



# Hyperglycemia at admission, comorbidities, and in-hospital mortality in elderly patients hospitalized in internal medicine wards: data from the RePoSI Registry

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## Abstract

**Aims** The association between hyperglycemia at hospital admission and relevant short- and long-term outcomes in elderly population is known. We assessed the effects on mortality of hyperglycemia, disability, and multimorbidity at admission in internal medicine ward in patients aged  $\geq 65$  years.

**Methods** Data were collected from an active register of 102 internal medicine and geriatric wards in Italy (RePoSi project). Patients were recruited during four index weeks of a year. Socio-demographic data, reason for hospitalization, diagnoses, treatment, severity and comorbidity indexes (Cumulative Illness rating Scale CIRS-SI and CIRS-CI), renal function, functional (Barthel Index), and cognitive status (Short Blessed Test) and mood disorders (Geriatric Depression Scale) were recorded. Mortality rates were assessed in hospital 3 and 12 months after discharge.

**Results** Of the 4714 elderly patients hospitalized, 361 had a glycemia level  $\geq 250$  mg/dL at admission. Compared to subjects with lower glycemia level, patients with glycemia  $\geq 250$  mg/dL showed higher rates of male sex, smoke and class III obesity. These patients had a significantly lower Barthel Index ( $p = 0.0249$ ), higher CIRS-SI and CIRS-CI scores ( $p = 0.0025$  and  $p = 0.0013$ , respectively), and took more drugs. In-hospital mortality rate was 9.2% and 5.1% in subjects with glycemia  $\geq 250$  and  $< 250$  mg/dL, respectively ( $p = 0.0010$ ). Regression analysis showed a strong association between in-hospital death and glycemia  $\geq 250$  mg/dL (OR 2.07; [95% CI 1.34–3.19]), Barthel Index  $\leq 40$  (3.28[2.44–4.42]), CIRS-SI (1.87[1.27–2.77]), and male sex (1.54[1.16–2.03]).

**Conclusions** The stronger predictors of in-hospital mortality for older patients admitted in general wards were glycemia level  $\geq 250$  mg/dL, Barthel Index  $\leq 40$ , CIRS-SI, and male sex.

**Keywords** Elderly · Hyperglycemia · Diabetes · Disability · Comorbidity · Mortality

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## Introduction

The number of adults with diabetes is raised from 108 million of 1980 to 422 million of 2014, worldwide [1]. This dramatic rise is largely due to the development of the “Diabesity” epidemic (type 2 diabetes and obesity) that represents the largest global epidemic in human history [2]. Diabetes directly caused 1.5 million of death in 2012, and 2.2 million of deaths were imputable to hyperglycemia [1].

Hyperglycemia is found in approximately 38% of hospitalized patients admitted to general wards, and diabetes represented the fourth most common disease mentioned on hospital discharges [4]. The association between increased glucose values and adverse hospital outcomes in hospitalized subjects with and without diabetes are well known [3]. The same results are well defined, when a different marker for poor glycemic control, such as glycemic variability was considered. A significant association between high glycemic variability and longer length of hospital stay and increased 3-month mortality in non-critical ill patients has been found [5]. However, the beneficial effects of better glycemic control in patients hospitalized in internal medicine wards are not clearly established and contribute to the efforts to achieve a better glycemic control in this setting. According to Ena and colleagues, there is an important gap between the clinical guidelines and both the management and the grade of glycemic control of diabetic subjects hospitalized in Spanish internal medicine wards [6]. Contrasting data are available about association between hyperglycemia at admission and main outcomes in subjects hospitalized in internal medicine wards.

A recent observational retrospective study did not find any correlation between mean glucose level, glycemic variability or persistent hyperglycemia and in-hospital mortality in elderly patients with community acquired pneumonia or chronic obstructive pulmonary disease admitted to internal medicine services [7] as well as according to the RePoSi register disability and not diabetes was a strong predictor of mortality in hospitalized elderly patients with pneumonia [8]. On the contrary, an association between admission glucose levels and in-hospital mortality and long of hospital stay was found in patients admitted to tertiary care hospital [9].

