Time to death in a prospective cohort of 252 patients treated for fracture of the proximal femur in a major hospital in Portugal

Tempo até a morte após fratura do fêmur proximal: uma coorte prospectiva de 252 doentes tratados no segundo maior hospital em Portugal

Tiempo transcurrido hasta la muerte después de una fractura de cadera: una cohorte prospectiva de 252 pacientes del segundo mayor hospital de Portugal

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Abstract

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The objectives were to analyze one-year survival and mortality predictors in patients with fracture of the proximal femur (low/moderate trauma). A prospective cohort was formed by inviting all patients hospitalized in the Orthopedic Ward of the second largest hospital in Portugal (May 2008-April 2009). Survival was assessed at 3, 6, 9, and 12 months after fracture and related to demographic factors, lifestyle, and clinical history, as well as to data from medical records (fracture type, surgery date, surgical treatment, and preoperative risk). Of the 340 patients hospitalized, 252 were included (78.9% women). Mortality at 3, 6, 9, and 12 months was 21.2%, 25%, 28.8%, and 34.6% for men and 7.8%, 13.5%, 19.2%, and 21.4% for women, respectively. Predictors of death were male gender (HR = 2.54; 95%CI: 1.40-4.58), ASA score III/IV vs. I/II (HR = 1.95; 95%CI: 1.10-3.47), age (HR = 1.06; 95%CI: 1.03-1.10), and delay in days to surgery (HR = 1.07; 95%CI: 1.03-1.12). Factors related to death were mainly related to patients' characteristics at admission.

Hip Fractures; Survival; Mortality

Resumo

Os objetivos foram analisar a sobrevivência após um ano e os fatores associados para doentes com fratura do fêmur proximal (baixo impacto). Foi constituída uma coorte com todos os doentes hospitalizados no serviço de ortopedia do segundo maior hospital de Portugal (maio de 2008 a abril de 2009). A sobrevivência foi avaliada aos 3, 6, 9 e 12 meses após a fratura e relacionada com fatores demográficos, estilo de vida, história clínica e fatores médicos (tipo de fratura, data da cirurgia, tratamento e risco pré-operatório). Dos 340 doentes hospitalizados, 252 (78,9% mulheres) foram incluídos. Mortalidade aos 3, 6, 9 e 12 meses de seguimento foi 21,2%, 25%, 28,8%, 34,6% para homens e 7,8%, 13,5%, 19,2%, 21,4% para mulheres. Os fatores associados com a mortalidade foram: sexo masculino (HR = 2,54; IC95%: 1,40-4,58), escore da American Society of Anesthesiologists mais elevado, III/IV vs. I/II (HR = 1,95; IC95%: 1,10-3,47), idade (HR = 1,06; IC95%: 1,03-1,10) e dias de atraso na cirurgia (HR = 1.07: IC95%: 1.03-1.12). Fatores associados com a mortalidade estão na maioria relacionados com as características do doente na admissão.

Fraturas do Quadril; Sobrevivência; Mortalidade

Introduction

Proximal femur fractures (PFF) create a public health burden due to their negative impact on the well-being of patients and families as well as on health systems, due to the need for care during the acute and recovery phases 1,2. In Portugal, a retrospective study based on hospital admissions reported 77,083 PFF from 2000 to 2008, or an average of 8,500 such fractures per year 3.

Most patients with PFF are older women (> 75 years) with underlying bone fragility and increased fracture risk 4. However, mortality is higher in men, although on average they are younger than women at the time of fracture 5,6. Risk of death remains high in the first 3 to 6 months after the fracture in both men and women, declining thereafter. However, mortality risk does not return to the same levels observed in the general population matched for age and gender but without PFF 7,8,9.

Early identification of patients at increased risk can help promote measures to reduce mortality 10,11. Factors such as age, gender, mobility prior to the fracture, fracture type, time to surgery, preexisting clinical conditions and their severity, medical complications following the fracture, and surgical treatment can influence the patient's risk of death after a PFF 12,13,14,15.

As with incidence, survival after PFF differs between countries 9. Predictors of mortality have received little research attention in Portugal. Available information on mortality after PFF in Portugal has relied on small patient samples from small hospitals 16,17,18.

