Original article

Poor nutritional status but not cognitive or functional impairment per se independently predict 1 year mortality in elderly patients with hip-fracture

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ARTICLE INFO

SUMMARY

Background & aims: Hip fractures are strongly associated with mortality in the elderly. Studies investigating predisposing factors have suggested a negative impact of poor nutritional, cognitive and functional status on patient survival, however their independent prognostic impact as well as their interactions remain undefined. This study aimed to determine whether poor nutritional status independently predicts 1 year post-fracture mortality after adjusting for cognitive and functional status and for other clinically relevant covariates.

Methods: 1211 surgically treated hip fracture elderly (age ≥ 65) patients consecutively admitted to the Orthopaedic Surgery Unit of the "Azienda Sanitaria Universitaria Integrata Trieste" (ASUITs), Cattinara Hospital, Trieste, Italy and managed by a dedicated orthogeriatric team. Pre-admission nutritional status was evaluated by Mini Nutritional Assessment (MNA) questionnaire, cognitive status by Short Portable Mental Status Questionnaire (SPMSQ) and functional status by Activity of Daily Living (ADL) questionnaire. All other clinical data, including comorbidities, type of surgery, post-operative complications (delirium, deep vein thrombosis, cardiovascular complications, infections, need for blood transfusions) were obtained by hospital clinical records and by mortality registry.

Results: Poor nutritional status (defined as MNA ≤23.5), increased cognitive and functional impairment were all associated with 3-, 6- and 12 month mortality (p < 0.001). Both cognitive and functional impairment were associated with poor nutritional status (p < 0.001). Logistic regression analysis demonstrated that the association between nutritional status and 3-, 6- and 12- month mortality was independent of age, gender, comorbidities, type of surgery and post-operative complications as well as of cognitive and functional impairment (p < 0.001). In contrast, the associations between mortality and cognitive and functional impairment were independent (p < 0.001) of demographic (age, gender) and clinical covariates but not of malnutrition. Kaplan-Meier analysis showed a lower mean survival time (p < 0.001) in patients with poor nutritional status compared with those well-nourished.

Conclusions: In hip fracture elderly patients, poor nutritional status strongly predicts 1 year mortality, independently of demographic, functional, cognitive and clinical risk factors. The negative prognostic impact of functional and cognitive impairment on mortality is mediated by their association with poor nutritional status.

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1. Introduction

Although many factors involved in declined function and independence have been identified and documented in the elderly, osteoporotic hip fractures have gained great attention because of their high prevalence [1] and adverse clinical outcomes [2,3]. In

geriatric patients hip fractures are notably associated with increased mortality, poor functional recovery, impaired rehabilitation, disability and dependency, leading to institutionalization [2–4]. Therefore, early detection and management of modifiable factors with potential impact on the prognosis after hip fracture are essential.

Malnutrition is highly prevalent in elderly with hip fracture [5–11], with a reported prevalence of over 50% in some studies [5,9]; in these patients it is a marker of poor functional prognosis, increased morbidity, mortality and healthcare costs [12–17]. Furthermore malnutrition is often associated with other predictors of adverse clinical outcomes in elderly patients. Specifically, the clustering of poor nutritional status, cognitive impairment and functional dependence, which is commonly observed in elderly patients marks a situation of extreme fragility, with high risk of hospital-related complications, permanent disability and death after fracture [10,13,16,18]. The interaction between malnutrition, cognitive and functional impairment is very complex and not yet fully understood [19]. While many studies have assessed the isolated effect of malnutrition on hip fracture mortality [8,14,20,21], very few of them have considered the concomitant influence of other confounding factors, primarily functional and cognitive impairment on overall mortality in a comprehensive assessment. Moreover, when these aspects were included in the analysis either a single field (functional capacity or cognitive impairment) was analyzed in the association with malnutrition [22,23] or only selected aspects of cognitive and functional performance were assessed [24], or relatively short follow up periods were considered [16,25].