For this reason, the aim of this study was to determine if the identification of a simple measure of elevated glucose level at the time of admission was associated with comorbidities and outcomes in a cohort of elderly hospitalized in Internal Medicine and Geriatric Wards participating to the RePoSI (REgistro per lo studio delle POLipatologie e politerapie SIMi) register study.

## Materials and methods

We retrospectively analyzed the data collected in the frame of the RePoSI (REgistro Politerapie SIMI) project. RePoSI is an independent and ongoing collaborative register, organized by the Mario Negri Institute for Pharmacological Research and the Italian Society of Internal Medicine (SIMI). It encompassed the setting-up of a network of 102 internal medicine and geriatric wards that collected information about polytherapy on elderly patients with several diseases. Patients' eligibility criteria were: (1) admission to one of the attendee's wards during the four index weeks chosen for recruitment (one in February, one in June, one in September, and one in December); (2) age  $\geq 65$  years; (3) having given informed consent. At least ten consecutive eligible patients were enrolled during each index week, recording data on socio-demographic details, the main reason for admission and comorbidities, diagnoses, treatment (including all drugs taken at hospital admission and recommended at discharge), clinical events during hospitalization and outcome. All participating centers had to complete the registration of all patients admitted, indicating those who were consecutively enrolled. For patients who were excluded, the reason had to be given. Also, data on mortality or any new hospitalization were collected, with a telephone interview to the patient or his/her relatives, 3 and 12 months after hospital discharge. Then, a final database was created and checked by the Mario Negri Institute for Pharmacological Research. The RePoSI study was approved by the Ethics Committee of each participating centre. All the details of database and population characteristics may be found in previous publication [10]. The project's design is accessible at the related website (<https://repositi.org/>).

The dataset relating to 4714 patients was used for all the analyses. Socio-demographic variables such as age class, marital status, living arrangement and hospital admissions were all considered. As clinical characteristics, we evaluated: disease distribution at hospital admission (classification was based on the International Classification of Diseases-Ninth Revision), cognitive status tested with the Short Blessed Test (SBT) [11], mood disorders assessed by the Geriatric Depression Scale (GDS) [12], functional status at hospital admission (measured by means of the Barthel Index) [13], kidney function by means of eGFR (calculated using the Chronic Kidney Disease Epidemiology Collaboration formula) [14], severity and comorbidity indexes (evaluated, respectively, by the Cumulative Illness rating Scale CIRS-SI and CIRS-CI) [15] and in-hospital, 3-month and 1-year mortality rates.

## Statistical analysis

Data were reported as percentages for categorical variables and as means (95% confidence intervals) for quantitative variables. A Barthel Index score of  $\leq 40$  was used to select patients with significant disability according to our population characteristic. According to ROC curve analysis, a 250 mg/dL glycemic threshold was chosen as the best cut-off value for predicting mortality in our dataset. The comparison between groups was made using Fisher's exact-test for contingency tables and the z-test for comparison of proportions. The non-parametric Mann–Whitney-U-test was used for comparison of quantitative variables. A multivariate logistic analysis was used to explore the relationship between variables and outcomes (in-hospital and 3-month follow-up mortality). Odds ratios and 95% confidence-intervals were computed. Variables were chosen according to the Hosmer–Lemeshow methodology [16]: after univariate analysis, only variables with a  $p < 0.20$  were included in the final model; then, through a backward process, variables were excluded until a significance level of  $p < 0.20$  was reached for each variable. A two-tailed  $p < 0.05$  was considered statistically significant. Stata Statistical Software 2016, Release 14 (Stata-Corp, College-Station, TX-USA) was used for database management and all the analyses.

## Results

This analysis included 4714 elderly inpatients admitted to the internal and geriatric wards of RePoSi project. Among 361 patients with a glycemia level  $\geq 250$  mg/dL, 54.8% were male and the mean age was 79.3 years (78.5–80.0), 7.3% were previously institutionalized and more than one third of patients were previously hospitalized. More than half of patients have a caregiver and were smokers or ex-smokers, and 3.4% were subjects with class III obesity (Table 1).