We conducted a prospective cohort study with one year follow-up to analyze time to death in patients over 49 years of age with a PFF caused by low/moderate trauma and to determine the main predictors of mortality.

Methods

Participants

All patients admitted to the Orthopedic Ward of the São João Hospital (SJ Hospital) for treatment of an acute PFF caused by low/moderate trauma from May 1st, 2008, to April 30th, 2009, were invited to participate. The local ethics committee approved the study on April 11th, 2005 (protocol n. 4/2005), and participants provided informed written consent in compliance with the Helsinki Declaration.

The SJ Hospital, the second largest hospital in Portugal, is located in Porto, a city of approximately 240,000 inhabitants (2009) located in Northwest Continental Portugal. Since all suspected cases of PFF undergo x-ray to confirm the diagnosis, the registry of imaging tests (PACS -Picture Archiving and Communication System) was searched daily to identify patients treated at the emergency service for PFF. Hospitalization was confirmed by consulting the hospital database or the nurses' board in the Orthopedic Ward.

Data collection

A structured questionnaire adapted from the MEDOS Questionnaire 19 was used to record the patients' age, gender, marital status, and living arrangement and daily activities (housekeeping, grocery shopping, childcare, walking) before the fracture, history of previous fractures, comorbidities, and current medication. An open question for the description of the event that caused the fracture was also included. The same trained interviewer applied all the questionnaires during hospitalization. Patients were defined as cognitively impaired when they were unable to inform their age, date and place of birth, and place of residence. In such cases, whenever available the closest relative answered the questions (38% of respondents), otherwise the patient was excluded from the study.

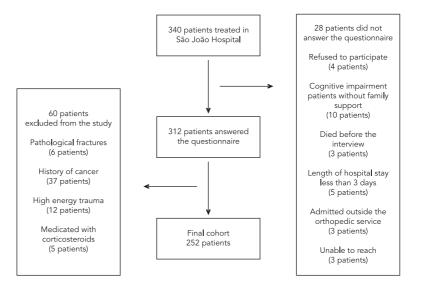
During the study period 340 patients were treated for PFF at SJ Hospital, but the final sample consisted of 252 patients. Figure 1 shows the patient flow and exclusion criteria. Patients answered the questionnaire four days after admission, on average.

To ensure accurate assessment of comorbidities, we analyzed hospital discharge records, which compile the following data on all discharges: gender, age, date of admission and discharge, cause of admission and main diagnosis (and up to 19 secondary causes and diagnoses) coded according to the International Classification of Diseases, version 9, Clinical Modification (ICD9-CM), procedures during hospitalization, and transfers to and from other hospitals. Based on the hospital discharge registry, we selected all cases of PFF (ICD9-CM codes 820.x) during the study period.

Additional data were obtained from patients' hospital records: type of fracture, surgical treatment, and preoperative risk as defined by the American Society of Anesthesiologists, or ASA score, which classifies patients in five categories: I normal healthy patient; II patient with mild systemic disease; III patient with severe systemic disease; IV patient with severe systemic disease that is a constant threat to life; and V moribund patient 20.

Figure 1

Flow chart of participants' selection (n = 252) and exclusion criteria of patients admitted to the Orthopedic Ward of São João Hospital, Portugal (May 1st, 2008, to April 30th, 2009).



Follow-up consisted of a short telephone interview with questions on mobility, place of residence after the fracture, independence in activities of daily living, and occurrence of new fractures. The questionnaire was applied at 3, 6, 9, and 12 months after the index fracture by the same interviewer that applied the initial questionnaire in the hospital. Survival time was measured in days starting from the day of admission to day of death or end of study (for patients that were alive at the end of the study). For patients that died, the exact date of death was requested from the respondent (closest family member). Twenty-four patients could not be reached by telephone at any of the follow-up times, so survival time was determined by the last contact: in some cases the discharge date (n = 7), in others the last appointment at any ward in the SJ Hospital (n = 16), or the last successful follow-up (n = 1).