Similarly, other predictive factors such as age, male gender, preexisting chronic diseases and post-surgical complications have all been associated with mortality following fracture [26–28]. Therefore, defining the independent predictive power of each individual factor on hip fracture mortality, specifically of malnutrition, in a comprehensive evaluation after an adequate follow-up period is extremely important particularly when addressing populations with mild cognitive and functional impairment and moderate malnutrition. In this case, active treatment of malnutrition may in fact result in a reduced rate of hospital-related complications and a better functional recovery [12,21,29].

Thus, the aim of this study was to investigate whether malnutrition, as detected using a specific and standardized tool (Mini Nutritional Assessment-MNA) is a predictor of increased risk of 3-, 6- and 12-month mortality after hip fracture independently of demographics, functional and cognitive status, preexisting chronic diseases and complications. Results from this study are relevant because malnutrition is a potential target condition for prevention or intervention with impact on rehabilitation [9,12,29].

2. Materials and methods

2.1. Study population

1211 consecutive elderly (age≥65) patients diagnosed with hip fracture and admitted for treatment to the Orthopaedic Surgery Unit of the "Azienda Sanitaria Universitaria Integrata Trieste" (ASUITs), Cattinara Hospital, Trieste, Italy directly from the Emergency Service from July 2014 to September 2016 were evaluated. All patients were evaluated and treated before and after surgery by an experienced orthogeriatric team. Criteria of exclusion were presentation to hospital >48 h after injury, presence of active oncologic disease, artificial nutrition and multiple fractures. The study was conducted according to the guidelines included in the Declaration of Helsinki involving human patients.

2.2. Clinical data

Upon admission, patient demographic information including age and sex was recorded. A clinical interview was administered to patients and/or caregivers to collect data on the following baseline and admission variables: clinical (previous diseases and treatments), functional (Activities of Daily Living −ADL) [30] and cognitive (Pfeiffer's Short Portable Mental Status Questionnaire (SPMSQ) [31]. Pre-admission nutritional status was recorded within 72 h from admission by Mini Nutritional Assessment (MNA) questionnaire [32], a validated, sensitive and reliable tool in grading the nutritional status of elderly patients. The MNA score distinguishes between elderly patients with adequate nutritional status (MNA >23.5) and patients at risk of malnutrition/malnourished (MNA≤23.5). Therefore, a cut-off of 23.5 was adopted to define inadequate nutritional status. Hypertension was defined as blood pressure ≥140/90 or use of antihypertensive drugs. All other data and diagnoses, including type of surgery, need for blood transfusions and postoperative complications (delirium, deep vein thrombosis, cardiovascular complications, infections, need for blood transfusions) were obtained by hospital clinical records. After 3-, 6- and 12 months data on mortality was obtained by mortality registry.

2.3. Statistical analysis

Data distributions for continuous variables was assessed by Shapiro–Wilk test and appropriate transformations used for non normal data. Associations between parameters were evaluated by Spearman correlation. Independency of the association between nutritional, functional and cognitive indexes and 3-, 6-, and 12 month mortality was tested by stepwise multiple logistic regression analysis. Factors showing a significant association with mortality were included in regression models. Survival analysis was performed using Kaplan Meier method. P values < 0.05 were considered statistically significant. Analysis was performed by SPSS v.17 software (SPSS, Inc., Chicago, IL). Continuous variables are presented as mean ± standard deviation.

3. Results

3.1. Patient clinical and demographical data

A total of 1211 patients (259 men and 952 women) were included in the study. Demographic, functional and clinical characteristics of the study population are shown in Table 1. The average age of participants was 84.7 years and about three-quarters were women. Mean ADL score indicated partial dependency and results from SPQMS showed mild cognitive impairment. Average MNA score was 22.3.61.4% of patients were affected by hypertension and about half of the subjects received blood transfusions, mostly postoperative. The most represented postoperative complications were electrolyte imbalance (17.5%), delirium (10.5%) and exacerbation of heart failure (9.9%). 3- 6- and 12-month mortality rates were respectively 11.4, 17 and 23.5%.

