Andexanet alfa in Factor Xa inhibitor-associated acute major bleeding

Supplementary Appendix

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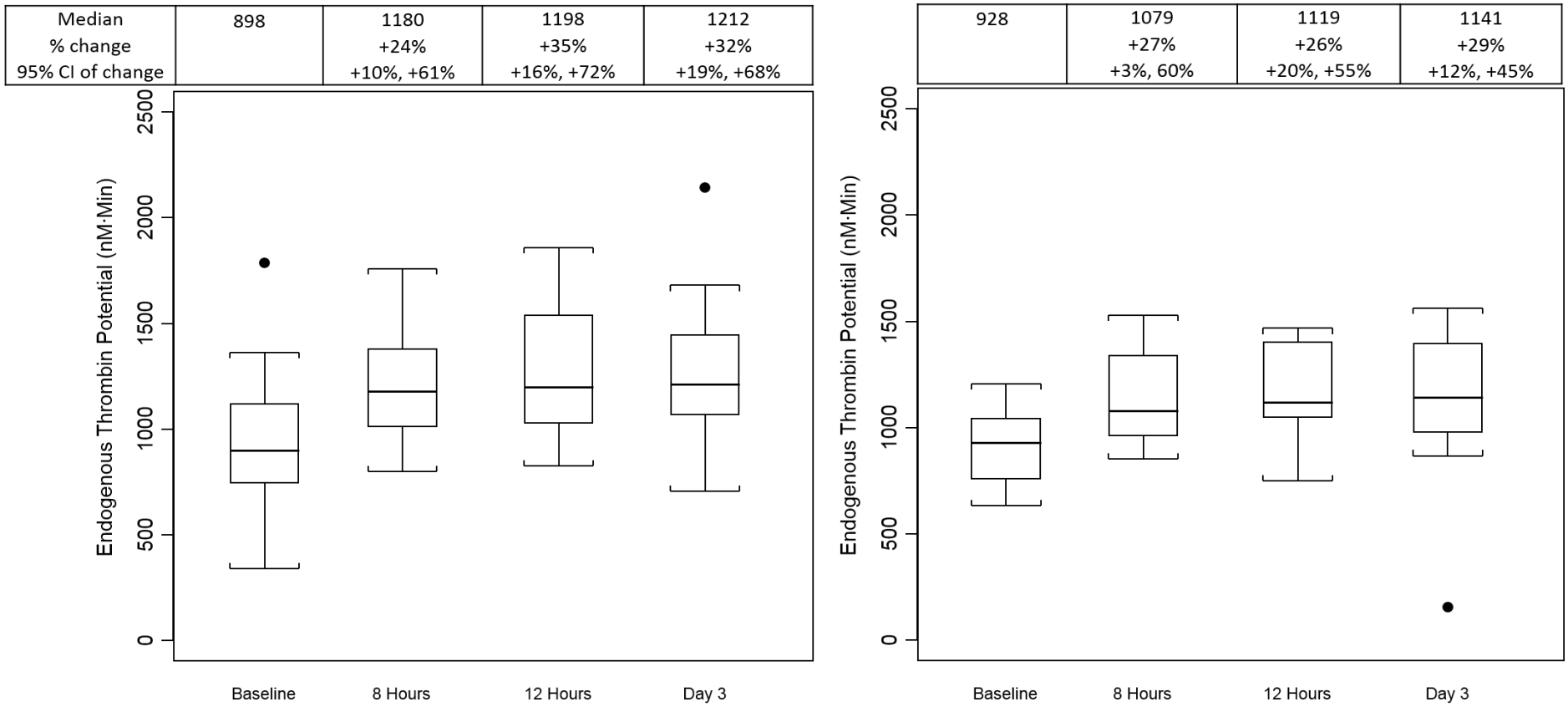
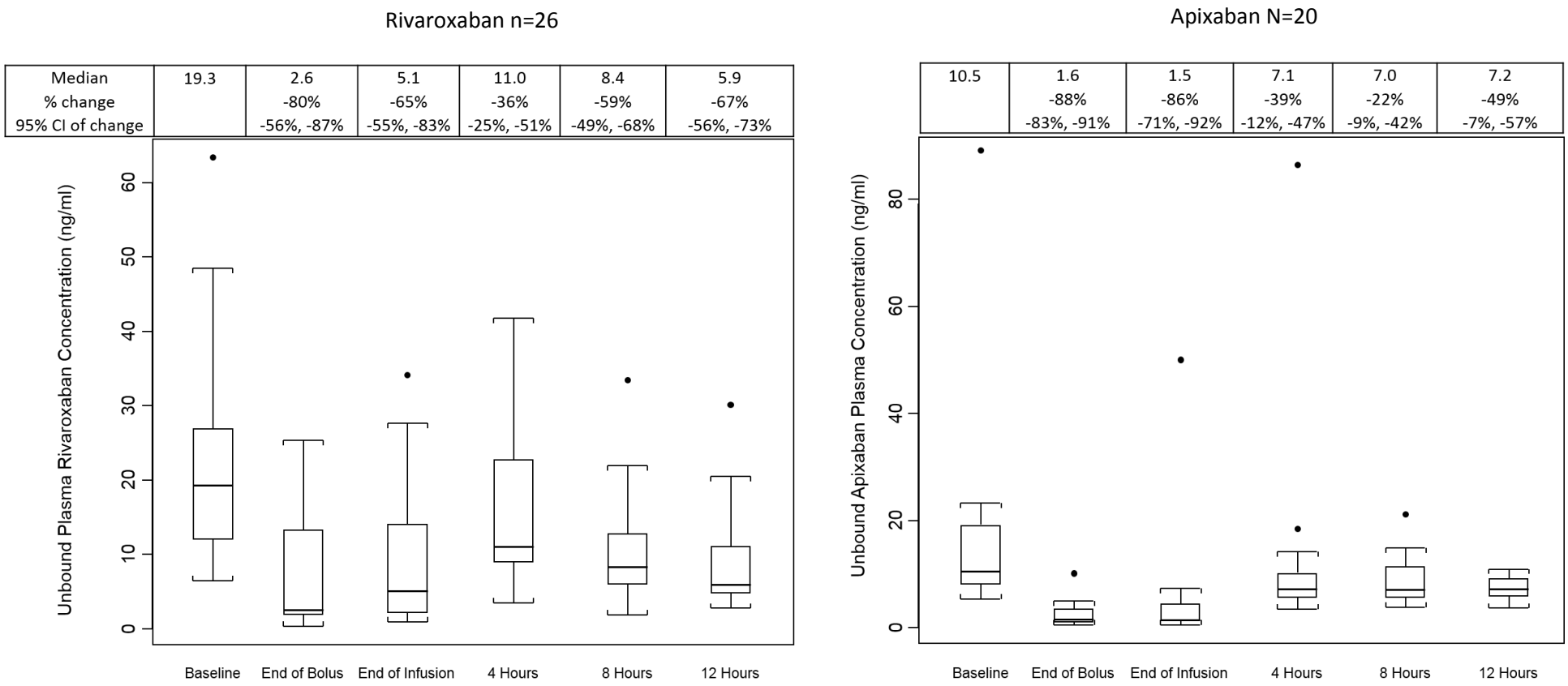
Figure S1. Endogenous Thrombin Potential