Laboratory and clinical characteristics of inpatients with glycemia level at admission  $\geq 250$  mg/dL and  $< 250$  mg/dL are shown in Table 2. Subjects with glycemia level  $\geq 250$  mg/dL had higher count of leucocytes ( $p < 0.0001$ ) and platelets ( $p = 0.0310$ ), a lower glomerular filtration rate ( $p < 0.0001$ ), and 15.3% of them had a severe low glomerular filtration rate ( $p = 0.0017$ ), than those with blood glucose  $< 250$  mg/dL. Moreover, inpatients with glycemia level  $\geq 250$  mg/dL had a lower Barthel Index ( $p = 0.0249$ ), 18.8% had a Barthel Index  $\leq 40$  ( $p = 0.0208$ ), and 30.8% need for urinary catheter ( $p < 0.0001$ ). Compared to subjects with blood glucose  $< 250$  mg/dL, the group of patients with glycemia level  $\geq 250$  mg/dL had a significantly high CIRS for the evaluation of both severity and comorbidity indexes ( $p = 0.0025$  and  $p = 0.0013$ , respectively) and they took more drugs at hospital admission ( $p = 0.0020$ ), at hospital discharge ( $p < 0.0001$ ), and

at 3-month ( $p < 0.0001$ ) and 1-year follow-up ( $p = 0.0099$ ). Overall, disease distribution showed that diabetes, hypertension, chronic renal failure, ischemic heart disease, heart failure, anemia, atrial fibrillation, chronic obstructive pulmonary disease (COPD), cancer and dementia were more frequent in patients with glycemia  $\geq 250$  mg/dL (Table 3). The in-hospital mortality rate of patients with glycemia  $\geq 250$  mg/dL was 9.2% ( $p = 0.0010$ ) (Table 4). At 3-month discharge, 8.1% of patients with glycemia  $\geq 250$  mg/dL were institutionalized ( $p = 0.0030$ ). Glycemia  $\geq 250$  mg/dL (OR 2.07, 95% CI 1.34–3.19), Barthel Index  $\leq 40$  (OR 3.28, 95% CI 2.44–4.42), CIRS-SI (OR 1.87, 95% CI 1.27–2.77), and male sex (OR 1.54, 95% CI 1.16–2.03) were strong predictors of mortality at in-hospital mortality (Figure 1) while diabetes mellitus and glomerular filtration rate were protective. Moreover, age was an independent prognostic factor for in-hospital mortality. It is worth emphasizing that in the final model we verified that an interaction between rapid and long acting insulin was not present, and we did not report neither rapid and long-acting insulin neither anti-diabetic drugs because they were not statistically significant.

## Discussion

This study has investigated the possible association between elevated glucose levels at the time of admission and comorbidities along with short- and long-term mortality in hospitalized elderly people. Patients admitted to internal medicine wards have some risk for mortality independently of admitting diagnosis. This study highlighted the crucial role of glycemia upon admission  $\geq 250$  mg/dL that significantly increases the risk of death regarding in-hospital mortality in elderly subjects with and without a prior diagnosis of diabetes mellitus. Our results are in line with previous analysis that found an increased in-hospital mortality in subjects with the admission glucose level between 100 and 200 mg/dL [17]. On the other hand, in patients brought to the emergency room a plasma glucose level greater than 162 mg/dL (9 mmol/L) was a predictor of in-hospital mortality [18]. A very recent analysis of emergency visits from a Swedish hospital showed that in-hospital mortality was significantly higher only for subjects with severe hyperglycemia and significant higher percentage of 30-day mortality (4.0–4.5%) occurred in modest ( $> 126$  mg/dL  $\leq 200$  mg/dL,  $> 7 \leq 11.1$  mmol/L) and severe hyperglycemia ( $\geq 200$  mg/dL,  $\geq 11.1$  mmol/L) irrespective of diagnosis and treating medical specialty [19]. Our findings are in agreement with previous studies that found a strong association between severe hyperglycemia (glucose  $> 200$  mg/dL) and 30-day risk of mortality in critical ill patients with sepsis [20]. In this regard, some evidence showed that subjects with newly diagnosed hyperglycemia were more severe ill than patients