3.2. Association between the clinical and demographic parameters and 3- and 6-months mortality

Correlation analysis between 3-, 6- and 12 month mortality and clinical and demographic parameters is also shown in Table 1. Both 3-, 6- and 12 month mortality was negatively (p < 0.001) associated with MNA and ADL scores and positively (p ≤ 0.05) with male gender, age, SPMSQ score, preexisting diseases (preoperative COPD and CKD), need for preoperative blood transfusions and

Table 1
Demographical and clinical profile and its association with mortality.

	Mean or frequency	3-months mortality		6-months mortality		1-year mortality	
		ρ	p	ρ	p	ρ	p
Gender (M/F)	259/952	0.167	<0.001	0.201	<0.001	0.209	<0.001
Age (y)	84.7 ± 7.4	0.162	<0.001	0.178	<0.001	0.212	<0.001
3-months mortality (%)	11.4						
6-months mortality (%)	17.0						
1-year mortality (%)	23.5						
ADL	4.4 ± 1.9	−0.168	<0.001	−0.231	<0.001	−0.252	<0.001
SPMSQ	3.8 ± 3.5	0.176	<0.001	0.206	<0.001	0.224	<0.001
MNA	22.3 ± 5.1	−0.219	<0.001	−0.280	<0.001	−0.293	<0.001
Hypertension (%)	61.4	0.021	0.472	0.013	0.661	0.024	0.412
Stroke events (%)	10.3	−0.027	0.347	−0.034	0.246	−0.047	0.099
Chronic heart failure (%)	13.7	0.072	0.013	0.040	0.175	0.056	0.051
COPD (%)	12.0	0.062	0.032	0.081	0.005	0.059	0.039
Asthma (%)	0.5	0.049	0.091	0.005	0.857	0.016	0.571
CKD (%)	16.8	0.114	<0.001	0.147	<0.001	0.158	<0.001
Liver cirrhosis (%)	1.5	−0.023	0.431	−0.017	0.564	−0.020	0.488
Diabetes (%)	18.8	−0.004	0.894	0.010	0.743	0.012	0.685
Use of corticosteroids (%)	2.5	0.127	<0.001	0.074	0.011	0.049	0.087
Smoke (%)	11.6	−0.047	0.101	−0.044	0.129	−0.030	0.292
Alcohol consumption (%)	25.5	−0.025	0.381	−0.015	0.612	−0.032	0.273
Transfusions pre-surgery (%)	41.9	0.059	0.040	0.089	0.002	0.085	0.003
Surgery (Endop-/Arthrop)	521/690	0.026	0.362	0.027	0.354	0.002	0.958
Delirium (%)	10.5	0.021	0.463	−0.007	0.819	−0.019	0.506
DVT (%)	0.1	−0.010	0.720	−0.013	0.651	−0.016	0.578
Pneumonia (%)	2.7	0.152	<0.001	0.119	<0.001	0.090	0.002
Respiratory failure (%)	4.1	0.187	<0.001	0.147	<0.001	0.119	<0.001
Electrolyte imbalance (%)	17.5	0.026	0.369	0.017	0.565	0.061	0.034
Heart failure exacerbation (%)	9.9	0.144	<0.001	0.121	<0.001	0.124	<0.001
Sepsis (%)	0.9	0.130	<0.001	0.168	<0.001	0.152	<0.001
Transfusions post-surgery (%)	54.3	0.052	0.072	0.098	<0.001	0.106	<0.001

Continuous variables are reported as mean ± SD. ρ: Spearman's coefficient; ADL: Activities of Daily Living; SPMSQ: Short Portable Mental Status Questionnaire; MNA: Mini Nutritional assessment; CHF: chronic heart failure; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; DVT: deep vein thrombosis.

postoperative development of pneumonia, respiratory failure, exacerbation of heart failure and sepsis. The positive associations (p = 0.013 and p < 0.001) of other preexisting conditions (CHF and use of corticosteroids respectively) with 3-month mortality were progressively lost at 12 months. Postoperative need for blood transfusions was positively associated (p < 0.001) with 6- and 12 month mortality.

