is scored on 3 levels and converted into a global score ranging from 0 (death) to 1 (perfect health) using an index [20].

Covariates

Sociodemographic and anthropometric data were collected from the participants' medical records at baseline. Based on the literature, some of these data were considered as potential confounding factors: age, sex, level of education, number of medications, number of medical histories, waist circumference and body mass index (BMI) [21–24].

Statistical analysis

Quantitative variables that were normally distributed were expressed as mean and standard deviation (SD), and quantitative variables that were not normally distributed were expressed as median and percentiles (P25-P75). Normality was assessed using the Shapiro–Wilk test, the observation of the Q–Q plot and histogram. The association between patient characteristics and mortality was first examined using the T-Student or Mann–Whitney test and in a bivariate hazard model, expressed as a hazard ratio (HR) and its confidence interval (CI). To retain only those factors most strongly associated with mortality, factors with a p -value $< 0,05$ were entered into a stepwise proportional hazards model, with variables removed if they did not reach the significant α -level of 0.05. The HR and CI were derived from this model. All analyses were performed with SAS version 9.4 (SAS Institute, Cary, North Carolina).

Results

Of the 622 patients included in the cohort at baseline, 58 were excluded because 2 NHs refused to continue the study. During the different times of follow-up, some participants were lost to follow-up because of relocation ($n = 41$) or refusal to continue the study ($n = 30$) and therefore no survival data were available for them. After considering these losses to follow-up, this study was performed on a final sample of 533 participants from the SENIOR cohort (Fig. 1).

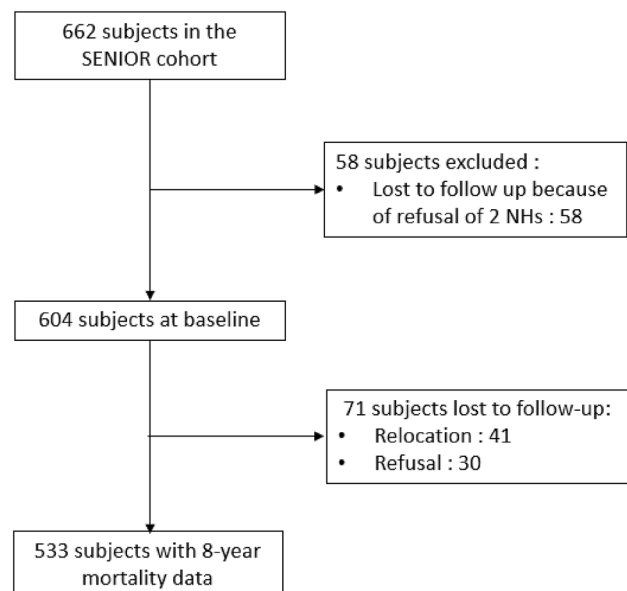


Fig. 1 Flowchart of the study

Table 1 shows the characteristics of the participants included in this study. The mean age was 83.4 ± 8.82 years and 86 (73%) were women. The mean number of medications per day was 10.0 ± 4.2 and the mean number of medical histories was 5.6 ± 3.9 . Most of the participants had completed secondary education (67.45%). In this sample, 138 (26%) were considered frail and the majority had a normal nutritional status (65%). As shown in the Kaplan–Meier curve (Figure S1 in the supplementary material), median survival time from entry into the cohort to death was 4.0 (1.9–6.9) years.

The results of the univariate Cox regression model are detailed in Table 2. A large proportion of the variables included in the analysis were associated with mortality 8 years after cohort entry. On the one hand, some factors appear to be negatively predictive of mortality, namely, age (HR = 1.05 (1.04–1.06)), pre-frail (HR = 1.39 (1.03–1.87)) and frail phenotype (HR = 2.16 (1.56–3.00)) compared with robust, risk of malnutrition (HR = 1.51 (1.22–1.85)) and malnutrition (HR = 3.26 (2.05–5.18)) compared to

normal nutritional status and the Katz score (HR = 1.07 (1.04–1.10)). On the other hand, higher BMI (HR = 0.96 (0.94–0.98)), higher waist circumference (HR = 0.99 (0.98–1.00)), higher grip strength (HR = 0.98 (0.97–0.99)), higher MMSE score (HR = 0.97 (0.95–0.99)), higher SSPB score (HR = 0.90 (0.88–0.93)), higher EQ-5D index (HR = 0.50 (0.34–0.76)) and higher Tinetti score (HR = 0.95 (0.93–0.97)) were associated with decreased probability of mortality in the cohort.