**Table 1** Socio-demographic characteristics and some modifiable risk factors of the REPOSI elderly population according to fasting glucose  $\geq 250$  mg/dL categorization

Variables	Inpatient with Fasting glucose > 250 mg/dL	Inpatient with Fasting glucose $\leq 250$ mg/dL	<i>p</i>
N° of subjects	361	4353	/
Men (%)	54.8	48.6	0.0222
Age*	79.3 (78.5—80.0)	79.5 (79.3—79.7)	0.5866
<b>Marital status (%)</b>			0.806
<i>Married</i>	54.0	53.9	
<i>Widow</i>	35.1	36.9	
<i>Separated</i>	1.1	1.3	
<i>Divorced</i>	1.4	1.3	
<b>Living arrangement (%)</b>			
<i>Alone</i>	20.1	23.0	0.016
<i>Spouse</i>	43.5	45.1	
<i>Sons</i>	15.7	15.0	
<i>Spouse and sons</i>	6.7	8.9	
<i>Other</i>	8.5	10.0	
Previously Institutionalized (%)	7.3	5.5	0.1633
Previously Hospitalized (%)	34.0	37.0	0.4157
<b>Caregiver (%)</b>	54.8	52.3	0.3749
<i>Spouse (%)</i>	33.5	33.2	0.646
<i>Brother/Sister (%)</i>	4.7	3.1	
<i>Son/Daughter (%)</i>	44.5	46.9	
<i>Son/Daughter in law (%)</i>	2.0	1.2	
<i>Grandson (%)</i>	2.6	3.8	
<i>Other (%)</i>	12.5	11.5	
Never Smoked (%)	47.9	55.0	0.038
ex-Smoker (%)	42.4	36.3	
Smoker (%)	9.5	8.5	
Never Alcohol (%)	55.2	56.7	0.189
Alcohol (%)	26.4	28.7	
ex-Alcohol (%)	8.4	5.8	
Casual Drinking (%)	9.8	8.6	
BMI*	26.48 (25.84—27.11)	25.85 (25.69—26.01)	0.1813
Underweight patients (%) <sup>†</sup>	3.8	4.0	0.8412
Optimal weight patients (%) <sup>†</sup>	40.7	41.1	0.9015
Overweight patients (%) <sup>†</sup>	34.8	35.3	0.8448
Class I obesity (%) <sup>†</sup>	11.0	12.9	0.3220
Class II obesity (%) <sup>†</sup>	4.7	3.0	0.1024
Class III obesity (%) <sup>†</sup>	3.4	1.4	0.0035

\* Data are reported as mean (95% Confidence Interval). <sup>†</sup>Underweight – BMI (Body Mass Index) < 18.5 kg/m<sup>2</sup>, Optimal weight 18.5 to 24.9 kg/m<sup>2</sup>; overweight – BMI  $\geq 25$  to 29.9 kg/m<sup>2</sup>; class I obesity – BMI 30.0–34.9 kg/m<sup>2</sup>; class-II obesity – BMI: 35.0–39.9 kg/m<sup>2</sup>; class-III obesity – BMI  $\geq 40.0$  kg/m<sup>2</sup>

with known diabetes or normoglycemic [3]; hyperglycemia represents an increase in blood glucose levels in response to physical stress and could indicate a more severe condition [21, 22]. On the other side, hyperglycemia could produce a more severe disease instead of being a marker of disease. In support of this hypothesis, intensive insulin therapy in critical ill patients results in a reduction in mortality and morbidity [23].

The importance of hyperglycemia could depend on the underlying medical condition and the level of stress. A slight hyperglycemia could represent an essential response producing beneficial effects [24]. Prolonged high levels of glucose could represent a maladaptive response determining the increase of reactive oxygen species and consequently cellular injury, intracellular and extracellular dehydration, electrolyte abnormalities, and depressing immune function [25].

