Plasma fibrinogen levels and all-cause and cause-specific mortality in an italian adult population: results from the Moli-sani study

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ABSTRACT

Epidemiological data on the association between fibrinogen levels and mortality are scarse and controversial. Longitudinal analyses were performed, separately by sex, on 17,689 individuals from the Moli-sani study [53% women, ≥35 years, free from cardiovascular disease (CVD) or cancer at enrolment], to evaluate the association between plasma fibrinogen and all-cause and cause-specific mortality. Over a median follow-up of 11.2 years, 1,058 deaths (34.7% CVD, 36.3% cancer) were ascertained. Both in the lowest (1.12-2.64 g/L) and highest (≥3.62 g/L) fibrinogen quintiles, women had an increased all-cause mortality hazard, when compared with third quintile (2.97-3.23 g/L). Dose-response analyses showed a U-shaped relationship in women (P overall <0.0001; P non-linear association <0.0001), but a positive linear association for all-cause mortality in men (P overall 0.0038; P non-linear association 0.76). Similar trends for a U-shaped association were observed for CVD mortality, while no association was observed with cancer deaths. A U-shaped association of fibrinogen levels with other-cause mortality was also found in both sexes. This study shows that not only higher but also lower fibrinogen levels represent hazard for mortality when compared to normal levels; U-shaped curves being prevalently observed in women.

INTRODUCTION

Fibrinogen is a glycoprotein synthesized by the liver that plays a key role in the coagulation cascade. In healthy individuals, the plasma levels of this protein range from 2 to 4 g/L, with a normal half-life of 3-5 days.[1,2]

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Contributions: RP and SC contributed equally. BdL, SC, and LI contributed to the conception and design of the work and interpretation of data; RP, and SC wrote the paper and performed the statistical analyses; BdL and RdLK performed fibrinogen measurements; SC, ADiC, ADeC, TP and MP, managed data collection; CC, MBD, GdG, and LI, originally inspired the Moli-sani Study and critically reviewed the manuscript. All Authors gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

Conflict of interest: BdL and RdLK are employees of Synapse Research Institute, part of Diagnostica Stago SAS. The other Authors report no conflict of interest.

Data availability: The data underlying this article will be shared on reasonable request to the corresponding Author. The data are stored in an institutional repository (https://repository.neuromed.it) and access is restricted by the ethical approvals and the legislation of the European Union.

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Circulating fibrinogen promotes hemostasis as the soluble fibrin precursor, but also by bridging activated platelets and enabling a correct location of erythrocytes, macrophages and fibroblasts at the site of a wound.[2,3] Fibrinogen thus contributes not only to stop bleeding, but also to enhance wound healing and promote tissue regeneration.[3] In addition, fibrinogen levels play an important role in atherosclerosis development and blood viscosity.[4] The potential causal role of increased fibrinogen levels in the pathophysiology of coronary artery and cardiovascular disease (CVD) can be attributed to several mechanisms, including increased blood viscosity and platelet aggregation, altered fibrin clot structure and enhanced red blood cell attachment to thrombi.[2,5-10] In one of the earliest meta-analyses on this subject, high plasma fibrinogen levels were associated with an increased risk of CVD to the same extent in healthy subjects and high-risk individuals with previous CVD events.[11]

Fibrinogen is also involved in the inflammatory response. Indeed, it is an acute phase reactant and, during episodes of inflammation, the synthesis of this protein is enhanced, making it a biomarker of systemic inflammation.[12] Due to its inflammatory function, fibrinogen is also implicated in the pathophysiology of several chronic diseases, including cancer.[5,13,14] Recent studies, show that elevated plasma levels of this coagulation factor are associated with an increased risk of colorectal, lung, breast,[13] and digestive tract cancers.[14]

CVD and cancer are responsible for the majority of morbidity, mortality and disabilities worldwide.[15] Since traditional risk factors alone do not adequately identify individuals at risk for these disorders,[16] fibrinogen has been used as a predictive biomarker of fatal and non-fatal CVD and of different types of cancer,[4,5,8,9,11,13,14,17-19] even if the majority of these studies have been performed in individuals already affected by these disorders.

In contrast, the association between fibrinogen levels and all-cause and cause-specific mortality has been less frequently studied in general populations and results are still controversial.[5,17-19] In a Japanese-American general population of elderly men (N=3,571, age range 71-93 years), the rise of one standard deviation of fibrinogen levels (0.64 g/L) was associated with an increased risk of all-cause, cardiovascular and cancer mortality.[19] Similar findings were reported in a nationwide study in the US (N=5,054, 47% men, mean age = 57 years), which showed a positive association across quartiles of fibrinogen (from lower to higher) with all-cause mortality and heterogeneous associations with cardiovascular mortality.[5] In addition, the potential effect of other inflammatory and hemostasis biomarkers on the association of fibrinogen levels and cause-specific mortality still remains poorly investigated.

The main purpose of the present study was to evaluate the association between plasma levels of fibrinogen and all-cause and cause-specific mortality in an Italian adult population, apparently healthy at enrolment. Furthermore, it was assessed if the mortality hazards associated to plasma fibrinogen levels could be modified by inflammatory and hemostatic biomarkers.

MATERIALS AND METHODS

Study population and study design

The Moli-sani study is an ongoing prospective cohort study that enrolled 24,325 individuals (48.1% men, aged ≥35 years, mean age ± SD: 55.8±12.0 years) randomly recruited from the general population of Molise region in Southern-central Italy between 2005 and 2010.[20] Thirty percent of subjects refused to participate; these were generally older and had a higher prevalence of CVD and cancer than the Moli-sani participants.