3.3. Association between demographic variables and nutritional, functional and cognitive status

We next determined potential associations between nutritional, cognitive and functional parameters. Linear regression analysis demonstrated significant (p < 0.001) correlations between age, MNA, ADL and SPMSQ scores. In contrast, no significant association was observed between gender, MNA, ADL and SPMSQ scores (Table 2).

Table 2
Association analysis between age, gender and nutritional, functional and cognitive indexes.

	MNA		ADL		SPMSQ	
	ρ	p	ρ	p	ρ	p
Gender (M/F)	0.032	0.223	0.020	0.446	−0.032	0.227
Age (y)	−0.286	<0.001	−0.304	<0.001	0.355	<0.001
MNA	−	−	0.615	<0.001	−0.605	<0.001
ADL	0.615	<0.001	−	−	−0.552	<0.001
SPMSQ	−0.605	<0.001	−0.552	<0.001	−	−

MNA: Miny Nutritional Assesment; ADL: Activities of Daily Living; SPQMS: Short Portable Mental Status Questionnaire (SPMSQ) in the study group. ρ: Spearman's coefficient.

3.4. Modeling the associations between nutritional, functional and cognitive status and mortality

Factors associated with mortality in correlation analysis were then included in multiple regression analyses. The results are reported in Tables 3–5. The negative association between nutritional status and 3-, 6- and 12-month mortality was significant (p < 0.001) independently of age, gender, comorbidities, functional and cognitive status, type of surgery and complications (Table 3). The results of Kaplan–Meier survival analysis are presented in Fig. 1. At 1 year after surgery, the cumulative survival rate was higher (p < 0.001) in well-nourished elderly patients compared with patients malnourished or at risk of malnutrition. Indeed, also functional status was negatively associated (p < 0.001) with 3-, 6- and 12 month mortality independently of demographics, type of surgery, chronic preexisting conditions and complications, but when nutritional status was included in the model, these associations were no longer significant (Table 4). Correspondingly, in a comprehensive model including demographics, comorbidities and complications in addition to nutritional status no significant associations were detected between mortality at different time-points and functional status (Table 4). Superimposable results were obtained when considering cognitive impairment (Table 5).

4. Discussion

The current study investigated the interactions between mortality and nutritional, demographic, clinical, functional and cognitive variables in hip fracture elderly patients. The main findings show that: 1) in correlation analysis better nutritional, functional and cognitive status are negatively correlated with 3-, 6- and 12 months mortality; 2) nutritional, functional and

Table 3
Multiple logistic regression analysis regression between Mini Nutritional Assessment (MNA) and 3- 6- and 12- month mortality in different statistical adjustment models.

Model	MNA											
	3-month mortality				6-month mortality				1 year mortality			
	B	SE	z	p	B	SE	z	p	B	SE	z	p
1	−0.124	0.019	45.075	<0.001	−0.143	0.017	71.367	<0.001	−0.133	0.0150	78.977	<0.001
2	−0.123	0.019	44.194	<0.001	−0.143	0.017	70.512	<0.001	−0.134	0.0151	79.246	<0.001
3	−0.124	0.019	43.182	<0.001	−0.142	0.017	67.617	<0.001	−0.134	0.0152	77.140	<0.001
4	−0.117	0.024	24.322	<0.001	−0.128	0.021	35.332	<0.001	−0.115	0.0193	35.669	<0.001
5	−0.122	0.019	41.071	<0.001	−0.140	0.017	64.636	<0.001	−0.133	0.0154	74.228	<0.001
6	−0.107	0.024	18.536	<0.001	−0.118	0.022	28.110	<0.001	−0.112	0.020	31.499	<0.001