**Table 2** Laboratory and clinical characteristics of the REPOSI population at hospital admission according to fasting glucose  $\geq 250$  mg/dL categorization

Variables	Inpatient with Fasting glucose $\geq 250$ mg/dL	Inpatient with Fasting glucose $< 250$ mg/dL	<i>p</i>
Systolic blood pressure (mm Hg)*	131.1 (128.9—133.3)	131.9 (131.2—132.5)	0.5243
Diastolic blood pressure (mm Hg)*	73.1 (71.9—74.4)	73.5 (73.1—73.8)	0.6331
Heart rate (bpm)*	78.6 (76.6—80.5)	79.0 (78.5—79.5)	0.6200
Body temperature (°C)*	36.86 (36.66—37.06)	37.84 (36.07—39.60)	0.3157
Creatinine (mg/dL)*	1.43 (1.32—1.53)	1.25 (1.22—1.28)	$< 0.0001$
GFR*	53.0 (50.4—55.6)	59.7 (59.0—60.4)	$< 0.0001$
Mild decrease in GFR	33.1	41.1	0.0055
Moderate decrease in GFR	40.8	35.8	0.0800
Severe decrease in GFR	15.3	9.8	0.0017
Kidney Failure	4.1	3.5	0.5424
Hemoglobin (mg/dL)*	11.79 (11.54—12.05)	11.82 (11.75—11.89)	0.7198
Leucocytes (cells per microliter) ( $\times 10^3/\mu\text{L}$ ) *	13.51 (9.00—18.02)	9.58 (9.14—10.03)	$< 0.0001$
Platelets (cells per microliter) ( $\times 10^3/\mu\text{L}$ ) *	244.58 (230.42—258.75)	229.47 (226.19—232.74)	0.0310
Cholesterol (mg/dL)*	156.4 (149.5—163.3)	159.8 (158.2—161.3)	0.4724
Short Blessed Test score *	9.3 (8.4—10.2)	9.1 (8.8—9.3)	0.4924
Overt Cognitive impairment (Short Blessed Test score $\geq 10$ ) (%)	43.1	44.0	0.7615
Need for urinary catheter (%)	30.8	21.8	0.0001
Barthel index score*	73.6 (70.2—77.1)	77.8 (76.9—78.7)	0.0249
Clinically significant disability (Barthel index $\leq 40$ ) (%)	18.8	14.2	0.0208
Geriatric Depression Scale score* Probable Depression (Geriatric Depression Scale score $> 2$ ) (%)	1.47 (1.32—1.62)	1.38 (1.34—1.42)	0.2236
N° of drugs at hospital admission*	6.3 (6.0—6.7)	5.7 (5.6—5.8)	0.0020
N of in-hospital drugs*	8.6 (8.0—9.2)	7.8 (7.7—8.0)	0.0411
N of drugs at hospital discharge*	8.7 (8.2—9.2)	7.6 (7.5—7.7)	$< 0.0001$
N of drugs at follow-up 3 months*	7.6 (7.1—8.1)	6.5 (6.4—6.6)	$< 0.0001$
N of drugs at follow-up 1 year*	7.9 (6.7—9.1)	6.4 (6.1—6.7)	0.0099
Severity index (by CIRS) *	1.72 (1.68—1.76)	1.66 (1.65—1.67)	0.0025
Comorbidity index (by CIRS) *	3.38 (3.17—3.59)	3.02 (2.96—3.07)	0.0013

\* Data are reported as mean (95% Confidence Interval) BMI = Body Mass Index; CIRS = Cumulative Illness Rating Scale