All significant variables from the univariate regression were entered into the multivariate Cox regression model with stepwise selection. These variables were added to and removed from the model until the model that best explained mortality in the cohort was obtained. The model obtained included 4 variables associated with mortality: age, BMI, MMSE score and SPPB score. Older age was associated with poorer survival, with each additional year increasing the risk of death in the 8 years after the cohort entry by 4%. (HR = 1.04 (1.03–1.06)). A one-unit increase in BMI and a one-point increase in MMSE and SPPB scores decreased

Table 1 Characteristics of the sample at baseline according to 8-year survival status

		Total	8-year survival status		p-value
			Deceased (n = 422)	Alive (n = 111)	
Age, years	n = 533	83.42 ± 8.82	84.99 ± 7.76	77.50 ± 10.04	< 0.0001
Gender, women	n = 531	391 (73.36%)	305 (72.3%)	86 (77.5%)	0.27
BMI, kg/m ²	n = 527	26.13 ± 5.41	25.77 ± 5.58	27.48 ± 4.52	0.0008
Medication, number	n = 524	10.01 ± 4.23	10.05 ± 4.19	9.87 ± 4.43	0.71
Medical history, number	n = 498	5.60 ± 3.89	5 (3–8)	5 (2–7)	0.22
<i>Educational level</i>	n = 510				0.44
None or primary education		66 (12.94%)	52 (12.3%)	12 (11.0%)	
Secondary education		344 (67.45%)	268 (63.5%)	76 (68.5%)	
Tertiary education		100 (17.2%)	81 (19.2%)	19 (16.3%)	
Waist circumference, cm	n = 519	99.43 ± 14.72	98.70 ± 14.84	102.12 ± 14.02	0.03
Grip strength, kg	n = 527	18.74 ± 10.27	18.19 ± 9.67	20.79 ± 12.09	0.04
ALMI, kg/m ²	n = 211	8.9 ± 4.88	8.64 ± 2.92	10.06 ± 9.47	0.20
<i>Fried phenotype</i>	n = 531				0.0002
Robust		78 (14.69%)	52 (12.4%)	26 (23.4%)	
Prefail		315 (59.32%)	244 (58.1%)	71 (64.0%)	
Frail		138 (25.99%)	124 (29.5%)	14 (12.6%)	
MMSE, /30 points	n = 528	24.10 ± 4.58	23.88 ± 4.69	24.90 ± 4.03	0.03
<i>MNA</i>	n = 524				0.002
Normal		341 (65.08%)	255 (61.7%)	86 (77.5%)	
Risk of malnutrition		163 (31.11%)	138 (33.4%)	25 (22.5%)	
Malnutrition		20 (3.82%)	20 (4.8%)	0 (0%)	
SPPB, /12 points	n = 527	5.52 ± 3.15	5.14 ± 3.06	6.94 ± 3.07	< 0.0001
Katz, /30 points	n = 522	11.27 ± 3.37	11.59 ± 3.50	10.09 ± 2.47	< 0.0001
EQ-5D	n = 523	0.57 ± 0.23	0.56 ± 0.24	0.62 ± 0.21	0.01
Tinetti, /28 points	n = 514	22.68 ± 5.66	22.10 ± 5.72	24.83 ± 4.90	< 0.0001

P-values in bold are less than 0.05

BMI Body Mass Index, ALM Appendicular Lean Mass, MMSE Mini Mental State Examination, MNA Mini Nutritional Assessment, SPPB Short Physical Battery Test, EQ-5D EuroQol-5 Dimension

Table 2 Crude and adjusted Hazard ratio of 8-year mortality

	Crude HR (95% C.I.)	Adjusted HR (95% C.I.)
Age, years	1.05 (1.04–1.06)	1.04 (1.03–1.06)
Gender, women	0.84 (0.68–1.04)	
BMI, kg/m ²	0.96 (0.94–0.98)	0.96 (0.94–0.98)
Medication, number	1.01 (0.98–1.03)	
Medical history, number	1.02 (0.99–1.04)	
<i>Educational level</i>		
None or primary education	Ref	
Secondary education	0.85 (0.63–1.14)	
Tertiary education	0.90 (0.64–1.27)	
Waist circumference, cm	0.99 (0.98–1.00)	
Grip strength, kg	0.98 (0.97–0.99)	
ALMI, kg/m ²	0.97 (0.93–1.02)	
<i>Fried phenotype</i>		
Robust	Ref	
Prefrail	1.39 (1.03–1.87)	
Frail	2.16 (1.56–3.00)	
MMSE, /30 points	0.97 (0.95–0.99)	0.97 (0.94–0.99)
<i>MNA</i>		
Normal	Ref	
Risk of malnutrition	1.51 (1.22–1.85)	
Malnutrition	3.26 (2.05–5.18)	
SPPB, /12 points	0.90 (0.88–0.93)	0.93 (0.90–0.97)
Katz, /30 points	1.07 (1.04–1.10)	
EQ-5D	0.50 (0.34–0.76)	
Tinetti, /28 points	0.95 (0.93–0.97)	

P-values in bold are less than 0.05

HR Hazard ratio, *CI* confidence interval, *BMI* Body Mass Index, *ALM* Appendicular Lean Mass, *MMSE* Mini-Mental State Examination, *MNA* Mini Nutritional Assessment, *SPPB* Short Physical Battery Test, *EQ-5D* EuroQol-5 Dimension

mortality by reducing the risk of death by 4, 3, and 7%, respectively (HR = 0.96 (0.94–0.98), HR = 0.97 (0.94–0.99), HR = 0.93 (0.90–0.97)).