Time (delay) to surgery was measured as the difference in calendar days between surgery date and admission date. For patients that suffered an additional fracture, survival time was measured from the second fracture.

Statistical analysis

Summary statistics on participants' characteristics were computed (mean and standard deviation or SD). Chi-square or Fisher's exact test was used to assess associations between categorical variables, and independent sample t-test to compare quantitative variables between two groups (after normality verification for groups less than 30). Statistical significance was set at p < 0.05.

No adjustment was conducted for history of previous fractures.

Survival analysis was conducted using time from fracture either to death or to end of study for patients that survived. Univariate analysis was performed using Kaplan-Meier curves and log-rank test for the following independent variables: gender (female, male), marital status (widowed, married, single/divorced), living arrangement (with someone, alone, institution), daily activities before fracture (< 4 hours and ≥ 4 hours per day), number of comorbidities (0-1, 2-4, or ≥ 5), type of surgery (osteosynthesis or arthroplasty), type of fracture [intracapsular (femoral neck) or extracapsular (trochanteric or subtrochanteric)], ASA score [low operative risk (ASA I-II) vs. high operative risk (ASA III-IV), with no patients classified as ASA V], and comorbidities (hypertension, heart disease, anemia, diabetes mellitus, respiratory disease, cerebrovascular disease, renal disease, and dementia). Medication was analyzed using both a dichotomous variable (yes/no) as well as categories according to the Anatomical Therapeutic Chemical classification system 21.

Proportional hazard assumption was verified using plot option, and residual analysis was conducted to assess the model's quality. Interactions between variables were also tested.

The final multivariate Cox's proportional hazards model included all independent variables (described previously) with significant p-value after age adjustment (p < 0.05). The results are presented using hazards ratios (HR) and respective 95% confidence intervals (95%CI).

Results

Baseline

Table 1 shows the participants' characteristics. Of the 252 patients included in the study, 78.9% were women, with mean age 80.3 years (SD 9.1) vs. 76.0 years (SD 11.5) in men (p = 0.015). Slightly more than half of the patients (52.8%) were over 80 years old (range 50-105). All patients were admitted on the day of the fracture.

Only 7.5% of patients had no comorbidities. Fifty patients (19.8%) reported a history of previous fractures (hip, wrist, or spine); 48 were women. Six patients (one man) sustained a second fracture on the contralateral hip during the study period, one of which while hospitalized.

Table 1 Baseline characteristics of patients (n = 252) admitted to the Orthopedic Ward of São João Hospital, Portugal (May 1st, 2008, to April 30th, 2009).

Characteristics	Wo	men	IV	len	p-value
	n	%	n	%	
Marital status					
Widowed	114	57.3	13	24.5	
Married	56	28.1	28	52.8	
Single/Divorced	29	14.6	12	22.6	< 0.001
Living arrangement					
With someone	132	66.3	37	69.8	
Alone	51	25.6	13	24.5	
Institution	16	8.0	3	5.7	0.815
Daily activities (hours per day)					
≥ 4	63	31.7	22	41.5	
< 4	136	68.3	31	58.5	0.178
Number of comorbidities					
0-1	44	22.1	13	24.5	
2-4	93	46.7	23	43.4	
≥ 5	62	31.2	17	32.1	0.895
Comorbidities					
Hypertension	111	55.8	20	37.7	0.019
Heart disease	64	32.2	16	30.2	0.784
Anemia	50	25.1	11	20.8	0.509
Diabetes mellitus	46	23.1	11	20.8	0.715
Respiratory disease	25	12.6	19	35.8	< 0.001
Thyroid disease	12	6.0	0	0.0	-
Cerebrovascular disease	26	13.1	7	13.2	0.974
Renal disease	13	6.5	5	9.4	0.367
Dementia	38	19.1	9	17.0	0.725

(continues)

Table 1 (continued)