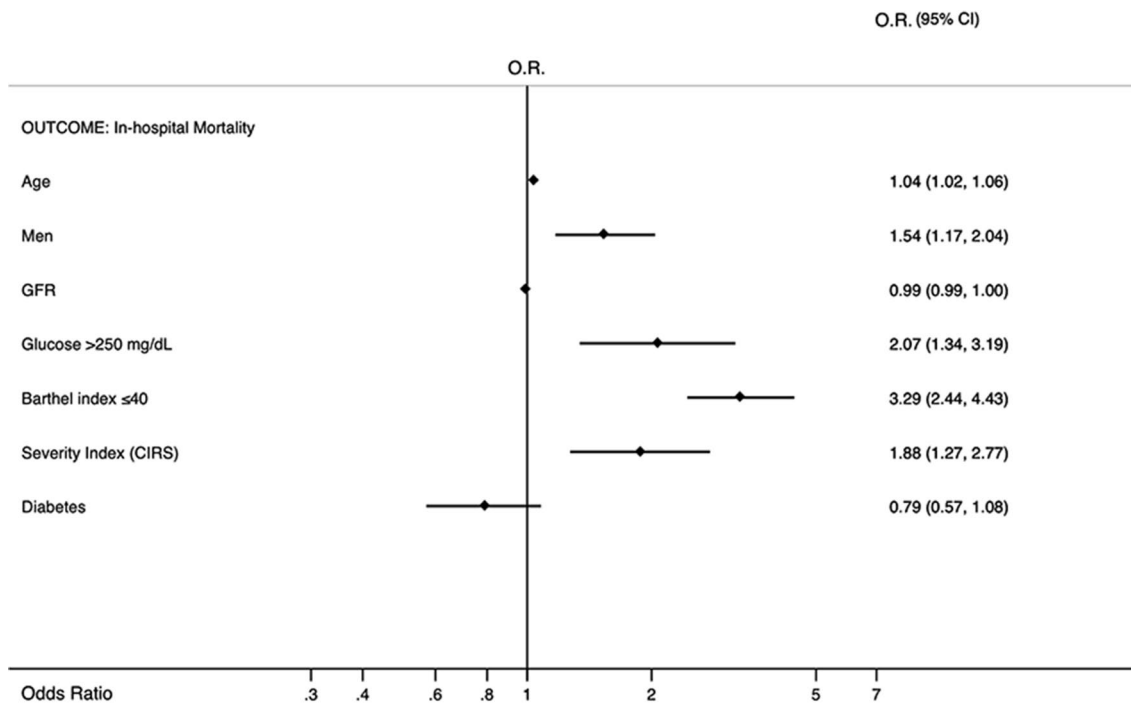
**Table 3** The most frequent clinical diagnoses (as percentage) in the REPOSI population according to fasting glucose  $\geq 250$  mg/dL categorization (the table only shows the diagnoses which frequency was more than 10%)

Comorbidities	Inpatient with Fasting glucose $\geq 250$ mg/dL	Inpatient with Fasting glucose $< 250$ mg/dL	<i>p</i>
Diabetes	56.8	27.0	$< 0.0001$
Hypertension	54.3	59.5	0.0526
Chronic renal failure	27.4	19.6	0.0004
Ischemic heart disease	26.6	22.5	0.0766
Heart Failure	23.4	19.8	0.0984
Anemia	22.8	20.3	0.2638
Atrial fibrillation	21.0	24.9	0.0998
COPD	19.7	19.9	0.9280
Cancer	16.7	19.1	0.2966
Dementia	15.2	14.9	0.8769

**Table 4** Length of hospital stay, destination at hospital discharge, in-hospital and at follow-up mortality of the whole REPOSI population according to fasting glucose  $\geq 250$  mg/dL categorization

Variables	Inpatient with fasting glucose $\geq 250$ mg/dL	Inpatient with fasting glucose $< 250$ mg/dL	p
Length of hospital stay* (days)	11.4 (10.4—12.3)	11.8 (11.4—12.3)	0.7463
In-hospital mortality (%)	9.2	5.1	0.0010
3-month mortality (%)	7.0	9.6	0.2254
12-month mortality (%)	13.5	14.0	0.9300
<i>Destination at discharge (3-month)</i>			
Home (%)	84.8	89.6	0.0413
Home care (%)	3.3	3.2	0.9659
Institution (%)	8.1	3.7	0.0030
Rehospitalization (%)	3.8	3.5	0.8237
<i>Destination at discharge (12-month)</i>			
Home (%)	96.9	88.5	0.1421
Home care (%)	0.0	2.9	0.3310
Institution (%)	3.1	5.7	0.5310
Rehospitalization (%)	15.8	15.0	0.8951