Tukey boxplot values are expressed as the median (horizontal line in each box) with the 25th (lower quartile, Q1) and 75th (upper quartile, Q3) percentiles (top and bottom of each box) and whiskers (top and bottom of each I bar) drawn to the lowest datum lowest datum still within 1.5 times (Q3 – Q1) of the lower quartile and the highest datum still within 1.5 times (Q3 – Q1) of the upper quartile. Outliers are shown as dots. The patients included in these plots are those in the efficacy population. The bolus was delivered over 15-30 minutes and the infusion lasted 2 hours. Subsequent time points are measured from the end of infusion. One patient in the efficacy population was receiving enoxaparin and is not included in these plots. The numbers at the top of the figures show the median value, the percentage change in median value from baseline and the 95% confidence interval of this change. In healthy volunteers (appendix), before fXA inhibitor dosing, the mean ETP was 1269 nM·Min (standard deviation (SD) 230) and on apixaban or on rivaroxaban (before andexanet) was 583 nM·Min (SD 158) and 434 nM·Min (SD 193), respectively.

Figure S2. Unbound Plasma Concentrations



Tukey boxplot values are expressed as the median (horizontal line in each box) with the 25th (lower quartile, Q1) and 75th (upper quartile, Q3) percentiles (top and bottom of each box) and whiskers (top and bottom of each I bar) drawn to the lowest datum lowest datum still within 1.5 times (Q3 – Q1) of the lower quartile and the highest datum still within 1.5 times (Q3 – Q1) of the upper quartile. Outliers are shown as dots. The patients included are those in the efficacy population. The bolus was delivered over 15-30 minutes and the infusion lasted 2 hours. Subsequent time points are measured from the end of infusion. The scales of the y-axes of the two plots are different to allow proper demonstration of outliers. One patient in the efficacy population was receiving enoxaparin and is not included in these plots. The numbers at the top of the figures show the median value, the percentage change in median value from baseline and the 95% confidence interval of this change.

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Table S1. Detailed Inclusion and Exclusion Criteria

INCLUSION CRITERIA
1. Either the patient or his or her medical proxy has been adequately informed of the nature and risks of the study and has given written informed consent prior to Screening
2. The patient must be at least 18 years old at the time of Screening
3. The patient must have an acute overt major bleeding episode requiring urgent reversal of anticoagulation Acute major bleeding requiring urgent reversal of anticoagulation is defined by at least ONE of the following: • Acute overt bleeding that is potentially life-threatening, e.g., with signs or symptoms of hemodynamic compromise, such as severe hypotension, poor skin perfusion, mental confusion, low urine output that cannot be otherwise explained; • Acute overt bleeding associated with a fall in hemoglobin level by ≥2 g/dL, OR a Hb ≤ 8 g/dL if no baseline Hb is available OR, in the opinion of the investigator that the patient's hemoglobin will fall to ≤ 8 g/dL with resuscitation; • − Acute symptomatic bleeding in a critical area or organ, such as, retroperitoneal, intra-articular or pericardial, intracranial or intramuscular with compartment syndrome.
4. The patient, for whom the bleeding is intracranial must have undergone a head CT or MRI scan demonstrating the intracranial bleeding Note: Patients with bleeding at non-intracranial locations do not require a head CT or MRI
5. Patient received or believed to have received one of the following within 18 hours prior to andexanet administration: apixaban, rivaroxaban, edoxaban or enoxaparin (dose of enoxaparin ≥1 mg/kg/d)
EXCLUSION CRITERIA
1. The patient is scheduled to undergo surgery in less than 12 hours with the exception of minimally invasive surgery/procedures
2. A patient with ICH has any of the following: • Glasgow coma score < 7 • Estimated intracerebral hematoma volume >60 cc as assessed by the CT or MRI
3. The patient has an expected survival of less than 1 month
4. The patient has a recent history (within 2 weeks) of a diagnosed thrombotic event (TE) as follows: myocardial infarction, disseminated intravascular coagulation (DIC), cerebral vascular accident, transient ischemic attack, unstable angina pectoris hospitalization, or severe peripheral vascular disease within 2 weeks prior to Screening

5. The patient has severe sepsis or septic shock at the time of Screening

6. The patient is pregnant or a lactating female

7. The patient has received any of the following drugs or blood products within 7 days or Screening:

   a) Vitamin K antagonist (VKA) (e.g., warfarin)
   b) Dabigatran
   c) Prothrombin Complex Concentrate products (PCC, e.g., Kcentra®) or recombinant factor VIIa (rfVIIa) (e.g., NovoSeven®)
   d) Whole blood, plasma fractions

   Note: Administration of platelets or packed red blood cells (PRBCs) is not an exclusion criterion;

8. The patient was treated with an investigational drug <30 days prior to Screening

9. Planned administration of PCC, fresh frozen plasma (FFP), or rfVIIa from Screening until within 12 hours after the end of the andexanet infusion

Table S2. Dosing and Administration of Andexanet alfa

Andexanet is administered as an IV bolus, immediately followed by a continuous infusion.

fXa Inhibitor	IV Bolus	IV Infusion
All patients receiving apixaban and those patients who received rivaroxaban >7 hours ago	400 mg at a target rate of 30 mg/min	480 mg @ 4 mg/min for 120 minutes
Patients who received enoxaparin, edoxaban, or a dose of rivaroxaban within ≤ 7 hours or at an unknown time* Patients who are believed to have received a fXa inhibitor but it is uncertain which one *if there is a delay between medical presentation and start of andexanet of more than 7 hours, the patient should receive the dose for rivaroxaban >7 hours ago	800 mg at a target rate of 30 mg/min	960 mg @ 8 mg/min for 120 minutes