Model 1: Age + gender.
Model 2: Age + gender + type of surgery.
Model 3: Age + gender + chronic conditions.
Model 4: Age + gender + ADL and SPMSQ.
Model 5: Age + gender + complications.
Model 6: Age + gender + chronic conditions + ADL and SPMSQ + complications.
ADL: Activities of Daily Living; SPMSQ: Short Portable Mental Status Questionnaire; B: Coefficient, SE: Standard Error; z: Wald test.
Chronic conditions: CKD, CHF, COPD, chronic use of corticoisteroids, need for blood transfusion before surgery.
Complication: need for blood transfusion after surgery, respiratory failure, heart failure reacutization, sepsis.

Table 4
Multiple regression analysis of the association between functional status and 3-, 6- and 12 month mortality in different statistical adjustment models.

Model	ADL											
	3-month mortality				6-month mortality				1 year mortality			
	B	SE	z	p	B	SE	z	p	B	SE	z	p
1	−0.190	0.048	15.898	<0.001	−0.252	0.042	36.717	<0.001	−0.246	0.036	43.493	<0.001
2	−0.188	0.048	15.537	<0.001	−0.251	0.042	36.244	<0.001	−0.246	0.037	43.486	<0.001
3	−0.196	0.049	16.235	<0.001	−0.258	0.042	37.093	<0.001	−0.252	0.038	44.374	<0.001
4	−0.189	0.049	14.795	<0.001	−0.248	0.043	33.758	<0.001	−0.245	0.038	41.260	<0.001
5	0.002	0.058	0.001	0.979	−0.060	0.051	1.403	0.236	−0.066	0.046	2.078	0.150
6	−0.017	0.061	0.076	0.783	−0.080	0.053	2.314	0.128	−0.078	0.047	2.678	0.102

Model 1: Age + gender.
Model 2: Age + gender + type of surgery.
Model 3: Age + gender + chronic conditions.
Model 4: Age + gender + complications.
Model 5: Age + gender + MNA.
Model 6: Age + gender + chronic conditions + complications + MNA.
ADL: Activities of Daily Living; SPMSQ: Short Portable Mental Status Questionnaire; MNA: Mini Nutritional assessment; B: Coefficient; SE: Standard Error; z: Wald test.
Chronic conditions: CKD, CHF, COPD, chronic use of corticosteroids, need for blood transfusion before surgery.
Complications: need for blood transfusion after surgery, respiratory failure, heart failure reacutization, sepsis.

Table 5
Multiple regression analysis of the association between cognitive function and 3-, 6- and 12 month mortality in different statistical adjustment models.

Model	SPMSQ											
	3-month mortality				6-month mortality				1 year mortality			
	B	SE	z	p	B	SE	z	p	B	SE	z	p
1	0.120	0.027	19.887	<0.001	0.124	0.023	28.541	<0.001	0.125	0.021	35.989	<0.001
2	0.119	0.027	19.746	<0.001	0.124	0.023	28.378	<0.001	0.125	0.021	35.955	<0.001
3	0.134	0.028	23.250	<0.001	0.134	0.024	31.241	<0.001	0.133	0.021	38.987	<0.001
4	0.120	0.028	18.691	<0.001	0.123	0.024	26.409	<0.001	0.125	0.021	34.042	<0.001
5	0.027	0.032	0.684	0.408	0.018	0.028	0.450	0.502	0.026	0.025	1.021	0.312
6	0.047	0.034	1.903	0.168	0.033	0.029	1.318	0.251	0.035	0.026	1.815	0.178