Discussion

This study aimed to identify potential factors associated with 8-year mortality in the SENIOR cohort composed of more than 600 NH residents. Our study showed that among the various factors covering different aspects of health, age, BMI, cognitive level and physical performance level were independently associated with mortality in this cohort. Many studies have focused on short-term mortality in NHs and within our SENIOR cohort some short-term associations have been highlighted. In particular, 1-year mortality was associated, notably, with sarcopenia (which includes a component of physical performance) [22], a lower risk of 2-year mortality was associated with higher BMI [25] and higher 3-year mortality was associated with a decline in physical

performance [26]. However, the investigation of long-term mortality is a necessary concern to adapt the care of NH residents.

A recent study exploring 9-year mortality in NHs in Poland showed a median survival time of 2.4 years and that 17% of NH residents survived for 8 years or more [27]. In our study, we found a median survival time of 4 years and 20.8% of the participants were still alive after 8 years. The difference in median survival time can be partly explained by the fact that the population included was substantially different; the Polish study focused on NH residents with chronic diseases and a certain level of dependency. As the presence of chronic diseases is associated with mortality, shorter life expectancy could be one way of explaining this difference [28]. Furthermore, due to the inclusion criteria in the SENIOR cohort, in particular mobility, the sample in this study might be in better general health than the Polish sample with a longer life expectancy in the SENIOR cohort.

We highlighted that each year of age increased the mortality by 4% in the cohort. This finding is consistent with

the literature where age is a well-known non-modifiable factor decreasing the probability of survival [29]. The association between age and mortality in NH residents reported in other studies varies with hazard ratios between 0.5 and 4% depending on the time of observation.

In our study, a higher BMI was associated with a 4% decrease in 8-year mortality. This finding is consistent with a meta-analysis constituted of more than 19,000 NH residents worldwide, which showed an inverse association between BMI and mortality [30]. At first glance, this seems paradoxical because in the general population, overweight and obesity are associated with an increased risk of disability and decreased survival [31]. However, in the geriatric population, there is a J-shaped curve for BMI, observable with a protective effect of overweight and obesity [30, 32]. Although on the one hand, a higher BMI is associated with improved survival, on the other hand, obesity can lead to potential negative health effects, especially on comorbidities, disability, physical performance and malnutrition [32]. However, within our cohort, the mean BMI of the participants was 26, which corresponds to an overweight status and a lower risk of adverse outcomes.

Few studies have investigated the association between cognition level and mortality in NHs. Our results are in concordance with an observational study that showed a higher number of deaths in NH residents with lower cognitive level after 3 years [33]. A Chinese study also conducted on NHs showed a decreased risk of death within 5 years with higher cognitive level (HR = 0.95 (0.92–0.99)) [12]. These findings are consistent with studies carried out outside NHs, which have shown a robust and significant association between cognitive status and survival. This association could be explained by three possible explanations: cognitive decline is an early indicator of dementia, which can reduce life expectancy; the presence of comorbidities, which can affect both cognition and mortality; and the difficulty of maintaining a healthy diet or physical activity in the presence of cognitive impairment [34]. Finally, we showed that physical performance level was significantly associated with mortality. This finding was also reported in a 5-year survival study conducted in Brazil, where the risk of death was 2.7 times higher in people with low physical performance assessed by the SPPB (HR = 2.77 (1–40, 5.50)) [11]. However, an analysis of 1-year mortality using a composite score of the SPPB showed no association with survival [35]. Obviously, the shorter follow-up time may partly explain the different results obtained. Indeed, a meta-analysis conducted by Pavasini et al., regrouping no less than 16,000 older people, showed that an SPPB score below 10 was associated with an increased risk of all-cause mortality [36]. In addition, in the NH resident population, a decline in SPPB score over time has also been shown to be associated with

an increased risk of mortality, providing some consistency with the findings of our study [26].