Table S3. Rating System for Effective Hemostasis

Bleed Type	Excellent (effective)	Good (effective)	Poor/none (not effective)
Visible	Cessation of bleeding ≤ 1 hour after end of infusion and no plasma, coagulation factor or blood products (excludes pRBCs).[1]	Cessation of bleeding between > 1 and ≤ 4 hours after end of infusion and ≤ 2 units plasma, coagulation factor or blood products (excludes pRBCs).[4]	Cessation of bleeding > 4 hours after end of the infusion and /or >2 units plasma, coagulation factor or blood products (excludes pRBCs).[5]
Muscular/skeletal	pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding ≤1 hour after the end of infusion; and the condition has not deteriorated during the 12-hour period	pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding >1 and ≤4 hours after end of infusion; and the condition has not deteriorated during the 12-hour period	No improvement by 4 hours after end of infusion and/or condition has deteriorated during the 12-hour period
Intracerebral hematoma	≤20% increase in hematoma volume compared to baseline on a repeat CT or MRI scan performed at both the 1 and 12 hour post infusion time points	>20% but ≤35% increase in hematoma volume compared to baseline on a repeat CT or MRI scan at +12-hour time point	>35% increase in hematoma volume on a CT or MRI compared to baseline on a repeat CT or MRI scan at +12-hour time point
Subarachnoid bleed	≤20% increase in maximum thickness using the most dense area on the follow-up vs baseline at both the 1 and 12 hour post infusion time points	>20% but <35% increase in maximum thickness using the most dense area on the follow-up at +12h vs baseline	>35% increase in maximum thickness using the most dense area on the +12h vs at baseline
Subdural hematoma	≤20% increase in maximum thickness at both the 1 and 12 hour post infusion assessments compared to baseline	>20% but < 35% increase in maximum thickness at +12h compared to baseline	>35% increase in maximum thickness at +12h compared to baseline
Pericardial	No increase in the size of pericardial effusion on repeat echocardiogram done within 12 hours of the end of infusion	<10% increase in the size of pericardial effusion on repeat echocardiogram done within 12 hours of the end of infusion	10% or more increase in the size of pericardial effusion on repeat echocardiogram done within 12 hours of the end of infusion
Intra-spinal	No increase in hematoma size on repeat CT or MRI scan done within 12 hours of the end of infusion	<10% increase in hematoma size on repeat CT or MRI scan done within 12 hours of the end of infusion	10% or more increase in hematoma size on repeat CT or MRI scan done within 12 hours of the end of infusion
GI, Urinary or non-visible bleeding not described above	≤10% decrease in both corrected hemoglobin/hematocrit at 12 hours[2,3] compared to baseline	>10 % to ≤20% decrease in both corrected hemoglobin/hematocrit at 12 hours compared to baseline[2,3]	>20% decrease in both corrected hemoglobin/hematocrit[2,3]

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Additional Factors to be Considered During Adjudication

1. Any additional diagnostic data for a particular bleeding site (e.g., nasogastric tube, ultrasound, GI endoscope, echocardiogram, or CT/MRI scans) will be taken into account for the overall assessment.
2. Any uncontrolled bleeding that did not respond to andexanet and was related to an underlying disease will be taken into account for the overall assessment.
3. Pain, swelling, and signs of bleeding are considered to be typical symptoms of musculoskeletal bleeding and are expected to be present at baseline.

[1]For all types of bleeding: no additional plasma, blood products (whole blood products not including packed red blood cells [PRBCs]) and/or coagulation factor products required after initial treatment with andexanet.

[2]The smallest percentage decrease in hemoglobin or hematocrit should be used to determine the efficacy rating of excellent, good, or poor/none. The net change is defined as the difference between the corrected hemoglobin or hematocrit value at baseline and 12 hours after infusion

[3] For the adjusted hemoglobin and hematocrit calculation, it will be assumed that for each unit of PRBC transfusion there is an increase of 1 g/dL in hemoglobin and a 3% increase in hematocrit.