Model 1: Age + gender.
Model 2: Age + gender + type of surgery.
Model 3: Age + gender + chronic conditions.
Model 4: Age + gender + complications.
Model 5: Age + gender + MNA.
Model 6: Age + gender + chronic conditions + complications + MNA.
SPMSQ: Short Portable Mental Status Questionnaire; MNA: Mini Nutritional Assessment; B: Coefficient, SE: Standard Error; z: Wald test.
Chronic conditions: CKD, CHF, COPD, chronic use of corticosteroids, need for blood transfusion before surgery.
Complications: need for blood transfusion after surgery, respiratory failure, heart failure reacutization, sepsis.

cognitive status are interrelated; 3) in multiple regression analysis, however, only nutritional status remains associated with mortality independently of functional and cognitive status, as well as of demographics, comorbidities and complications.

Compared with previous literature [8,14,16], this is the first study, to our knowledge, to show the independent predictive role of malnutrition on long-term mortality (1 year) in hip fracture patients in a comprehensive assessment incorporating clinical,
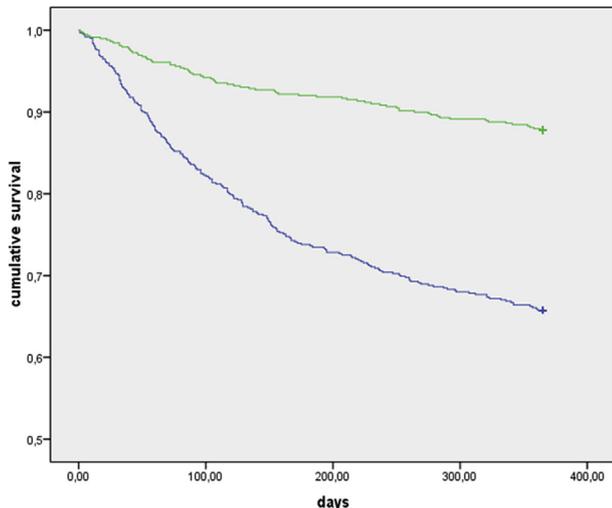
Fig. 1. Kaplan Meier survival analysis curves for non malnourished/at risk of malnutrition (MNA ≤23.5, blue line) and well-nourished (green line) patients. P < 0.001 for 3-, 6-and 12 month mortality between groups.

anthropometric, demographic, functional and cognitive predictive factors.

High prevalence of malnutrition among older patients with hip fracture has been well documented [5–11], as well as its consequences on length of hospital stay [12], functional recovery [9,14,16], disability [9] and mortality [8,14,16]. However, nutritional status is seldom included among the variables predictive of mortality after hip fracture [21,33] and validated screening tools to identify high-risk patients hardly ever include malnutrition/risk of malnutrition [28,34]. On the other hand, inclusion of malnutrition among predictors of mortality in hip fracture patients may improve outcomes and healthcare efficiency [35]. Our study demonstrates the strong adverse impact of malnutrition on mortality in this population and strengthens the rationale for extensive preoperative nutritional screening. In line with our findings, Gumiero et al. [14], showed that MNA, but not other risk assessment tools commonly used in these patients such as The American Society of Anesthesiologists Physical Status Score (ASA) is associated with mortality 6 months after hip fracture.

When nutritional status is included among the preoperative indicators of mortality, the relative impact of each prognostic factor is unknown [36]. This information is important, because it allows clinicians to identify high risk patients and to target treatment to the most effective intervention. In addition to malnutrition, associations of demographics, comorbidities, postoperative complications, functional and cognitive status with mortality were observed. Increased mortality has been previously reported in older hip fracture patients with higher rates in people over 85 years old [37]. We found that the direct association between age and mortality was lost in multivariate analysis; however, compared with other study populations [37], our cohort was much larger. In addition, management of patients by an experienced orthogeriatric team could have contributed to minimize the detrimental effects of age on mortality following hip fracture surgery. Also the relations of gender and comorbidities with mortality risk found in correlation analysis were lost in multiple regression models. Although the former association has not been unequivocally demonstrated in the literature [38,39] studies pointing at male gender as a risk factor after hip fracture were conducted in vulnerable populations, characterized by multiple comorbidities and high functional dependence [21]. This is not the case of our study cohort, whose preoperative functional status was only mildly impaired, suggesting that in this setting, other risk factors may have a heavier