The Moli-sani study complies with the Declaration of Helsinki and was approved by the Catholic University Ethical Committee, Rome, Italy. All the participants enrolled provided written informed consent. Details of the Moli-sani study have been previously described.[20]

For the purpose of this study, participants with missing measurements of plasma fibrinogen, as well as individuals with an un-known cause of death were excluded from the analysis. In addition, participants with incomplete medical and dietary questionnaires and a previous history of cancer or CVD were omitted. The final study sample consisted of 17,689 individuals (53% women; mean age 54.2±11.3 years, Supplementary Figure S1).

Supplementary Figure S2 reported the distribution of fibrinogen levels (means and standard error) in individuals excluded according to history of cancer or CVD and in the final study cohort, separately by sex, showing that men with a history of CVD or cancer had fibrinogen levels significantly higher than those selected for the final study sample.

Plasma fibrinogen measurements

Fibrinogen levels were measured in citrated plasma samples by the Clauss method,[21] using the STA Liquid FIB reagent on the STA-R Max (Diagnostica Stago, France) according to the manufacturers recommendations at the Department of Functional Coagulation, Synapse Research Institute, Maastricht, the Netherlands.[22] Both intra- and inter-assay coefficients of variation were 2.1% for samples within the normal range of fibrinogen specified to be 2-4 g/L in healthy subjects.

In Appendix S1 venous blood sample collection, storage, shipment and quality check procedures are reported. Additionally, a detailed description of the common risk factors' assessment is provided in Appendix S2.

Outcome ascertainment

The subjects of the whole original Moli-sani cohort were monitored for mortality until December 31st 2018. All-cause and cause-specific mortality were assessed by the Italian mortality registry (ReNCaM registry), validated by Italian death certificates (ISTAT form), and coded according to the 9th version of International Classification of Diseases (ICD-9).

The primary outcome was all-cause mortality. Additionally, cardiovascular mortality included deaths from diseases of the circulatory system, when the underlying cause of death included ICD-9 codes 390-459. Cancer death was considered when the underlying cause of death included ICD-9 codes 140-208. Noncardiovascular/noncancer causes of death were included in the "other-cause mortality" group.

Statistical analysis

Data are presented as median values and interquartile range (IQR) or as frequencies in the case of categorical variables. Plasma fibrinogen levels were classified into quintiles.

Differences in the distribution of baseline characteristics in women (N=9,355) and in men (N=8,334), according to plasma fibrinogen quintiles were calculated using analysis of variance adjusted for age (CATMOD procedure for categorical variables and GLM procedure for continuous variables in SAS software, Table 1).

To estimate the association between plasma fibrinogen quintiles and all-cause and cause-specific mortality hazard, multiple imputation techniques were used (SAS PROC MI, N=10 imputed datasets; and PROC MIANALYZE) to maximize data availability. Hazard ratios (HR) and 95% CI for all-cause and cause-specific mortality according to plasma fibrinogen quintiles were calculated using Cox proportional hazard (unadjusted, age- and multivariable) models with time-on-study on the time scale, and considering the third quintile of fibrinogen as reference (Table 2). We conducted a cause-specific analysis of competing risks of deaths from other causes. Final multivariable models included the variables associated with both outcome and exposure with a P value (adjusted for age) <0.20 (Supplementary Table S1). Adjusted survival curves were constructed to show event rates during follow up by plasma fibrinogen quintiles (Figure 1).

Curvilinear relationships stratified by sex between plasma fibrinogen levels and all-cause and cause-specific mortality were tested via a multivariable Cox model using a restricted cubic spline with 3 knots at fixed percentiles (5th, 50th, and 95th percentiles of the plasma fibrinogen distribution; Supplementary Figure S3). Sensitivity and sub-group analyses were performed: i) considering a case complete analysis for all variables; ii) excluding early deaths (follow up time ≥2 years) and iii) stratifying by age classes (<65 years, ≥65 years).

To assess the potential influence of sex hormones in the association between fibrinogen levels and mortality in women, we also performed analyses stratified by menopausal status. Finally, it has been also investigated if the studied association could be influenced by other inflammatory and hemostasis biomarkers.

Multiplicative interaction between fibrinogen (continuous) and the potential effect modifiers was tested adding corresponding cross-product terms in the model. A two-sided P value <0.05 was considered as statistically significant. Data analysis used SAS/STAT software, version 9.4 (SAS Institute Inc., Cary, NC, USA).[23]

RESULTS

In the Moli-sani cohort, the median (IQR) value of plasma fibrinogen levels was 3.09 (2.72-3.50) g/L in women and 2.80 (2.48-3.19) g/L in men (Supplementary Figure S3).

Table 1 shows the distribution of the baseline characteristics according to quintiles of fibrinogen in women

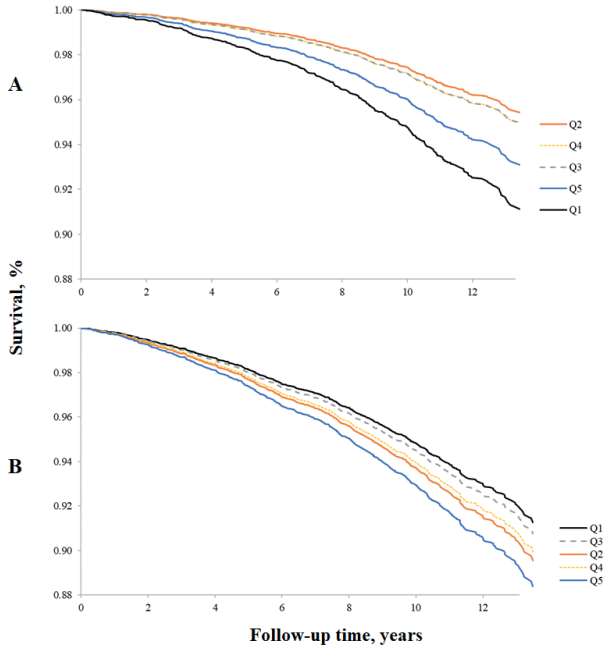

Figure 1. Multivariable survival estimates for all-cause mortality according to plasma fibrinogen quintiles in women (N= 9,355, panel A) and in men (N=8,334, panel B). Multivariable survival curves were obtained from the multivariable model adjusted for age, BMI, education, income, menopausal status, antihypertensive and diabetes medications in women and for age, BMI, education, smoking habit, physical activity, and diabetes medications in men, by using the first imputed dataset. The other imputed datasets are similar and thus omitted.