[4]For all types of bleeding, no more than two additional units of plasma or blood products and/or coagulation factor products required after initial treatment with andexanet. "Blood products" include whole blood but not PRBCs.

[5]For all types of bleeding, more than two additional units of plasma or blood products and/or coagulation factor products required after initial treatment with andexanet. "Blood products" include whole blood but not PRBCs.

Table S4. Thrombotic Event Definitions

Term	Definition
Transient ischemic attack	A transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, with signs or symptoms lasting < 24 hours and no evidence of new infarct on neuroimaging if performed.
Stroke	Stroke is an acute episode of neurological dysfunction consistent with a vascular cause. Stroke is defined as the rapid onset of signs and or symptoms of a new persistent neurological deficit consistent with an obstruction to cerebral blood flow or with cerebral hemorrhage with no apparent nonvascular cause (e.g., trauma, tumor, or infection). Signs or symptoms must last at least 24 hours or if systems less than 24 hours have neuroimaging evidence of new infarct. Available neuroimaging studies will be considered to support the clinical impression and to determine if there is a demonstrable lesion compatible with an acute stroke. Strokes will be classified as ischemic, hemorrhagic, or unknown. Ischemic stroke with hemorrhagic transformation will be primarily classified with ischemic stroke etiology. <u>For the diagnosis of stroke, the following 4 criteria should be fulfilled:</u> 1. Rapid onset* of a focal/global neurological deficit with at least one of the following: a. Change in level of consciousness b. Hemiplegia c. Hemiparesis d. Numbness or sensory loss affecting one side of the body e. Dysphasia/Aphasia f. Hemianopia (loss of half of the field of vision of one or both eyes) g. Amaurosis fugax (transient complete/partial loss of vision of one eye) h. Other new neurological sign(s)/symptom(s) consistent with stroke **If the mode of onset is uncertain, a diagnosis of stroke may be made provided that there is no plausible non stroke cause for the clinical presentation* 2. Duration of a focal/global neurological deficit ≥ 24 hours, OR The neurological deficit results in death, OR Neuroimaging evidence of new infarct 3. No other readily identifiable non stroke cause for the clinical presentation (e.g., brain tumor, trauma, infection, hypoglycemia, peripheral lesion) 4. Confirmation of the diagnosis by at least one of the following:* i. Specialist evaluation (consult notes) ii. Brain imaging procedure (at least one of the following): CT scan, MRI scan, Cerebral vessel angiography iii. Lumbar puncture (i.e., spinal fluid analysis diagnostic of intracranial hemorrhage) **if a stroke is reported but evidence of confirmation of the diagnosis by the methods outlined above is absent, the event will be discussed by the Blinded Stroke Advisory Committee, which is part of the Adjudication Committee. In such cases, the event may be adjudicated as a stroke on the basis of the clinical presentation alone.* <u>If the acute focal signs represent a worsening of a previous deficit, these signs must have either</u>: 1. Persisted for more than one week, or 2. Persisted for more than 24 hours and were accompanied by an appropriate new CT or MRI finding <u>Strokes are sub-classified as follows:</u> 1. Ischemic (Non hemorrhagic): a stroke caused by an arterial obstruction due to either a thrombotic