Characteristics	Wo	men	N	len	p-value
	n	%	n	%	
Medication *					
Yes	158	79.4	34	64.2	
No	15	7.5	10	18.9	0.009
Type of medications used					
Antihypertensive	84	42.2	20	37.7	0.713
Anxiolytics/Sedatives	63	31.7	8	15.1	0.021
Antithrombotic	44	22.1	14	26.4	0.393
Lipid modifying agents	38	19.1	4	7.5	0.054
Cardiac therapy	29	14.6	5	9.4	0.379
Antidepressants	27	13.6	2	3.8	0.054
Insulins and analogues	22	11.1	4	7.5	0.357
Blood glucose lowering drugs	16	8.1	4	7.5	0.619
Place of fall					
Home	163	81.9	34	64.2	
Outdoors	36	18.1	19	35.8	0.005
Type of fracture					
Intracapsular	82	41.2	20	37.7	
Extracapsular	117	58.8	33	62.3	0.647
Type of surgery **					
Osteosynthesis	133	67.9	36	69.2	
Arthroplasty	63	32.1	16	30.8	0.850
ASA score ***					
I-II	102	52.6	24	47.1	
III-IV	92	47.4	27	52.9	0.531

^{*} Data not available for 35 patients;

Treatment was surgical in 98.4% of the patients. Mean time from admission to surgery was 3.5 days (SD 4.4) in women versus 3.2 days (SD 2.8) in men (p = 0.74). Approximately one-third (36.7%) of patients underwent surgery on the day of admission or the following day, 22% on the second day after admission, and 41% on subsequent days. Time from admission to surgery in patients with ASA I or II was shorter (2.6 days; SD 2.8) than in patients with ASA III or IV (4.2 days; SD 5.1) (p = 0.003). However, gender differences were identified: in patients with ASA III or IV, 33.7% of women underwent surgery on the day of admission or the following day compared to 7.4% of men (p = 0.007).

In patients on antithrombotic medication prior to the fracture, waiting time for surgery was 4.8 days (SD 4.7) as opposed to other patients (3.1 days; SD 3.9) (p = 0.012). Men on antithrombotic medication prior to the fracture waited longer for surgery (5.2 days; SD 3.4) when compared to women on the same medication (4.7 days; SD

5.1) (p = 0.753), as well as compared to men not on prior antithrombotic medication (2.8 days; SD 2.5) (p = 0.035).

Among patients on antithrombotic medication prior the fracture, 84.6% of men were ASA score III or IV, compared to 47.7% of women in the same ASA group (p = 0.018).

Mean length of hospital stay was similar in women and men, namely 14.9 days (SD 12.4) for women and 13.3 days (SD 12.3) for men (p = 0.421). Length of stay was greater for patients living alone prior to the fracture when compared to those living with someone else, i.e., 18.5 days (SD 15.7) and 13.4 days (SD 10.9) respectively (p = 0.021).

Follow-up

One year after the fracture, 59 patients had died (41 women and 18 men), 13 of whom died during hospitalization (8 women and 5 men). Mortality in men was higher at 3, 6, 9, and 12 months

^{**} Patients not submitted to surgery n = 4;

^{***} Data not available for 3 patients with surgery.

follow-up, namely 21.2%, 25%, 28.8%, and 34.6%, compared to 7.8%, 13.5%, 19.2%, and 21.4% in women, respectively.

Survival analysis

Overall one-year survival was 75% (95%CI: 70%-81%). In men it was 64% (52%-78%) and in women 82% (73%-84%). Figure 2 shows Kaplan-Meier curves for statistically significant variables according to the log-rank test. After adjustment for age, the variables gender, ASA score, presence of comorbidities, and number of comorbidities remained as significant predictors of mortality (Table 2). No interaction between variable was identified.

