Data supplement

Logistic Regression Analysis

1. Missing data analysis

Logistic regression analysis is typically in patients with complete data sets. Missing data of the variables to be used in the regression analysis are depicted in **Table S1**. BMI, history of hypertension, FDP at 8 h, platelet count and CK had missing values (Only FDP and platelet count with >5% missing values, analyzed in Paragraph 2). **Figure S1, Panel A** depicts the summary, with the patterns in **Panel B and C**. There were no missing values in the outcome variables, or in sex, age, ancestry, treatment assignment, or dose.

Table S1

	Missing data analysis		
Variable	**Valid**	**Missing**	**%**
Sex	3339	0	0
Age	3339	0	0
BMI	3295	44	1.3
Ancestry	3339	0	0
History of Hypertension	3338	1	0.03
Treatment assignment	3339	0	0
rt-PA dose	3339	0	0
FDP at 8 h	**1667**	**1669**	**50.0**
Platelet count	**2860**	**479**	**14.4**
CK	3327	12	0.4
Adjudicated fatal or non-fatal bleeding	3339	0	0
Adjudicated bleeding or all-cause mortality	3339	0	0

Legend. BMI, body mass index; rt-PA, recombinant tissue-type plasminogen activator; FDP, fibrin(ogen) degradation products at 8 h; CK, creatine kinase. Valid and missing data columns are counts