\* Data are reported as means (95% Confidence Interval)



**Fig. 1** Multivariate Analysis in-hospital mortality OR = odds ratio; 95% CI = 95% confidence interval; GFR = Glomerular Filtration Rate calculated by CKD-EPI formula; CIRS = Cumulative Illness Rating Scale

In our patients, there was no association between diabetes and mortality. This finding is in agreement with previous studies which showed that critical ill patients with diabetes did not have an increased mortality and conversely had a decreased mortality [26]. Moreover, diabetes was not a strong predictor of mortality in hospitalized elderly patients with pneumonia [8]. A possible explanation could be due to the long-term

effect of antidiabetic treatment or alternatively to a more appropriately attention of clinicians to the hyperglycemic state of admitted patients. Another important finding of our analysis concerns the fundamental role of Barthel index and CIRS. Recent studies showed that hyperglycemia was an independent factor of functional outcomes of patients with acute ischemic stroke as measured with Barthel index [27]. Barthel Index was

a strong predictor of mortality at in-hospital, 3-month, and 1-year mortality. Functional disability represents a common risk factor in subjects aged  $\geq 75$  years [28]. The role of functional disability was found to be relevant to explain the risk of early readmissions in a cohort of patients aged 75 and older [29]. In addition, limited activities in daily living on hospital admission as measured by Barthel Index score less than forty-five were predictive of prolonged hospital stay independent of diagnosis. Whereas a Barthel Index less than sixty-five on admission predicts mortality within six months of discharge. Finally, functional disability on admission was predictive of institutionalization on discharge [30]. Higuchi et al. showed that the Barthel Index as indicator of activities of daily living may be a useful predictor for 1-year mortality in very elderly patients undergoing percutaneous coronary intervention for acute coronary syndrome [31].

Regarding the important role of CIRS-SI, our findings are in line with a recent analysis that showed that CIRS assessment of comorbidity burden is the more useful clinical tool for the evaluation of length of hospital stay and all-cause mortality in hospitalized elderly patients [32]. In addition, CIRS represents the instrument of choice for multimorbidity assessment in clinical trials and has the benefit to predict mortality hospital readmission and prolonged hospital stay [33, 34]. CIRS  $\geq 3$  at discharge was significantly associated with a risk of hospital readmission within 3 months [35].

CIRS-SI was a strong predictor of mortality at in-hospital and 1-year mortality. CIRS-SI and CIRS CI were higher in patients affected by pneumonia in comparison with people without pneumonia [8]. It is worth mentioning that a high index of comorbidity (CIRS index  $>3$ ) is significantly associated with gastrointestinal bleeding in elderly patients [36].

Another important finding was the role of male sex. Our data are consistent with previous analysis that showed that male sex was more affected by cumulative illness burden [10, 37]. Moreover, male sex was associated with increased number of cardio-renal-metabolic diseases among patients with type 2 diabetes [38].

The major strength of the study is the multicentre design of the REPOSI register and the large number of internal medicine and geriatric wards involved that make the study representative of the real-world scenario. A major limitation of this study concerns the lack of clinical information, particularly nutritional status, that is beyond the purpose of the RePoSI study. Moreover, we did not collect data that could influence outcome such as glycemia levels measurements during hospitalization and hypoglycemic events.

## Conclusions

In conclusion, our analysis revealed that a single blood glucose level taken at the time of admission was associated with comorbidities and short-term outcomes in the real-world scenario of a cohort of elderly hospitalized in Internal Medicine and Geriatric Wards. Further studies are necessary to evaluate whether intervention and normalization of blood glucose levels in these individuals make a difference to short- and long-term outcomes.

## Appendix: Investigators and co-authors of the REPOSI (REGistro POLiterapie SIMI, Società Italiana di Medicina Interna) Study Group are as follows

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## Declarations

**Conflicts of interest** The authors declare that there is no conflict of interest.

**Ethical Standard Statement** The RePoSI study was approved by the Ethics Committee of each participating centre.

**Consent to Participants** Informed consent was obtained from all individual participants included in the study.

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