(N=9,355) and men (N=8,334) of the Moli-sani cohort. In both genders, individuals with higher levels of fibrinogen were older, with higher body mass index (BMI), D-dimer and hs-CRP levels, compared to those in the lower quintiles. In addition, subjects in the highest fibrinogen quintile were mostly non-smokers/former-smokers and were more frequently treated with medications for hypertension, diabetes and dyslipidaemia. Women with the higher levels of fibrinogen were mostly in menopausal status and were less frequently treated with hormonal contraception.

Table 1. Baseline characteristics in women (N=9,355) and men (N=8,334) of the Moli-sani cohort, according to plasma fibrinogen quintiles.

	Women					Men				
	Quintiles of Fibrinogen					Quintiles of Fibrinogen				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
	1,870	1,872	1,871	1,871	1,871	1,667	1,666	1,672	1,664	1,665
Fibrinogen, g/L										
Range (min-max)	1.12-2.64	2.65-2.96	2.97-3.23	3.24-3.61	3.62-6.38	1.10-2.41	2.42-2.68	2.69-2.94	2.95-3.30	3.31-7.17
Median	2.44	2.81	3.09	3.40	3.93	2.22	2.55	2.80	3.10	3.62
IQR range	2.27-2.55	2.72-2.89	3.03-3.16	3.32-3.50	3.76-4.21	2.08-2.33	2.48-2.62	2.74-2.86	3.01-3.19	3.43-3.93
Baseline characteristics										
Age, years	46.1 (41.4-52.9)	50.1 (43.5-58.0)	52.7 (45.3-61.6)	56.0 (47.8-64.5)	59.0 (51.5-67.9)	49.2 (43.0-58.2)	50.8 (43.6-59.4)	53.1 (45.2-61.6)	57.7 (46.6-63.2)	58.2 (49.2-67.4)
Residence										
Rural	33.0	35.2	32.2	31.5	32.1	36.5	34.5	35.1	32.5	34.6
Urban	67.0	64.8	67.8	68.5	67.9	63.5	65.5	64.9	67.5	65.4
Education										
Up to lower secondary school	42.8	48.4	53.2	57.2	60.8	49.1	46.9	49.2	50.1	51.3
High school or higher	57.2	51.6	46.8	42.8	39.2	50.9	53.1	50.8	49.9	48.7
Income										
<40000 €/year	57.0	56.6	53.0	57.6	54.5	62.8	60.0	58.6	60.0	57.5
≥40000 €/year	12.7	12.9	11.0	9.0	8.5	13.1	15.0	14.5	14.2	14.1
Not responders	30.3	30.5	36.0	33.4	37.0	24.1	25.0	26.9	25.8	28.4
Physical activity										
Low	33.5	31.8	33.6	33.3	34.4	28.7	33.4	35.0	34.0	35.5
Medium	29.3	33.1	34.3	35.0	34.9	29.8	30.5	30.3	36.2	39.8
High	37.2	35.1	32.1	31.7	30.7	41.5	36.1	34.7	29.8	24.7
Smoking habit										
Never smoker	62.0	64.3	64.0	66.2	67.8	37.9	37.2	33.6	30.0	27.1
Current smoker	24.6	23.6	22.0	19.7	18.2	22.4	23.5	27.3	30.7	32.8
Former smoker	13.4	12.1	14.0	14.1	14.0	39.7	39.3	39.1	39.3	40.1
BMI, kg/m^2										
Normal weight	56.6	41.9	32.1	26.5	17.5	26.9	23.9	21.1	17.9	15.1
Overweight	30.3	37.5	40.8	37.7	34.8	50.5	52.8	50.3	51.9	48.0
Obese	13.1	20.6	27.1	35.8	47.7	22.6	23.3	28.6	30.2	36.9
D-dimer, ng/dl	166 (128-210)	173 (134-216)	182 (140-224)	187 (143-232)	200 (154-255)	147 (110-185)	159 (119-193)	162 (123-200)	165 (123-201)	185 (142-233)
hs-CRP, mg/L	0.7 (0.4-1.3)	1.1 (0.6-2.0)	1.5 (0.8-2.6)	2.1 (1.1-3.8)	3.6 (1.8-6.9)	0.8 (0.5-1.4)	1.0 (0.6-1.7)	1.4 (0.8-2.2)	1.8 (1.0-3.1)	3.2 (1.7-6.3)
Antithrombotic medications	0.9	2.0	2.5	3.4	3.8	2.0	1.8	3.1	3.4	5.5
Liver disease	3.2	3.8	3.9	3.2	3.8	5.1	4.5	4.6	4.9	4.6
Anti-hypertensive medications	13.4	18.8	24.7	29.2	40.8	18.6	19.0	23.3	25.4	34.8
Diabetes medications	1.0	2.1	2.7	3.2	6.0	2.9	3.8	4.1	5.4	7.9
Dyslipidaemia medications	2.7	3.8	5.6	7.0	9.8	3.2	3.3	4.5	4.8	7.1
Menopausal status	30.8	44.4	55.3	65.3	75.5					
Hormonal contraception	38.0	34.5	29.7	25.2	20.7					
Hormonal replacement therapy	4.5	4.8	6.3	5.3	5.6					

Median values and interquartile range (IQR) are reported for continuous variables and percentage for categorical variables. P adjusted for age. BMI: body mass index; hs-CRP: high sensitivity C-reactive Protein.