Figure S1. Panel A. Overall summary of missing values

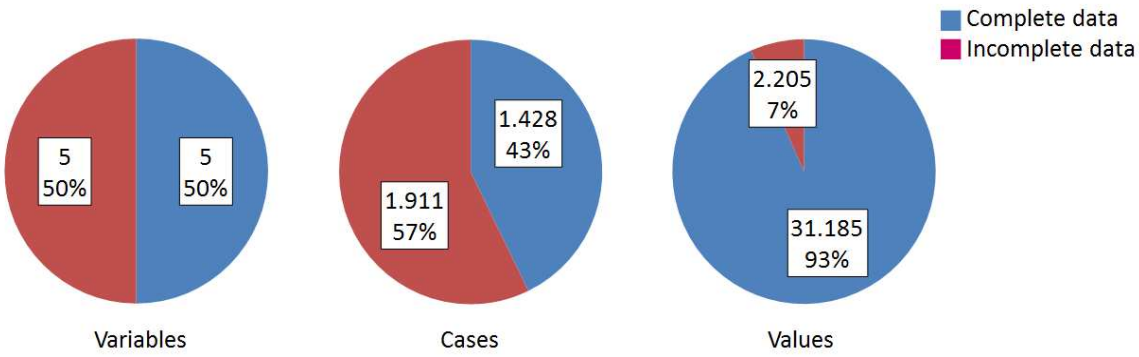


Figure S1. Panel B. Missing value patterns

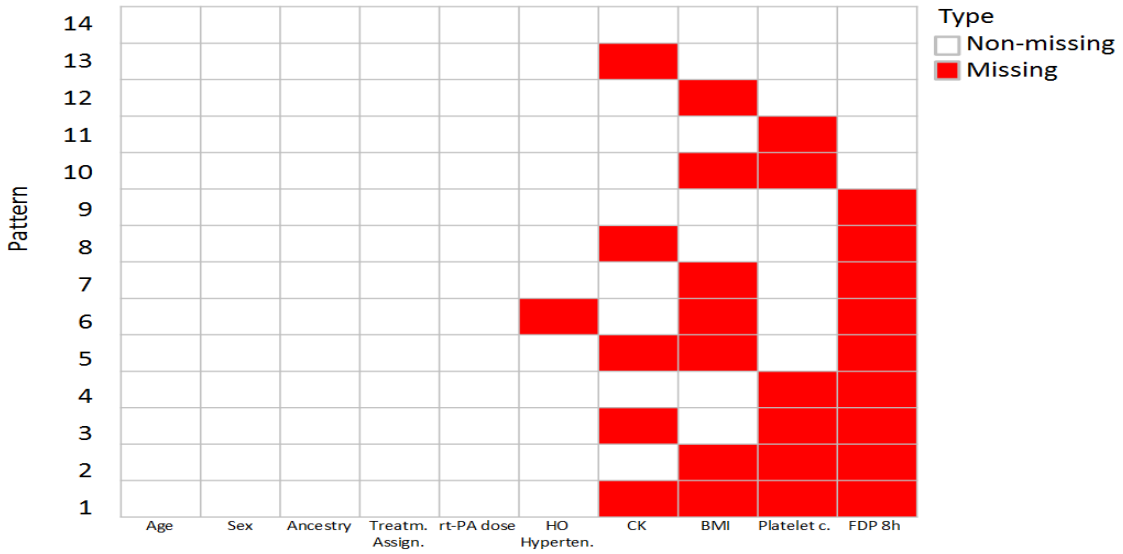
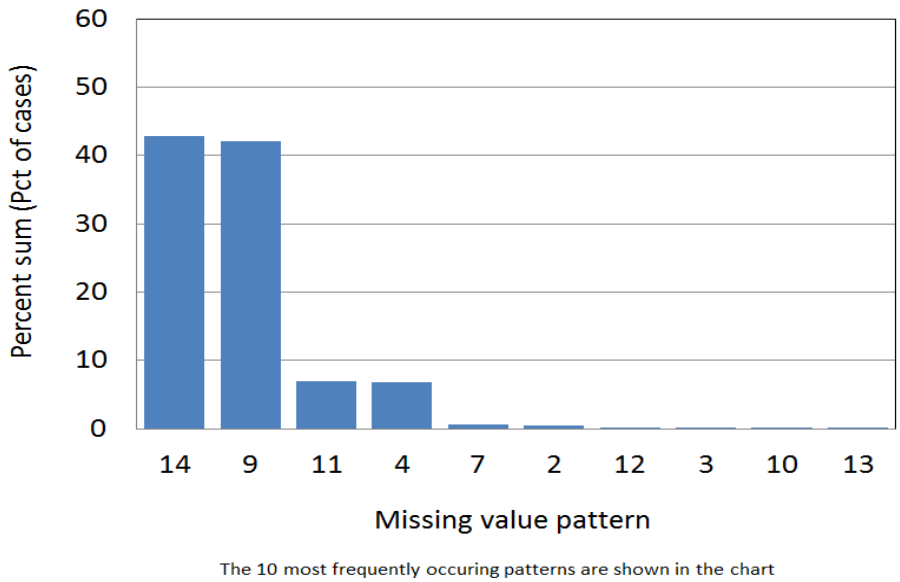


Figure S1. Panel C. Pattern frequency



Legend. Panel A indicates 50% of the 10 variables included in the model had one or more missing values, in 57% of the cases and 7% of the data values. The most frequent pattern was no missing data, followed by FDP at 8 hours missing. Treatm. Assign., treatment assignment; rt-PA, recombinant tissue-type plasminogen activator; HO Hyperten., history of hypertension; CK, creatine kinase; BMI; Body mass index; Platelet c., platelet count; FDP 8h, FDP, fibrin(ogen) degradation products at 8 h.

2. Analysis of variables with >5% missing data

Fibrin(ogen) degradation products (FDP) at 8 hours

The number of valid observations was 1667, with 1669 missing values. The distribution was not normal, with a range of 5 to 8800 microgram/ml, a mean of 291,9 (SE 16.7), median 69, mode 34, Skewness 6.1 (SE 0.06) and kurtosis of 53.9 (SE 0.12). The Kolmogorov-Smirnov test of normality (with Lilliefors significance correction) was 0.337 (df 1670), at p<0.001. To assess whether data were missing ad random, clinical data indicated that this parameter was collected on as many patients as possible at baseline, and at 50, 300, and 480 minutes after rt-PA infusion, and determined in a central Coagulation Core Laboratory. There was no systematic exclusion of participants reported. The distribution among participants with or without bleeding (primary outcome) is depicted in **Table S2.**