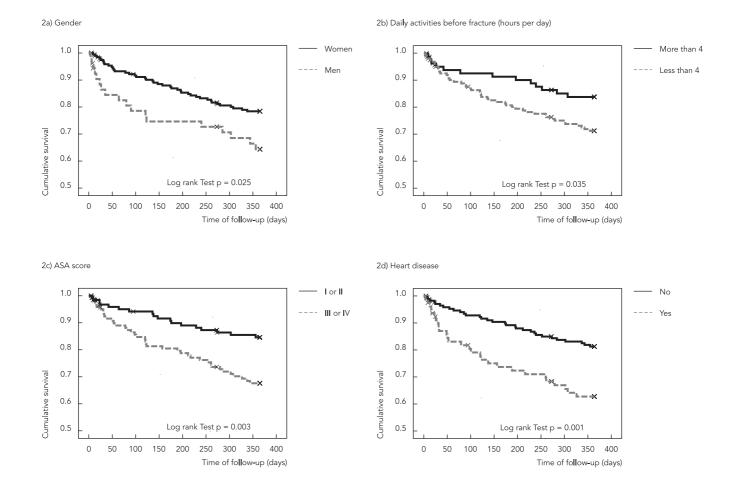
Table 3 shows the results of the multivariate Cox's proportional hazards model. Accordingly, male gender (HR = 2.54; 95%CI: 1.40-4.58), III/V ASA score (HR = 1.95; 95%CI: 1.10-3.47), age (HR = 1.06; 95%CI: 1.03-1.10), and delay to surgery (HR = 1.07; 95%CI: 1.03-1.12) were independent and statistically significant predictors of survival after PFF.

Discussion

Mortality after PFF creates a major clinical and public health burden. One-fourth of patients in our study had died after one year. Increased mortality was associated with increasing age,

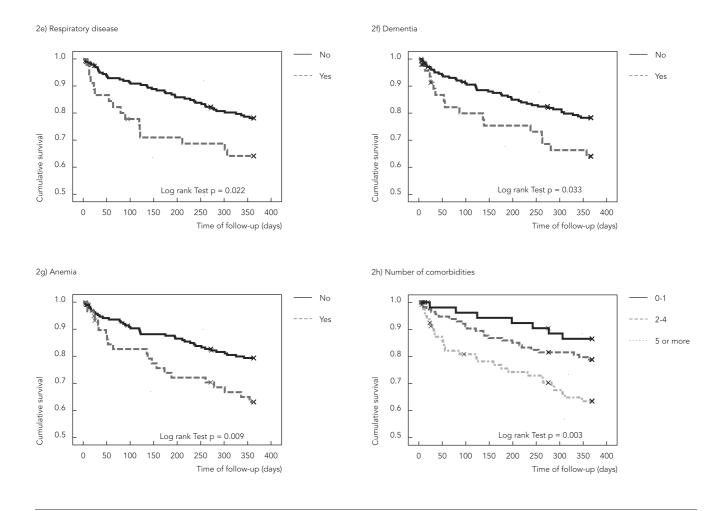
Figure 2

Kaplan-Meier curves with significant log-rank test for gender, daily activities before fracture, ASA score, heart disease, respiratory disease, dementia, anemia, and number of comorbidities. Cohort study of 252 patients admitted to the Orthopedic Ward of São João Hospital, Portugal (May 1st, 2008, to April 30th, 2009).



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Figure 2 (continued)



longer delay to surgery, male gender, and worse ASA score.

Overall one-year mortality in men (34.6%) was higher than reported in some European countries such as Ireland (30.1%), Norway (31%), and the Netherlands (33%). Meanwhile, in women, one-year mortality (21.4%) was higher than in Ireland and Norway, but lower than in the Netherlands 22,23,24. In Portugal, as far as we know only one other prospective cohort (patients > 65 years old) studied survival after PFF 17, and overall mortality in women was similar (22.2%), while in men our study showed lower overall mortality (48.3%). However, the results should be analyzed with caution, since this other study included patients more than 65 years of age.

Even after adjusting for age, ASA score, and delay to surgery, men showed 2.54 times higher mortality risk (95%CI: 1.40-4.58) in women. These results are similar to findings from a prospective cohort of 218 patients in Spain (2007), with a relative risk of 2.44 (95%CI: 1.01-5.93) after adjusting for age, type of fracture, living situation, functional status prior to the fracture, mental status, comorbidities, delirium during admission, and situation at discharge (patients discharged to the community versus to an institution) 25.