	(e.g., large vessel disease/atherosclerotic or small vessel disease/lacunar) or embolic etiology. This category includes ischemic stroke with hemorrhagic transformation. 2. Hemorrhagic: a stroke due to a hemorrhage in the brain as documented by neuroimaging or autopsy. This category will include strokes due to primary intracerebral hemorrhage (intraparenchymal or intraventricular), and primary subarachnoid hemorrhage. 3. Subdural hematoma will be classified as intracranial hemorrhage but will not be classified as either stroke or as intracerebral hemorrhage. 4. Unknown: the stroke type could not be determined by imaging or other means (e.g., lumbar puncture, neurosurgery, or autopsy) or no imaging was performed.
Myocardial infarction	The term acute myocardial infarction (MI) should be used when there is evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischaemia. Under these conditions any one of the following criteria meets the diagnosis for MI: 1. Detection of a rise and/or fall of cardiac biomarker values [preferably cardiac troponin (cTn)] with at least one value above the upper limit of normal (ULN) and with at least one of the following: • Symptoms of ischaemia • New or presumed new significant ST-segment–T wave (ST–T) changes or new left bundle branch block (LBBB) • Development of pathological Q waves in the ECG • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality • Identification of an intracoronary thrombus by angiography or autopsy. 2. Cardiac death with symptoms suggestive of myocardial ischaemia and presumed new ischaemic ECG changes or new LBBB, but death occurred before cardiac biomarkers were obtained, or before cardiac biomarker values would be increased. 3. Percutaneous coronary intervention (PCI) related MI is arbitrarily defined by elevation of cTn values (>5×ULN) in patients with normal baseline values or a rise of cTn values >20% if the baseline values are elevated and are stable or falling. In addition, either (i) symptoms suggestive of myocardial ischaemia or (ii) new ischaemic ECG changes or (iii) angiographic findings consistent with a procedural complication or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required. 4. Stent thrombosis associated with MI when detected by coronary angiography or autopsy in the setting of myocardial ischaemia and with a rise and/or fall of cardiac biomarker values with at least one value above the ULN. 5. Coronary artery bypass grafting (CABG) related MI is arbitrarily defined by elevation of cardiac biomarker values (>10×ULN) in patients with normal baseline cTn values. In addition, either (i) new pathological Q waves or new LBBB, or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Venous Thromboembolism	Venous thromboembolism is defined as symptomatic lower limb deep vein thrombosis or pulmonary embolism confirmed by objective testing. Criteria for the objective confirmation of deep vein thrombosis include: • A constant filling defect in two or more views on contrast venography in one or more proximal venous segments (iliac, common femoral, superficial femoral, popliteal) • New or previously undocumented non-compressibility of one or more venous segments on compression ultrasound • A clearly defined intraluminal filling defect on contrast enhanced computed tomography Criteria for the objective diagnosis of pulmonary embolism include: • An intraluminal filling defect on pulmonary angiography • Sudden contrast cut-off of one or more vessels more than 2.5 mm in diameter on a pulmonary angiogram • A high probability VQ scan (one or more segmental perfusion defects with corresponding normal ventilation) • An abnormal non-high VQ scan plus criteria for the diagnosis of DVT • An unequivocal, intra-arterial, un-enhancing filling defect in the central pulmonary vasculature (pulmonary trunk, main pulmonary arteries, anterior trunk, right and left interlobar and lobar arteries) on CT
Cardiovascular death	Cardiovascular death includes death resulting from an acute myocardial infarction (MI), sudden cardiac death, death due to heart failure (HF), death due to stroke, death due to cardiovascular (CV) procedures, death due to CV hemorrhage, and death due to other CV causes. Unwitnessed deaths and deaths of unknown cause will be considered cardiovascular.

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Thrombin Generation Levels in Older Healthy Volunteers

Thrombin generation was measured as previously described for the ANNEXA-A (apixaban) and ANNEXA-R (rivaroxaban) studies in older healthy volunteers[1]. In brief, thrombin generation was measured using Calibrated Automated Thrombogram (CAT) according to the manufacturer's instruction (Diagnostica Stago) and performed by Quest Diagnostics. CAT parameters analyzed include ETP, peak thrombin, lag time, time-to-peak, and velocity index. ETP was prospectively chosen as the endpoint because it reflects the overall thrombin generation potential.

Table S5 shows the composite ETP data from the ANNEXA-A and ANNEXA-R studies. Baseline ETP was calculated from the pre-anticoagulant assay results on all subjects (N=148) from the two studies. Pre-Andexanet values (just prior to the bolus of andexanet or placebo) were calculated separately for the apixaban subjects (N=65) and the rivaroxaban subjects (N=80) from all subjects in the safety population.

Table S5: Composite ETP data from the ANNEXA-A and ANNEXA-R studies

Parameter	Baseline	Apixaban Pre-Andexanet	Rivaroxaban Pre-Andexanet
	ETP (nM·min)		
N	148	65	80
Mean	1269	583	434
1 SD	230	158	192
2 SD	459	316	383

1. Siegal DM, Curnutte JT, Connolly SJ, Lu G, Conley PB, Wiens BL, Mathur VS, Castillo J, Bronson MD, Leeds JM, Mar FA, Gold A, Crowther MA. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. N Engl J Med. 2015 Dec