Platelet count

The number of valid observations was 2860 with 479 missing values. The distribution was not normal, with a range of 65 to 3370.10^9/L, a mean of 285 (SE 1.8), median 275, mode 264, Skewness 12.9 (SE 0.05) and kurtosis of 407.2 (SE 0.09). The Kolmogorov-Smirnov test of normality (with Lilliefors significance correction) was 0.092 (df 2860), at p<0.001. To address the question whether data were missing ad random, clinical data indicated that this parameter was determined locally at the participating centers at baseline. There was no systematic exclusion of participants reported. The distribution among participants with or without the primary outcome is depicted in **Table S2.**

It was concluded based on Table S2, the reported cause of missing values,[12-17] and the Little's test in SPSS, that cases with missing values are not systematically different from cases without missing values, and that data were missing completely at random.

Table S2. Missing data analysis (variables with > 5% missing data)

	FDP 8h		Platelet Count	
Variable	**Valid**	**Missing**	**Valid**	**Missing**
Sex (men %)	81.9	82.3	82.1	82.3
Age (y)	56.7 (0.3)	56.9 (0.2)	56.8 (0.2)	56.5 (0.5)
BMI (kg/m^2)	27.5 (0.1)	27.9 (0.1)	27.6 (0.1)	28.0 (0.2)
Ancestry (white %)	88.4	88.2	88.0	90.0
History of hypertension (%)	36.3	40.3	38.0	40.3
Treatment assignment (invasive %)	51.4	49.3	50.6	48.9
IV rt-PA dose (mg/first 6h)	102.1 (0.3)	111.3 (0.6)	106.8 (0.4)	106.4 (0.9)
Adjudicated fatal or non-fatal bleeding (%)	30.8	28.2	29.6	29.0
Adjudicated bleeding or ACM (%)	34.4	34.4	34.4	35.9
CK (IU/L)	2330 (56.7)	2449 (59.0)	2407 (45.0)	2281 (95.5)
CK (times URL)	13.6 (0.3)	13.4 (0.3)	13.4 (0.3)	13.9 (0.6)

Legend. Data are rounded means with standard errors in brackets, unless indicated otherwise. BMI, body mass index, IV, intravenous; rt-PA, recombinant tissue-type plasminogen activator; FDP, fibrin(ogen) degradation products; ACM, all-cause mortality; CK, creatine kinase; CK times URL, CK expressed as times the upper reference limit

3. Handling of missing data: primary analysis and outcome

The primary outcome compared the odds of Adjudicated Fatal and Non-Fatal Bleeding vs no Bleeding. The subset of complete cases for this analysis (n=1034), is large enough for the logistic regression analysis with sufficient power.

Table S3. Primary outcome: Multivariable Prediction of Adjudicated Fatal and Non-Fatal Bleeding

					95% C.I.	
Variables	**B**	**S.E.**	**Wald**	**Odds Ratio**	**Lower**	**Upper**
Sex (vs. women)	−0.28	0.18	2.38	0.76	0.53	1.08
Age (y)	0.03	0.01	20.97	1.03	1.02	1.04
Ancestry (vs. White)	−0.07	0.21	0.12	0.93	0.62	1.40
BMI (kg/m2)	−0.07	0.02	16.98	0.94	0.91	0.97
rt-PA dose (mg)	−0.00	0.01	0.02	1.00	1.00	1.01
Platelet count (.10^9/L)	0.00	0.00	6.41	1.00	1.00	1.00
FDP 8 h (microgram/ml)	0.00	0.00	6.66	1.00	1.00	1.00
Treatment (vs. non-Invasive)	0.61	0.14	20.41	1.84	1.41	2.40
Hypertension history	0.04	0.14	0.09	1.04	0.79	1.38
CK (log, IU/L)*	**0.96**	**0.18**	**29.06**	**2.60**	**1.84**	**3.68**
Constant	−2.16	1.02	4.44	0.12		

Legend. Multivariable logistic regression of the initial model, adjudicated fatal and non-fatal bleeding vs no bleeding (reference category in brackets). BMI, body mass index; rt-PA, recombinant tissue-type plasminogen activator; FDP, fibrin(ogen) degradation products measured at 8 h after treatment initiation; CK, creatine kinase;*CKmax adjusted for the upper reference limit. Model parameters: omnibus test of model coefficients Chi-square 126.7 df 10; −2 Log likelihood 1281.3; Cox and Snell R square. 0.12; Nagelkerke R square. 0.16; Hosmer Lemeshow Chi square 10.00, df 8; classification overall percentage 64.3, n=1034 complete, unimputed cases. Bootstrapping OR 2.60 [1.84 to 3.68].