Plasma fibrinogen levels and all-cause mortality

The cohort of 17,689 participants was followed-up for a median of 11.2 years (IQR=10.2-12.2 years; 196,668.38 person-years) during which 1,058 deaths were ascertained; 367 (34.7%) subjects died from CVD and 384 (36.3%) from cancer.

Multivariable survival curves for all-cause mortality according to plasma fibrinogen quintiles, were well separated in both women and men (Figure 1, P=0.0001 in women and P=0.042 in men).

Table 2 shows hazard ratio and 95% confidence intervals for all-cause and cause-specific mortality according

Table 2. Hazard Ratios (95% confidence interval) for all-cause and casuse-specific mortality according to plasma fibrinogen quintiles, in women and men of the Moli-sani cohort.

	Women						Men					
	Quintiles of Fibrinogen						Quintiles of Fibrinogen					
	Q1	Q2	Q3	Q4	Q5	Pvalue	Q1	Q2	Q3	Q4	Q5	Pvalue
Fibrinogen Range, g/L	1.12-2.64	2.65-2.96	2.97-3.23	3.24-3.61	3.62-6.38		1.10-2.41	2.42-2.68	2.69-2.94	2.95-3.30	3.31-7.17	
All-cause mortality												
N events/ N total	48/1,870	45/1,872	57/1,871	106/1,871	163/1,871		64/1,667	96/1,666	107/1,672	142/1,664	230/1,665	
Death rate (95% CI)/ 10,000 Person Years	22.9 (17.3-30.4)	21.2 (15.8-28.4)	27.0 (20.8-35.0)	50.4 (41.7-61.0)	79.4 (68.1-92.6)		34.4 (26.9-43.9)	51.7 (42.3-63.2)	58.0 (48.0-70.1)	77.8 (66.0-91.7)	128.6 (113.0-146.3)	
HR Crude (95% CI)	0.99 (0.66-1.50)	0.60 (0.38-0.94)	Ref.	1.40 (1.01-1.95)	2.92 (2.18-3.91)	<.0001	0.57 (0.43-0.75)	0.88 (0.68-1.14)	Ref.	1.56 (1.22-2.00)	2.21 (1.74-2.80)	<.0001
HR_1 (95% CI)	2.05 (1.36-3.11)	0.89 (0.57-1.40)	Ref.	1.02 (0.74-1.42)	1.56 (1.16-2.09)	<.0001	0.87 (0.66-1.15)	1.01 (0.78-1.30)	Ref.	1.22 (0.95-1.56)	1.39 (1.09-1.77)	0.0026
HR_2 (95% CI)	1.98 (1.30-3.01)	0.90 (0.57-1.41)	Ref.	1.00 (0.72-1.39)	1.45 (1.08-1.96)	0.0001	0.94 (0.71-1.24)	1.07 (0.83-1.38)	Ref.	1.24 (0.97-1.58)	1.31 (1.03-1.67)	0.052
Cardiovascular mortality												
N events/ N total	13/1,870	15/1,872	18/1,871	46/1,871	69/1,871		14/1,667	28/1,666	32/1,672	53/1,664	79/1,665	
Death rate (95% CI)/ 10,000 Person Years	6.2 (3.6-10.7)	7.1 (4.3-11.7)	8.5 (5.4-13.5)	21.9 (16.4-29.2)	33.6 (26.6-42.6)		7.5 (4.5-12.7)	15.1 (10.4-21.8)	17.3 (12.3-24.5)	29.0 (22.2-38.0)	44.2 (35.4-55.1)	
HR Crude (95% CI)	0.74 (0.32-1.70)	0.95 (0.46-1.96)	Ref.	1.72 (0.96-3.08)	4.40 (2.62-7.39)	<.0001	0.42 (0.25-0.72)	0.84 (0.53-1.32)	Ref.	1.70 (1.11- 2.61)	2.44 (1.61-3.69)	<.0001
HR_1 (95% CI)	1.80 (0.78-4.19)	1.59 (0.77-3.28)	Ref.	1.15 (0.64-2.07)	2.04 (1.21-3.43)	0.014	0.70 (0.41-1.18)	0.98 (0.62-1.55)	Ref.	1.27 (0.83-1.95)	1.42 (0.94-2.16)	0.043
HR_2 (95% CI)	1.87 (0.80-4.36)	1.68 (0.81-3.46)	Ref.	1.10 (0.61-1.98)	1.80 (1.06-3.04)	0.056	0.74 (0.43-1.26)	1.03 (0.65-1.62)	Ref.	1.28 (0.83-1.96)	1.33 (0.88-2.02)	0.14
Cancer mortality												
N events/ N total	18/1,870	19/1,872	21/1,871	36/1,871	47/1,871		22/1,667	45/1,666	46/1,672	52/1,664	78/1,665	
Death rate (95% CI)/ 10,000 Person Years	8.6 (5.4-13.6)	9.0 (5.7-14.0)	9.9 (6.5-15.3)	17.1 (12.3-23.7)	22.9 (17.2-30.5)		11.8 (7.8-17.9)	24.2 (18.10-32.5)	24.9 (18.7-33.3)	28.5 (21.7-37.4)	43.6 (34.9-54.4)	
HR Crude (95% CI)	1.00 (0.53-1.86)	0.30 (0.12-0.73)	Ref.	1.27 (0.76-2.12)	1.92 (1.20-3.07)	<.0001	0.43 (0.27-0.66)	0.83 (0.57-1.21)	Ref.	1.18 (0.80-1.74)	1.55 (1.06-2.26)	<.0001
HR_1 (95% CI)	1.51 (0.80-2.84)	0.37 (0.15-0.90)	Ref.	1.05 (0.63-1.76)	1.31 (0.81-2.10)	0.032	0.60 (0.39-0.94)	0.93 (0.63-1.36)	Ref.	0.97 (0.66-1.42)	1.05 (0.72-1.54)	0.14
HR_2 (95% CI)	1.57 (0.83-2.97)	0.38 (0.16-0.93)	Ref.	1.03 (0.62-1.72)	1.17 (0.72-1.90)	0.053	0.69 (0.44-1.08)	1.00 (0.68-1.46)	Ref.	0.97 (0.66-1.43)	0.96 (0.65-1.41)	0.48
Other-cause mortality												
N events/ N total	17/1,870	11/1,872	18/1,871	24/1,871	47/1,871		28/1,667	23/1,666	29/1,672	37/1,664	73/1,665	
Death rate (95% CI)/ 10,000 Person Years	8.1 (5.0-13.0)	5.2 (2.9-9.4)	8.5 (5.4-13.5)	11.4 (7.7-17.0)	22.9 (17.2-30.5)		15.0 (10.4-21.8)	12.4 (8.2-18.6)	15.7 (10.9-22.6)	20.3 (14.7-28.0)	40.8 (32.4-51.3)	
HR Crude (95% CI)	1.26 (0.61-2.63)	0.70 (0.31-1.59)	Ref.	1.27 (0.67-2.41)	2.90 (1.66-5.06)	<.0001	1.09 (0.66-1.82)	1.06 (0.62-1.80)	Ref.	2.16 (1.31-3.56)	3.29 (2.03-5.33)	<.0001
HR_1 (95% CI)	2.85 (1.37-5.96)	1.09 (0.48-2.48)	Ref.	0.89 (0.47-1.68)	1.44 (0.82-2.51)	0.010	1.72 (1.03-2.86)	1.22 (0.72-2.08)	Ref.	1.66 (1.01-2.74)	2.02 (1.24-3.28)	0.032
HR_2 (95% CI)	2.60 (1.24-5.48)	1.07 (0.47-2.43)	Ref.	0.88 (0.46-1.66)	1.43 (0.81-2.51)	0.022	1.74 (1.04-2.91)	1.28 (0.75-2.18)	Ref.	1.73 (1.05-2.85)	2.00 (1.23-3.25)	0.042

