Supplementary material

MMP-9 release into collateral blood vessels before endovascular thrombectomy to assess the risk of major intracerebral haemorrhages and poor outcome for acute ischaemic stroke: a proof-of-concept study

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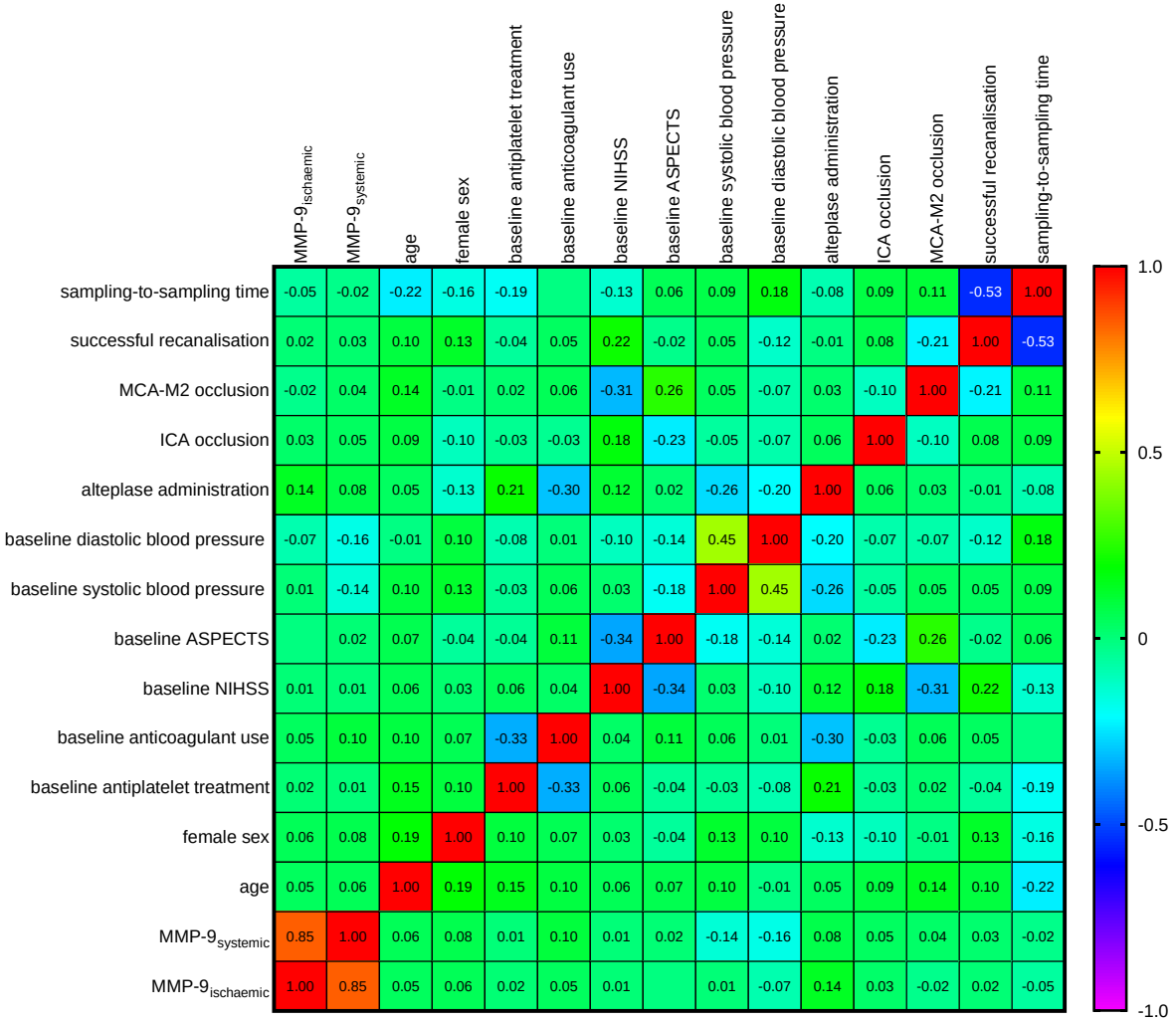
Figure S1: Correlation matrix between candidate variables for multivariable modelling