The odds ratio for all reported bleeding vs no bleeding (n=1428) was 2.0 per log CK increase [95% confidence interval, CI 1.6 to 2.7] and for combined bleeding + all-cause mortality (ACM) vs no bleeding and survival (n=1067) 3.1 [2.2 to 4.4], with similar results for the other variables in the model and model characteristics as depicted in Table S3.

When excluding puncture bleeding, the odds ratios were 2.2 [1.7 to 3.0] for all reported bleeding (n=1305); 3.6 [2.4 to 5.3] for fatal and non-fatal bleeding (n=911); and 4.2 [2.9 to 6.2] for bleeding + ACM (n=944), with similar results for the model characteristics as depicted in Table S3 (data not shown).

4. Outcomes with imputed data

Multiple imputation

Imputation specifications were: 5 imputations, automatic selection between a fully conditional specification or monotone methods, linear or logistic regression to estimate missing variables, and a maximum number of 100 parameters in the imputation model (Table S4.a and b).

Simple imputation with the median

The model after simple imputation with the median in variables with >5% missing values is in Table S4B.

Table S4.a. Multivariable Prediction of Adjudicated Fatal and Non-Fatal Bleeding (Multiple imputation)

					95% C.I.	
Variables	**B**	**S.E.**	**Wald†**	**Odds Ratio**	**Lower**	**Upper**
Sex (vs. women)	−0.48	0.12	-	0.63	0.49	0.79
Age (y)	0.03	0.01	-	1.03	1.02	1.04
Ancestry (vs. White)	−0.13	0.14	-	0.88	0.67	1.16
BMI (kg/m2)	−0.05	0.10	-	0.95	0.93	0.97
rt-PA dose (mg)	0.00	0.00	-	1.00	1.00	1.01
Platelet count (.10^9/L)	0.00	0.00	-	1.00	1.00	1.00
FDP 8 h (microgram/ml)	0.00	0.00	-	1.00	1.00	1.00
Treatment (vs. non-Invasive)	0.62	0.09	-	1.86	1.57	2.22
Hypertension history	0.16	0.10	-	1.18	0.98	1.42
CK (log, IU/L)*	**0.92**	**0.11**	**-**	**2.51**	**2.02**	**3.11**
Constant	−2.12	0.60	-	0.12		

Legend. Multivariable logistic regression with pooled data of 5 iterations, for the main outcome, adjudicated fatal and non-fatal bleeding (reference category in brackets). BMI, body mass index; rt-PA, recombinant tissue-type plasminogen activator; FDP, fibrin(ogen) degradation products measured at 8 h after treatment initiation; CK, creatine kinase;*CKmax adjusted for the upper reference limit. †Not calculated for pooled estimates of the iterations. Model parameters: omnibus test of model coefficients Chi-square 262.3 df 10; −2 Log likelihood 2959.8; Cox and Snell R square 0.1; Nagelkerke R square 0.1; Hosmer Lemeshow Chi square 10.3. df 8; classification overall percentage 65.8, n=2374.

Table S4.b. Outcomes compared with multiple imputation vs simple imputation with the median

Clinical parameter	**Multiple imputation**	**Median imputation**
IR Bleeding	2.1 [1.7 to 2.4]	2.0 [1.7 to 2.4]
Fatal and non-Fatal Bleeding*†	2.5 [2.0 to 3.1]	2.4 [1.9 to 3.0]
Bleeding + ACM†	2.2 [1.8 to 2.7]	2.5 [2.0 to 3.0]
IR NP Bleeding	2.1 [1.8 to 2.5]	2.0 [1.7 to 2.5]
NP Fatal and non-Fatal Bleeding*†	2.9 [2.3 to 3.7]	2.8 [2.2 to 3.5]
NP Bleeding + ACM†	2.4 [1.9 to 3.0]	2.8 [2.2 to 3.5]

Legend. Adjusted OR for bleeding per unit increase (peak log CK normalized for URL). *Primary outcome; †adjudicated bleeding events. IR bleeding, investigator reported hemorrhagic complication; ACM, adjudicated all-cause mortality; NP, non-puncture bleeding. (n=2108 to 3339 for multiple, and 2063 to 3287 for median imputation; with similar results for the other variables in the model as depicted in Table S4; data not shown).