impact on mortality than functional activities and comorbidities. Most studies have previously assessed the weight of coexisting chronic conditions on mortality by using cumulative scores. In this study, in correlation analysis we at first evaluated distinctly the impact of each disease. In line with previous evidence we found that COPD was associated with 1-year mortality [38] while the association between CHF and mortality demonstrated at 3 months was lost at 1 year. Although some pre- and postoperative complications were associated with mortality in correlation analysis, they were not involved in the relationship between functional and cognitive status and mortality, as assessed by multiple regression analysis. This is in agreement with previous studies showing that, among hospital complications only major events, such as myocardial infarction or intensive care unit stay were independent predictors of mortality following surgery for hip fracture repair [40]. The current study also confirmed a solid cross−sectional association between malnutrition, poor functional and cognitive status, which are often clustered in identifying a situation of increased frailty [10,41]. In fact, this pattern has been directly and causally involved in the increased rate of postoperative complications, mortality and impaired functional recovery after fragility hip fracture in the elderly [18]. Unlike previous studies which did not take into consideration cognitive and functional variables in the analysis of the association between mortality and malnutrition [8,14] or considered only selected aspects of cognition and functional capacity in this association but for short follow up periods [16], in a further effort to identify the role and relevance of nutritional status, we included this parameter in multiple regression analysis. Our results show that, at variance with comorbidities and complications, nutritional status mediates the relationship between preoperative functional and cognitive status and mortality. This finding holds true not only for short term- (3 months) but also for mid- (6 months) and long-term mortality (1 year). To our knowledge, this is the first study showing in an adequately powered cohort of elderly patients with moderate cognitive and functional disability and mildly compromised nutritional status the strong and independent burden of malnutrition on mortality far beyond hospitalization and discharge. Further studies will be required to investigate the mechanisms underlying the associations between nutritional status, mortality and functional parameters.

Considering the relevance of a correct identification of malnutrition in this population, the use of the most adequate screening tool should be warranted. In this study we used the MNA, whose predictive validity has been consistently documented when assessing mortality in this group of patients [14,16]. Recently, another study demonstrated that MNA is a better tool as compared with MUST and NRS-2002 to predict mortality in hip-fracture elderly patients, showing significantly higher mortality rates in the groups of malnourished/at risk of malnutrition as compared with well nourished patients [8].

This study has several strengths and avenues for future research. One strength is the large sample size and the number of collected variables, which allowed to define among a cluster of indicators negatively associated with clinical outcome, the precise and independent role for nutritional status in the prediction of mortality. Secondly, this result was obtained for the first time in an orthogeriatric setting. Finally, compared to other studies, collected data were not limited to those acquired at admission, but included hospital- and surgery-related complications. On the other hand, it should also be noted that, despite the large sample size, this study was conducted in a single center. By including more centers it is likely that these findings could be extended also to different clinical settings.

In conclusion, our study shows that impaired physical and mental function per se are not directly associated with mortality in elderly patients with hip fracture, and that malnutrition mediates

the general association between impaired functional and mental status and mortality. As a consequence, nutritional risk screening should be included in any risk stratification at admission in geriatric hip fractures.

Funding/support

None.

Authors contributions

Conceived and designed the study: RB, PDC.
Acquired, analyzed and interpreted the data: PDC, LM, CR, GC, GGC.
Drafted or revised the article: MZ, GGC, RB.
Final approval of the version to be published: MZ, GGC, PDC, CR, GC, LM, RB.

Conflict of interest

None declared.

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