Model 1: adjusted for age; Model 2 women: model 1 plus BMI, education, income, menopausal status, anti-hypertensive and diabetes medications; Model 2 men: model 1 plus BMI, education, smoking habit, physical activity and diabetes medications. BMI: body mass index; CI: confidence interval and HR: Hazard ratio.

to plasma fibrinogen quintiles stratified by sex. Women in the lowest (Q1: 1.12-2.64 g/L) and in the highest (Q5: ≥3.62 g/L) fibrinogen quintiles had an increased hazard for all-cause mortality, when compared with those in the third quintile (Q3: 2.97-3.23 g/L), (HR_{Q1vsQ3}: 1.98; 95% CI 1.30-3.01 and HR_{Q5vsQ3}: 1.45; 95% CI 1.08-1.96, respectively; model 2, Table 2).

Instead, only men in the highest (Q5: ≥3.31 g/L) quintile had an increased hazard of all-cause mortality as compared to those in the third (Q3: 2.69-2.94), (HR_{Q5vsQ3}: 1.31; 95% CI 1.03-1.67; model 2, Table 2).

Dose-response analyses between fibrinogen and all-cause mortality showed a U-shaped relationship in women (P value for overall association <0.0001 and P value for non-linear association <0.0001; Figure 2) and a positive linear association in men (P value for overall association = 0.0038 and P value for non-linear association = 0.76; Figure 2).

Case-complete analysis restricted to data without missing values for covariates (8,944 women and 8,006 men; Supplementary Table S2) and analysis performed with the exclusion of early deaths (follow-up ≥ 2 years, 9,329 women and 8,281 men; Supplementary Table S3) yielded very similar results.

To assess whether the results in women were affected by sex hormones, we performed the analyses also stratifying by menopausal status (Supplementary Table S4 and Figure S4).

In pre-menopause women, only a trend of association between fibrinogen levels and all-cause mortality was observed. In post-menopause women, instead, both with fibrinogen levels lower than 2.64 g/L (HR_{Q1vsQ3}: 2.15; 95% CI 1.36-3.41) and higher than 3.61 g/L (HR_{Q5vsQ3}: 1.40; 95% CI 1.03-1.92, Supplementary Table S4), an increased hazard for all-cause mortality was found.

Plasma fibrinogen levels and cause-specific mortality

For cardiovascular mortality, we observed a significantly increased hazard only in women with fibrinogen levels higher than 3.61 g/L (HR_{Q5vsQ3}:1.80; 95% CI 1.06-3.04; model 2, Table 2), when compared with those in the third one. A marginally curvilinear relationship was found between plasma fibrinogen levels and cardiovascular mortality in women (P value for overall association = 0.15 and P value for non-linear association = 0.12; Figure 3). In addition to previous results, the linear association was confirmed in men (P value for overall association = 0.016 and P value for non-linear association = 0.24; Figure 3).

No relevant associations were found between fibrinogen levels and cancer mortality in either women or men (Table 2 and Figure 3). However, dose-response analyses showed a U-shaped trend in women and a sex-interaction (P=0.045, Figure 3) describing different sex-effect of fibrinogen on cancer mortality.

On the other hand, non-linear but U shaped relationships between plasma fibrinogen levels and other-cause mortality were found in both women and men (Table 2 and Figure 3). The most frequent causes of death in this outcome were diseases of respiratory (26.5% in women and 25.8% in men) and gastro-intestinal (14.5% in women and 17.4% in men) systems (Supplementary Table S5). Unfortunately, further analyses specific for the different causes of death were unreliable due to the small number of events.