5. Parsimonious modeling

Instead of imputations, platelet count, FDP at 8 h, and rt-PA dose, with OR ≈1, were dropped from the model using backward elimination. The intermediate model retained factors previously reported to be associated with bleeding,[12-18] including invasive procedures, age, female sex, low body weight, and a history of hypertension, further eliminating ancestry and history of hypertension in the parsimonious model.

Table S5. Multivariable Prediction of Adjudicated Fatal and Non-Fatal Bleeding (Parsimonious model)

Variables					**95% C.I.**	
Intermediate model	**B**	**S.E.**	**Wald**	**Odds Ratio**	**Lower**	**Upper**
Sex (vs women)	−0.51	0.12	17.92	0.60	0.48	0.76
Age (y)	0.03	0.00	52.05	1.03	1.03	1.04
Ancestry (vs White)	−0.13	0.14	0.79	0.88	0.67	1.16
BMI (kg/m2)	−0.06	0.01	22.02	0.95	0.93	0.97
Treatment (vs non-Invasive)	0.63	0.09	49.91	1.89	1.58	2.24
Hypertension history	0.17	0.09	3.293	1.19	0.99	1.43
CK (log. IU/L)*	**0.89**	**0.11**	**64.38**	**2.43**	**1.96**	**3.03**
Constant	−1.55	0.45	11.97	0.21		
					95% C.I.	
Parsimonious model	**B**	**S.E.**	**Wald**	**Odds Ratio**	**Lower**	**Upper**
Sex (vs women)	−0.52	0.11	19.05	0.60	0.487	0.75
Age (y)	0.04	0.00	53.91	1.04	1.03	1.05
BMI (kg/m2)	−0.05	0.01	27.91	0.95	0.93	0.97
Treatment (vs non-Invasive)	0.63	0.09	49.92	1.88	1.58	2.24
CK (log. IU/L)*	**0.88**	**0.11**	**63.74**	**2.42**	**1.95**	**3.00**
Constant	−1.65	0.44	13.89	0.19		

Legend. Multivariable logistic regression of the primary outcome, adjudicated fatal and non-fatal bleeding (reference category in brackets). BMI, body mass index; rt-PA, recombinant tissue-type plasminogen activator; FDP, fibrin(ogen) degradation products; CK, creatine kinase;*Adjusted for the upper reference limit. Model parameters for respectively the intermediate building and parsimonious model, omnibus test of model coefficients Chi-square 250.6 df 7 (246.7 df 5); −2 Log likelihood 2914.1 (2918.0), Cox and Snell R square. 0.1 (0.1); Nagelkerke R square. 0.1 (0.1); Hosmer Lemeshow Chi square 6.4, df 8 (5.6, df 8); classification overall percentage 65.1 (65.0), n=2329 (2329). Bootstrapping odd ratio for the parsimonious model: 1.42[1.95 to 3.00].

The odds for all reported bleeding vs no bleeding (n=3287) was 2.0 per log CK increase [95% CI 1.7 to 2.4]; and for combined bleeding + all-cause mortality (ACM) vs no bleeding and survival (n=2396), this was 2.5 [2.0 to 3.1], with similar results for the other variables in the model and model characteristics as depicted in Table S5.

When excluding puncture bleeding, the odds ratios were 2.1 [1.7 to 2.5] for all reported bleeding (n=3021); 2.8 [2.2 to 3.6] for fatal and non-fatal bleeding (n=2063); and 2.8 [2.2 to 3.5] for bleeding + ACM (n=2130), with similar results for the model characteristics as depicted in Table S5 (data not shown).