The gender differences in mortality are difficult to explain, although it appears that men with PFF tend to be more ill and frailer than women, making them more vulnerable to postoperative complications 26. A recent study on cause of death in patients after PFF found that men were more likely to die from respiratory diseases, malignant neoplasms, and cardiovascular diseases ²⁷. In our study, although complications and causes of death were not studied, we identified a higher prevalence of respiratory diseases

Table 2

Age-adjusted hazard ratios (HR) for one-year mortality after proximal femur fracture. Cohort study of 252 patients admitted to the Orthopedic Ward of São João Hospital, Portugal (May 1st, 2008, to April 30th, 2009).

Variable	Crude HR (95%CI)	Age-adjusted HR (95%CI)
Gender		
Female	1.00	1.00
Male	1.85 (1.06-3.21)	2.44 (1.38-4.31)
Daily activities before fracture (hours per day)		
≥ 4	1.00	1.00
< 4	1.88 (1.02-3.48)	1.54 (0.82-2.90)
ASA score		
ASA I-II	1.00	1.00
ASA III-IV	2.38 (1.36-4.17)	2.27 (1.29-3.98)
Comorbidities		
Without heart disease	1.00	1.00
With heart disease	2.31 (1.39-3.86)	2.06 (1.23-3.44)
Without respiratory disease	1.00	1.00
With respiratory disease	2.03 (1.14-3.61)	2.11 (1.19-3.76)
Without dementia	1.00	1.00
With dementia	1.83 (1.03-3.25)	1.82 (1.02-3.22)
Without anemia	1.00	1.00
With anemia	1.91 (1.12-3.26)	1.72 (1.01-2.96)
Number of comorbidities		
0-1	1.00	1.00
2-4	1.70 (0.73-3.95)	1.66 (0.71-3.84)
≥ 5	3.32 (1.45-7.60)	3.08 (1.34-7.06)
Delay to surgery	1.08 (1.03-1.12)	1.08 (1.03-1.12)

95%CI: 95% confidence interval.

in men, and more men were on antithrombotic medication prior to the fracture, placing them at greater risk of respiratory complications and thromboembolic events.

Age increased the risk of mortality after a PFF, with a 6% increase in mortality for each year (HR = 1.06; 95%CI: 1.03-1.10) in our study. A similar prospective cohort study in Italy with 3,707 patients > 50 years of age found a similar risk (HR = 1.08; 95%CI: 1.06-1.09) 28.

A controversial factor affecting mortality after PFF is time between admission and surgery ^{29,30,31}. Although the optimal time for surgery after PFF in the elderly is not clear, most authors report that early surgical intervention (< 24, < 48, or < 72 hours) is associated with better prognosis and improved health status 15,31,32. Delay to surgery prolongs hospital stay and increases the risk of pulmonary embolism, deep venous thrombosis, heart failure, urinary infection, and pressure sores 33,34,35, thus delaying rehabilitation and increasing the risk of death.

Table 3

Final Cox's proportional hazards analysis. Predictors of one-year survival after proximal femur fracture. Cohort study of 252 patients admitted to the Orthopedic Ward of São João Hospital, Portugal (May 1st, 2008, to April 30th, 2009).

Variable	Crude	Adjusted * HR (95%CI)	
	HR (95%CI)		
Gender			
Female	1.00	1.00	
Male	1.85 (1.06-3.21)	2.54 (1.40-4.58)	
ASA score			
ASA I/II	1.00	1.00	
ASA III/IV	2.38 (1.36-4.17)	1.95 (1.10-3.47)	
Age	1.05 (1.02-1.08)	1.06 (1.03-1.10)	
Delay to surgery	1.08 (1.03-1.12)	1.07 (1.03-1.12)	

95%CI: 95% confidence interval; HR: hazard ratio.

^{*} HR adjusted for gender, age, delay to surgery, and ASA score.

In our study, each day of delay to surgery was associated with a 7% increase in risk of death. Time to surgery can help explain gender differences in survival. In our study, men were less likely than women to undergo surgery on the day of admission or on the following day. Surgery is often postponed to stabilize patients' clinical condition and optimize their hemodynamic status, since most patients admitted after a PFF are clinically unstable, dehydrated, anemic, and malnourished 36. In addition, patients on antithrombotic medication prior to the fracture (highly common in the elderly for preventing thrombosis and atrial fibrillation) normally have their surgery delayed to prevent excessive intraoperative bleeding 37,38. However, according to our findings a simple analysis of the use of antithrombotic medication does not appear to result in differences in time to surgery, since men on antithrombotic medication prior to surgery waited an average of 0.5 days longer than women. On the other hand, when analyzing ASA score and time to surgery, only 7% of men with ASA III or IV underwent surgery on the day of admission or the following day, compared to 34% of women with the same ASA score. This indicates that even if there is no difference in ASA between men and women, the comorbidities that contribute to higher ASA scores in men require longer time for stabilization, thus resulting in longer time to surgery.