On the other hand, some other factors have been associated with medium-or long-term mortality in NHs in the literature, but not in our study. In the study by Kantoch et al., gender and nutritional status were significantly associated with long-term survival (i.e., 9 years) [27]. In the same vein, Ozturk et al. recently highlighted the association of MNA-SF with mortality up to 7 years [37]. Regarding nutritional status, some differences in our methodology could explain the different results obtained. In our univariate analysis nutritional status was significantly associated with mortality but not in the stepwise proportional hazards model. In fact, in the case of two highly correlated variables, this statistical method only retains one of them [38]. This is likely to be the case for nutritional status, which has been reported in the literature to be associated with several variables included in our analyses, such as cognitive status and BMI [39, 40]. Surprisingly, our study found no association between gender and survival. It has been known for years that women have a longer life expectancy than men [41]. Logically enough, gender should have been significantly associated with mortality in our study. The rationale we can put forward to explain why this is not the case is the gender distribution within our cohort. In fact, the proportion of women was 73%, which possibly leads to a lower statistical power of this variable. Furthermore, no association between mortality and frailty was highlighted in this study, in contrast to the meta-analysis conducted by Zhang et al. which included 9076 NH residents and in which frailty appeared to be a strong predictor of mortality (HR = 1.88 (1.57, 2.25)) [42]. There are several possible explanations for this difference. Firstly, thirteen of the fourteen studies included in Zhang's meta-analysis had a time span of 2 years or less compared to the follow-up of 8 years of our study. Secondly, it was shown that the ability to discriminate between frail and non-frail patients, as well as the likelihood of negative outcomes such as mortality, varied according to the definition of frailty used [43, 44]. At last, frailty appeared to be significantly associated with mortality in our univariate analysis. It is possible that, in the SENIOR cohort, frailty was a weaker factor associated with mortality as it did not remain significant in the multivariate analysis.

This study has several limitations. First, there is some evidence that changes in health status over time could influence survival, as reported in a Belgian study that used data on physical performance over time to identify evolutionary trajectories that were significantly associated with mortality [26]. However, we have only used baseline data, which is a non-dynamic reflection of the health status of the participants. Second, survival probabilities should not be limited to health elements in old age. In fact, conditions throughout life can have an impact on mortality, as shown by this recent Italian study, which looked at mortality in a cohort of men

during a follow-up period of 61 years. It appeared that some factors such as literacy, Mediterranean diet, early maternal death were associated with mortality in the very long term [45]. Unfortunately, we did not have the opportunity to collect these data and, therefore, to include them in this study. Third, several factors could not be included in this analysis. This is particularly the case for preventive factors, such as pneumococcus or influenza vaccinations, social engagement and depressive disorders which have been associated with mortality in NH residents [46–49]. Fourth, it is important to consider the fact that some of the mortality data in this study were collected during the Covid-19 pandemic, which severely affected NHs. As in many other countries, excess mortality was reported in Belgium. Up to October 2021, half of all COVID-19 deaths in Belgium involved nursing home residents, representing 7.9% of the nursing home population (out of 162,700 residents) [50]. Then, we did not measure comorbidities with a standardised and validated tool, but as a “medical history” consisting of all the participants’ antecedents. Furthermore, our results must be considered cautiously. Indeed, the population included in our cohort in 2013 may no longer correspond to the actual population in NHs. In fact, the living habits of older people have been changing for several years. Particularly in Europe, many aids have been developed to help older people stay at home despite their daily difficulties. This has led to people being admitted to NHs later and in poorer health than before [51]. Finally, due to refusal to continue the study and relocation, we were unable to include data from one-fifth of the patients enrolled at baseline. This significant proportion of missing data may have influenced the results.

On the other hand, this study has some strengths. First, mortality in the SENIOR cohort has been explored at different follow-up times with some consistent results. In particular, a significant association was observed between lower mortality rate and lower BMI, decline in physical performance and age [22, 25, 26]. We can hypothesise that these factors are strong predictors of mortality over time, which reinforces the robustness of the results obtained. Secondly, the cohort consisted of a relatively large sample size which contributes to the robustness of the statistical analyses. Finally, the 8-year period with a large inclusion of health components provided a unique opportunity to observe factors associated with mortality in the long term.

This study has some interesting implications. The first is to encourage further studies to explore the long-term survival in NHs to confirm our findings. The second is to encourage the promotion of physical activity in nursing homes. Indeed, physical activity seems to be a good lever to act on both physical performance and cognitive abilities, in addition to improve, notably, quality of life [52, 53]. The third is to encourage interventional studies to assess the

impact of physical activity on the levels of physical performance and cognitive abilities in NH residents.

In conclusion, our study shows that in addition to age, which is a non-modifiable risk factor, certain modifiable factors related to physical or mental health contribute to increased 8-year survival in NHs. In addition to the assessment and screening with regular follow-up of these factors, the promotion of physical activity in nursing home residents should be a public health priority.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40520-023-02579-5>.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest All authors declare no competing interest.

Ethical approval This study has been approved by the Ethics Committee of the Teaching Hospital of the University of Liège (reference 2013/178) with an amendment related to this study in July 2022.

Informed consent All participants signed written informed consent in accordance with the declaration of Helsinki.

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