6. Summary and conclusion

A primary outcome of an association of CK with fatal or non-fatal bleeding, with an odds ratio of 2.60, 95% CI 1.84 to 3.68 (per log CK increase adjusted for the URL) was found with modeling of the TIMI 2 data. Data are missing (>5%) completely at random in 2 variables, platelet count and FDP at 5 h, but the unbiased inclusion of complete datasets in the multivariable binomial logistic regression had a sufficient sample size. For bleeding + ACM, the outcome was 3.11 [2.21 to 4.38]. This indicates, that with repetitive re-analysis of the population the sample is taken from, 95% of the estimates for fatal and non-fatal bleeding are expected to be between 1.58 and 3.68 (2.21 to 4.38 for bleeding+ACM) vs non-bleeding and survival.

In the sensitivity analysis, the association between CK and bleeding/death is robust, with around 2 to 4 times greater odds (per unit log CK) increase compared to not bleeding/survival (Table S6). The sample sizes were sufficient, between 911 and 3339 per analysis, applying different choices of inclusion criteria for variables, of imputation strategies (with multiple imputation currently the most accepted method, but a scarcity of tools to check its adequacy), and of patients, in clinical bleeding assessment (all bleeding, only adjudicated bleeding, or adjudicated bleeding+ ACM) and types of bleeding (puncture bleeding).

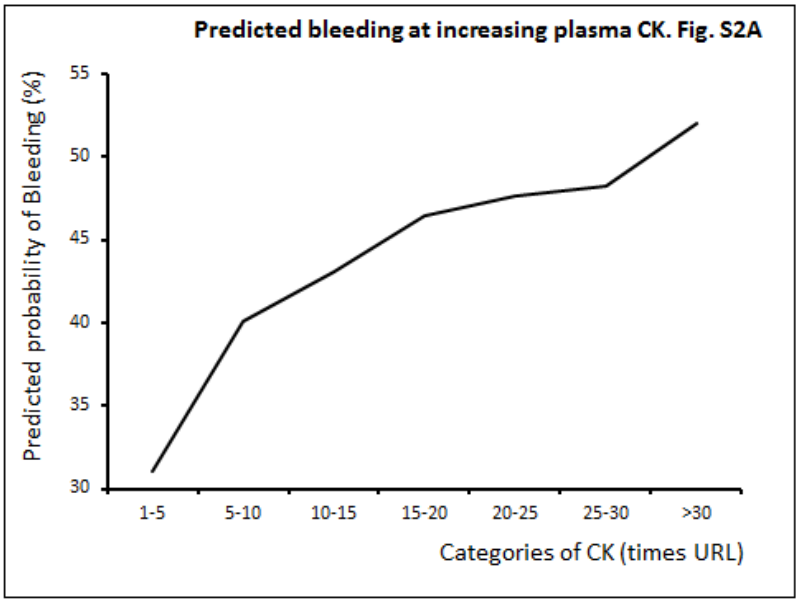
Table S6. Odds ratio matrix of sensitivity analyses of the odds for bleeding (and death) vs no bleeding (and survival)/per unit (log) CK increase