In our final Cox's proportional hazards analysis, ASA score, which takes severity of comorbidities into consideration, was found to be useful in predicting mortality in patients with a PFF, corroborating other studies 10,39.

The study presents some limitations. Participants' mental status was not tested objectively. However, we evaluated their orientation in time and space based on their answers on age, date and place of birth, and place of residence. Contrary to other studies 40,41, we did not find an association between mortality and daily activities prior to hip fracture; however, this may have resulted from limitations in the way the variable was collected, by categories: < 4 hours versus ≥ 4 hours of daily activity, not taking into consideration previous limitation in walking or use of walking aids.

The study's strong points are related to the design, as a prospective cohort study conducted in the second largest hospital in Portugal. Data were collected directly from patients and from objective and standardized records, thereby increasing data quality. In addition, unlike other studies, the same interviewer applied all the questionnaires at baseline and follow-up, also enhancing data quality. Finally, diagnosis and treatment of PFF were confirmed by consulting imaging records, thereby avoiding misclassification of fractures, while type of surgery and comorbidities were assessed from patients' clinical charts, thus avoiding recall bias.

In conclusion, in the present study, increased risk of death after PFF was associated with increasing age, delay to surgery, male gender, and worse ASA score. Patients with PFF require a multidisciplinary approach and the use of clinical score systems (e.g., ASA score) together with data that can be easily and routinely collected from patients' charts (time to surgery, medications), thus facilitating early identification of high-risk patients and treatment adjustments. The high mortality rates identified in this setting also call for improvements in post-fracture treatment and care in Portugal.

Resumen

Los objetivos del estudio fueron analizar la supervivencia tras un año y los factores asociados para enfermos con fractura de la cadera (bajo impacto). Fue constituida una cohorte con todos los enfermos hospitalizados en el servicio de ortopedia del segundo mayor hospital de Portugal (mayo/2008 - abril/2009). La supervivencia fue evaluada a los 3, 6, 9 y 12 meses tras la fractura y relacionada con factores demográficos, estilo de vida, historia clínica y factores médicos (tipo de fractura, fecha de la cirugía, tratamiento y riesgo preoperatorio). De los 340 enfermos hospitalizados, 252 (78,9% mujeres) fueron incluidos. La mortalidad a los 3, 6, 8 y 12 meses de seguimiento fue de un 21,2%, 20%, 28,8%, 34,6% en hombres y un 7,8%, 13,5%, 19,2%, 21,4% en mujeres. Los factores asociados con la mortalidad fueron: sexo masculino (HR = 2,54; IC95%: 1,40-4,58), ASA puntuación más elevada, III/IV vs. I/II (HR = 1,95; IC95%: 1,10-3,47), edad (HR = 1,06; IC95%: 1,03-1,10) y días de retraso en la cirugía (HR = 1,07; IC95%: 1,03-1,12). Los factores están en su mayoría relacionados con las características del enfermo en la admisión.

Fracturas de Cadera; Supervivencia; Mortalidad

Contributors

S. Campos gathered the data from the interviews at admission and follow-up, conducted the analysis, discussion of the results, and wrote the draft. S. M. F. Alves participated in the analysis, discussion of the results, and writing of the final manuscript. M. S. Carvalho participated in the statistical analysis, discussion of the results, and final version of the manuscript. N. Neves participated in the interpretation and discussion of the results and final version of the manuscript. A. Trigo-Cabral participated in the discussion and interpretation of the results and writing of the final manuscript. M. F. Pina was responsible for the study design and conception, interpretation and discussion of the results, and writing of the final manuscript.

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