Sub-group analyses by age

No difference in the association between fibrinogen and all-cause and cause-specific mortality according to two age classes (<65 years and ≥65 years) was found (Supplementary Figure S5).

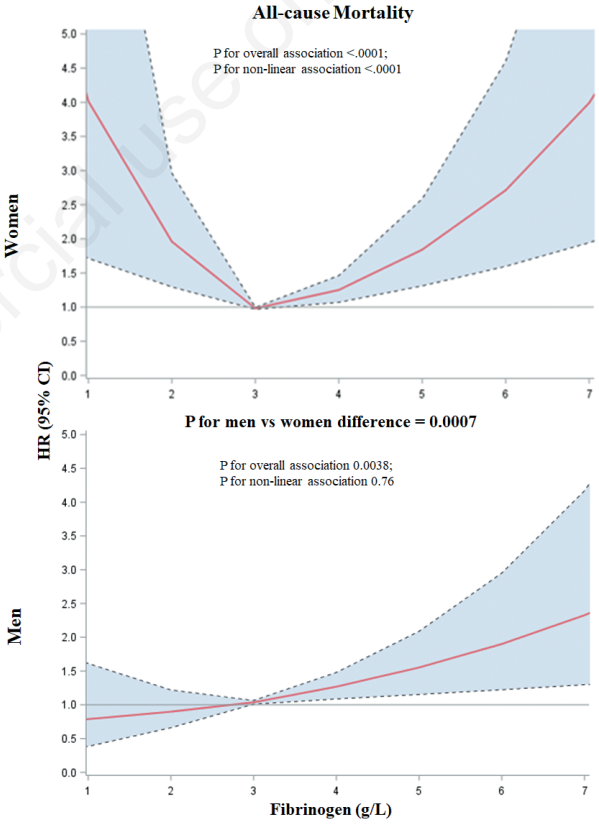

Figure 2. Dose-response curve of all-cause mortality according to plasma fibrinogen levels in women (N= 9,355) and in men (N= 8,334). The dose-response curves were obtained from multivariable model adjusted for age, BMI, education, income, menopausal status, anti-hypertensive and diabetes medications in women and for age, BMI, education, smoking habit, physical activity, and diabetes medications in men, by using the first imputed dataset. The other imputed datasets are similar and thus omitted. The reference value of the dose response association is the median value of fibrinogen distribution in women and men (median 3.09 g/L and 2.80 g/L, respectively). CI: confidence interval and HR: Hazard ratio.

Inflammatory and hemostasis biomarkers role assessment

Inclusion in the final adjusted model of a marker of inflammation (hs-CRP) and/or one of the hemostatic system (D-dimer) only marginally affected the associations of fibrinogen with all-cause mortality rates (Supplementary Table S6). According to a cut off that distinguishes between low and high hazard of all-cause mortality,[24] a modification of effect by D-dimer levels was found only in men (P for interaction = 0.022) showing that low and high fibrinogen levels were associated with increased hazard of all-cause mortality only in individuals with D-dimer ≥221 ng/dL (Figure 4).

Additionally, Supplementary Table S6 shows that the associations of fibrinogen levels with cardiovascular, cancer or other-cause mortality were independent from inflammatory (hs-CRP) and hemostasis (D-Dimer) markers.

DISCUSSION

The main findings of our study conducted in an Italian adult apparently healthy general population, show that both women and men in the highest quintile of fibrinogen, had an increased hazard for all-cause mortality, when compared with the median quintile.

Considering the wide range of normal values for fibrinogen (2-4 g/L), the median quintile of our cohort, which includes the median value found in healthy subjects, was used as reference group for our analyses. In this way it was possible to explore whether not only higher levels of fibrinogen but also lower ones could be associated with mortality hazard.

In particular, in women, but not in men, a U-shaped relationship between plasma fibrinogen levels and the hazard of all-cause mortality was observed. This finding in women is difficult to explain, but could be linked in some way to the influence of sex hormones on the hemostasis balance. It is widely known indeed that sex hormones in women can affect many physiological pathways, directly influencing basal levels of inflammatory and hemostasis biomarkers.[25-29] Of note, different associations have been observed considering endogenous or exogenous hormones and also according to the type of hormonal therapy used.[28,29] In this context, to assess whether results in women were affected by sex hormones, we stratified the analysis by menopausal status and found that the U shaped association was more evident in postmenopausal women.