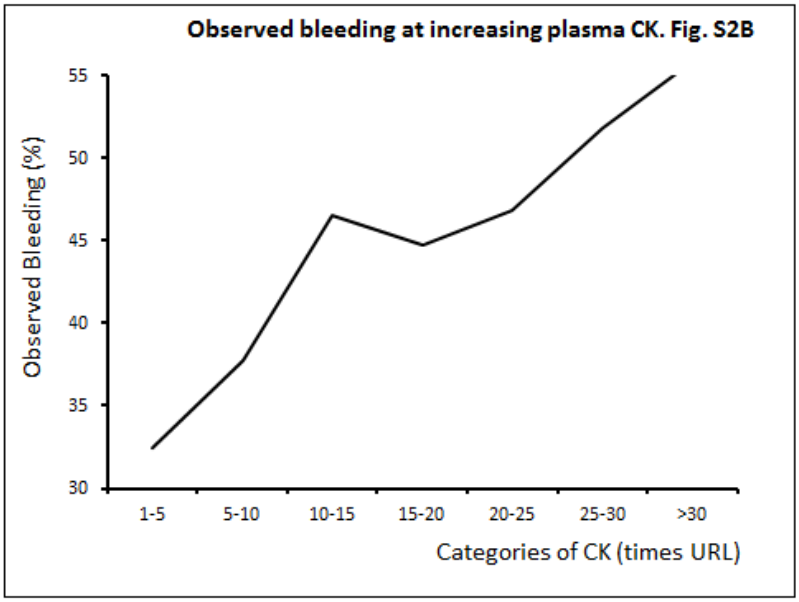
	Logistic Regression Models		
Clinical bleeding categories	**Main**	**Multiple Imputation**	**Parsimonious**
Including puncture bleeding			
All IR Bleeding	2.05 [1.55 to 2.70]	2.05 [1.73 to 2.44]	2.00 [1.68 to 2.38]
Fatal and non-Fatal Bleeding†	2.15 [1.58 to 2.94]	1.97 [1.64 to 2.37]	1.93 [1.59 to 2.35]
Fatal and non-Fatal Bleeding*‡	**2.60 [1.84 to 3.68]**	2.51 [2.02 to 3.11]	2.42 [1.95 to 3.00]
Bleeding + ACM†	2.62 [1.93 to 3.56]	1.82 [1.52 to 2.19]	2.03 [1.68 to 2.45]
Bleeding + ACM‡	3.11 [2.21 to 4.38]	2.22 [1.80 to 2.73]	2.49 [2.02 to 3.07]
Non-puncture bleeding			
All IR NP Bleeding	2.22 [1.66 to 2.97]	2.11 [1.77 to 2.52]	2.06 [1.70 to 2.47]
NP Fatal and non-Fatal Bleeding†	2.83 [1.97 to 4.07]	2.24 [1.80 to 2.79]	2.23 [1.79 to 2.78]
NP Fatal and non-Fatal Bleeding‡	3.55 [2.38 to 5.30]	2.88 [2.26 to 3.67]	2.80 [2.19 to 3.57]
NP Bleeding + ACM†	3.47 [2.44 to 4.92]	1.99 [1.62 to 2.43]	2.29 [1.86 to 2.82]
NP Bleeding + ACM‡	4.22 [2.86 to 6.22]	2.40 [1.92 to 3.01]	2.80 [2.23 to 3.53]

Legend. Adjusted odds ratios for bleeding per unit CK increase (peak log CK normalized for URL). All IR bleeding, all investigator reported hemorrhagic complications (vs none); ACM, adjudicated all-cause mortality; NP, non-puncture bleeding.*__Primary outcome__; †Adjudicated bleeding events vs non-adjudicated events (IR bleeding+no bleeding; n=1428 for the main; n=3339 for the imputed; and n=3287 for the parsimonious model; with similar model characteristics as other outcomes; data not shown). ‡Adjudicated bleeding events vs no bleeding (no investigator reported nor adjudicated bleeding).

The analysis is technically sound (model prediction in **Figure S2**), performed with rare daily data on CK in combination with investigator reported as well as adjudicated bleeding events, in a large sample of carefully studied patients with highly elevated CK after MI. However, new, prospective studies will be needed to determine whether CK is clinically useful to stratify risk for (fatal) bleeding in individual patients, or should guide dosing of thrombolytic and antithrombotic drugs.

Figure S2. Predicted and observed bleeding by CK





Legend Adjudicated fatal or non-fatal bleeding by CK categories (expressed as times the upper reference limit, URL). The highest value of the range is included in the respective CK category; Categories 1-5 (n=581); 5-10 (n=570); 10-15 (n=425); 15-20 (n=283); 20-25 (n= 172); 25-30 (n=110); and >30 (n=188). Panel A, probability of bleeding by CK categories as predicted by the adjusted binary logistic model (calculated as e**(a+bX)/(1+e**(a+bX); adjusted for sex, BMI, invasive treatment, and CK). Panel B, observed bleeding by CK categories. Kendall's tau correlation between observed and predicted data 0.8; n=2329.