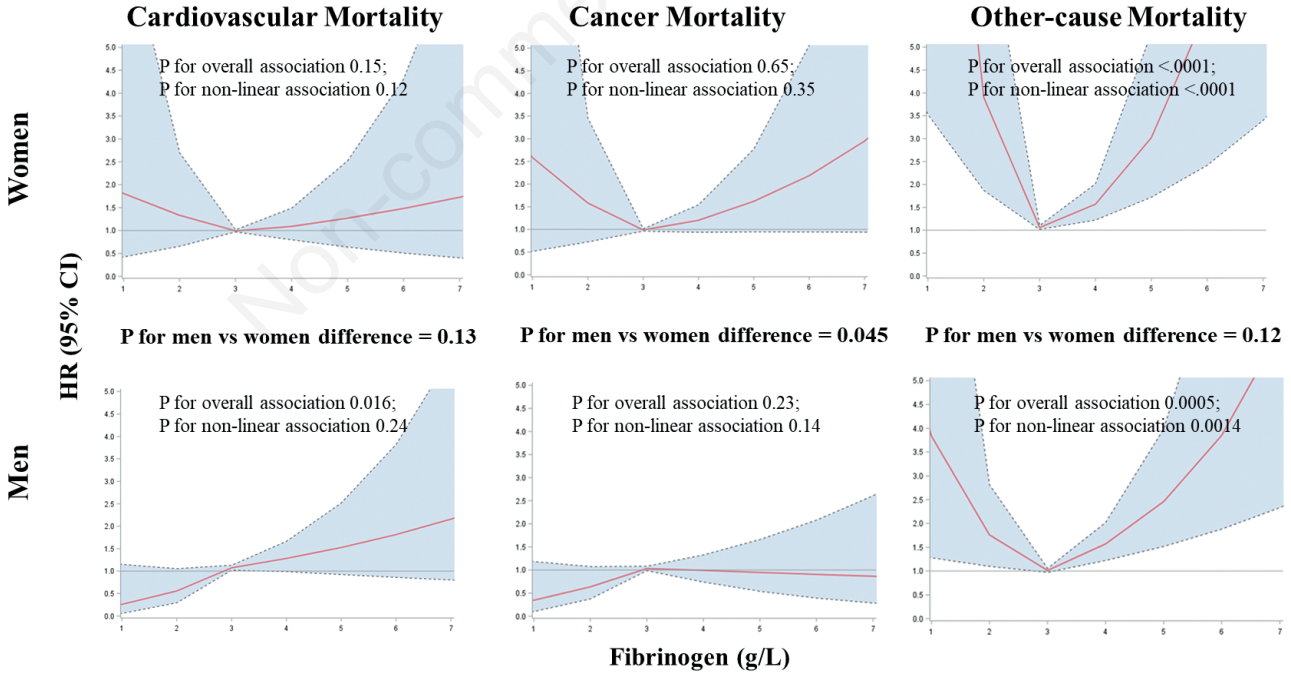

Figure 3. Dose-response curve of cause-specific mortality according to plasma fibrinogen levels in women (N= 9,355) and in men (N= 8,334). The dose-response curves were obtained from multivariable model in women adjusted for age, BMI, education, income, menopausal status, anti-hypertensive and diabetes medications and in men adjusted for age, BMI, education,smoking habit, physical activity, and diabetes medications; by using the first imputed dataset. The other imputed datasets are similar and thus omitted. The reference value of the dose response association is the median value of fibrinogen distribution in women and men (median 3.09 g/L and 2.80 g/L, respectively). CI: confidence interval and HR: Hazard ratio.

On the other hand, in dose-response analysis for cardiovascular mortality we observed only a marginally U-shaped association in women. However, the results confirmed the high hazard for CVD mortality in both genders. These findings for CVD mortality are consistent with the current data in the literature which reported that higher levels of this hemostatic factor are related both to the development of atherosclerosis and to increased risk of coronary artery and CVD.[4,8,11]

Furthermore, the role of this coagulation protein has been widely investigated in cancer clinical settings, showing that high pre-operative levels of fibrinogen were associated with a poor prognosis of overall/disease free survival in gastrointestinal and, in particular, colorectal cancer.[14,30,31] In addition, few studies from general adult population reported that high levels of fibrinogen were associated with increased risk of fatal and not fatal colorectal, breast and lung cancers.[13,32]

In the present study, no relevant associations were found with cancer mortality in either gender.

Concerning the absence of correlation of fibrinogen levels with cancer mortality, the latter is a heterogeneous outcome that included deaths for different types of cancer (solid tumours and blood cancers). Unfortunately, analyses for different types of specific cancer related death were unreliable due to the small number of events in the present study.

A curvilinear relationships between plasma fibrinogen levels and other-cause mortality were found in both women and men. Similarly to cancer mortality, also this outcome is not specific and includes all the noncardiovascular/noncancer deaths. Therefore, we don't know which one of the other-cause deaths is responsible for our findings.

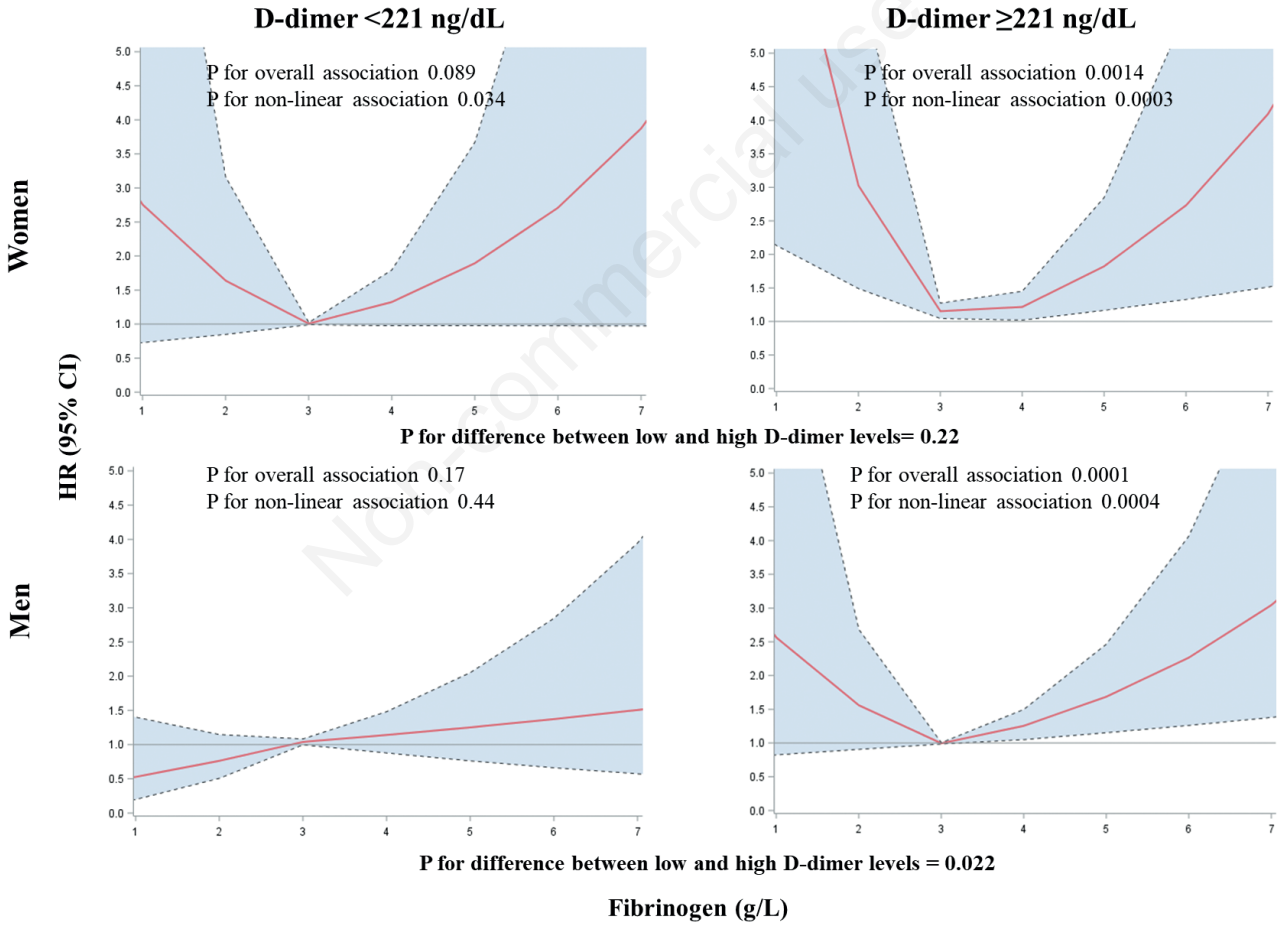

Figure 4. Dose-response curve of all-cause mortality according to plasma fibrinogen levels separated by sex and stratified by D-dimer levels (<221 ng/dL; ≥221 ng/dL). The dose-response curves were obtained from multivariable model in women adjusted for age, BMI, education, income, menopausal status, anti-hypertensive and diabetes medications and in men adjusted for age, BMI, education, smoking habit, physical activity, and diabetes medications; by using the first imputed dataset. The other imputed datasets are similar and thus omitted. The reference value of the dose response association is the median value of fibrinogen distribution (median 2.96 g/L). CI: confidence interval and HR: Hazard ratio.

For all these reasons, future studies are needed to better define the role of fibrinogen in relation to cause-specific mortality in the field of different types of tumors and non-cardiovascular deaths.

Concerning previous evidence from literature, in particular, our findings are in line with the study by Liu *et al*., that did not report any association with cancer mortality and partially with the EPIC-Norfolk study (N=16,850, 55% women, mean age 60 years)[5] showing that men, but not women, in the highest quintile of fibrinogen had increased risk for all-cause, CVD and other-cause mortality, compared to those in the lowest quintiles.[18]

When the potential effect of inflammatory and hemostasis biomarkers was investigated, it was found that the association between fibrinogen plasma levels and all-cause or cause-specific mortality was independent from any of these biomarkers. However, a modification of effect by D-dimer was found in men since curvilinear relationship between fibrinogen levels and all-cause mortality was observed at D-dimer levels ≥221 ng/dL. As demonstrated in a previous study,[24] values of D-dimer ≥ 221 ng/dL, represent a cut off that distinguishes between low and high hazard of all-cause mortality. For this reason we selected this parameter for our analyses.

In individuals with both high levels of fibrinogen and D-dimer, the increased hazard of all-cause mortality could be explained by the inflammatory condition underlying a wide range of chronic disorders.[33] In contrast, the high hazard observed in individuals with low levels of fibrinogen and high levels of D-dimers could be explained by a possible condition of subclinical activation of the coagulation, as suggested by the high levels of D-dimer with subsequent fibrinogen consumption. Therefore, an increased hazard of all-cause mortality may be revealed not only by high, but also by low levels of fibrinogen, when combined with high D-dimers levels.

In conclusion, the present study emphasizes the concept that inflammation and hemostasis are intermingled pathways and should be considered together in risk assessment; indeed fibrinogen, due to its dual role in inflammation and hemostasis, could be considered as an ideal candidate biomarker of mortality.

Of note, a public health implication of our findings could be to consider plasma fibrinogen as a marker, easy to test, to identify high-risk individuals in terms of survival in a general adult population. In particular, major attention should be paid to women for which, not only the highest but also the lowest levels of fibrinogen represent an increased mortality hazard.

Strengths and limitations

Major strengths of our study are its prospective design, the great number of apparently healthy individuals included and the availability of a wide number of lifestyle, anthropometric and biological variables, allowing for a better understanding of the predictive role of fibrinogen levels.

On the other side, a limitation is represented by the fact that fibrinogen levels have been assessed, for each participant, only in a single plasma sample obtained at the moment of recruitment of the cohort; therefore, indications on possible long-term variation of its baseline levels are lacking. Since the measurements were all performed on frozen samples thawed several years after their storage in liquid nitrogen in a dedicated biobank, this could have theoretically influenced the measurements. However, freezing and long-term storage appears to have little effect on fibrinogen levels. The changes observed in the study were minimal and are irrelevant for clinical interpretation.[34] Indeed, the plasma quality was assured experimentally by measuring the levels of coagulation factors FV and FVIII, known to be sensitive to freezing and thawing, which were found within the normal range.[35]

CONCLUSIONS

This study confirms that higher levels of fibrinogen represent a hazard for all-cause mortality in a general apparently healthy population. However, different results were obtained when considering the lowest fibrinogen levels separately between women and men, suggesting that further investigations are needed to better define the biological mechanisms explaining the fibrinogen dualistic role (biomarker of both inflammation and hemostasis) in mortality prediction.

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