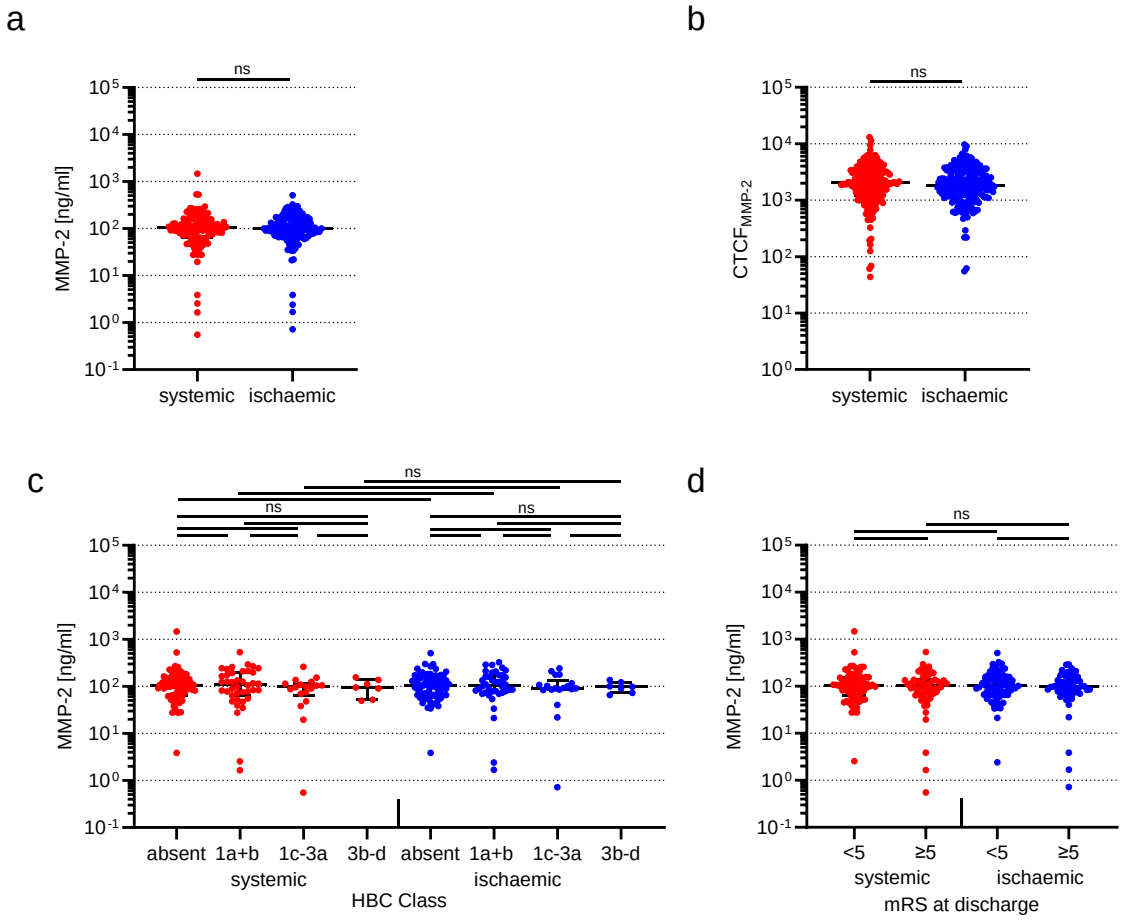
Green represents (near-)zero correlation whereas colour scale towards magenta or red refers to the degree of correlation towards -1 or +1, respectively.
ASPECTS, Alberta Stroke Program Early CT Score; ICA, internal carotid artery; MCA–M2, middle cerebral artery M2 segment; MMP-9, Matrix Metalloproteinase-9; NIHSS, National Institutes of Health Stroke Scale.

Table S1: Intracranial bleeding events by Heidelberg Bleeding Classification

Intracranial haemorrhage[a]	**Overall**[b] (n=132)
No haemorrhage, n (%)	67 (50.8)
HBC 1a, n (%)	22 (16.7)
HBC 1b, n (%)	18 (13.6)
HBC 1c, n (%)	11 (8.3)
HBC 2, n (%)	5 (3.8)
HBC 3a, n (%)	2 (1.5)
HBC 3b, n (%)	5 (3.8)
HBC 3c, n (%)	34 (25.8)
HBC 3d, n (%)	0 (0)

[a]Intracranial haemorrhages were anatomically categorised according to the Heidelberg Bleeding Classification (HBC): 1a, haemorrhagic infarction (HI1) – scattered small petechiae; 1b, HI2 – confluent petechiae; 1c, parenchymal haematoma (PH1) – haematoma within infarcted tissue, occupying <30%; 2, PH2 – haematoma occupying ≥30% of the infarcted tissue; 3a – PH remote from infarcted brain tissue; 3b – intraventricular haemorrhage; 3c – subarachnoid haemorrhage; 3d – subdural haemorrhage.
[b]Including multiple lesions per patient.

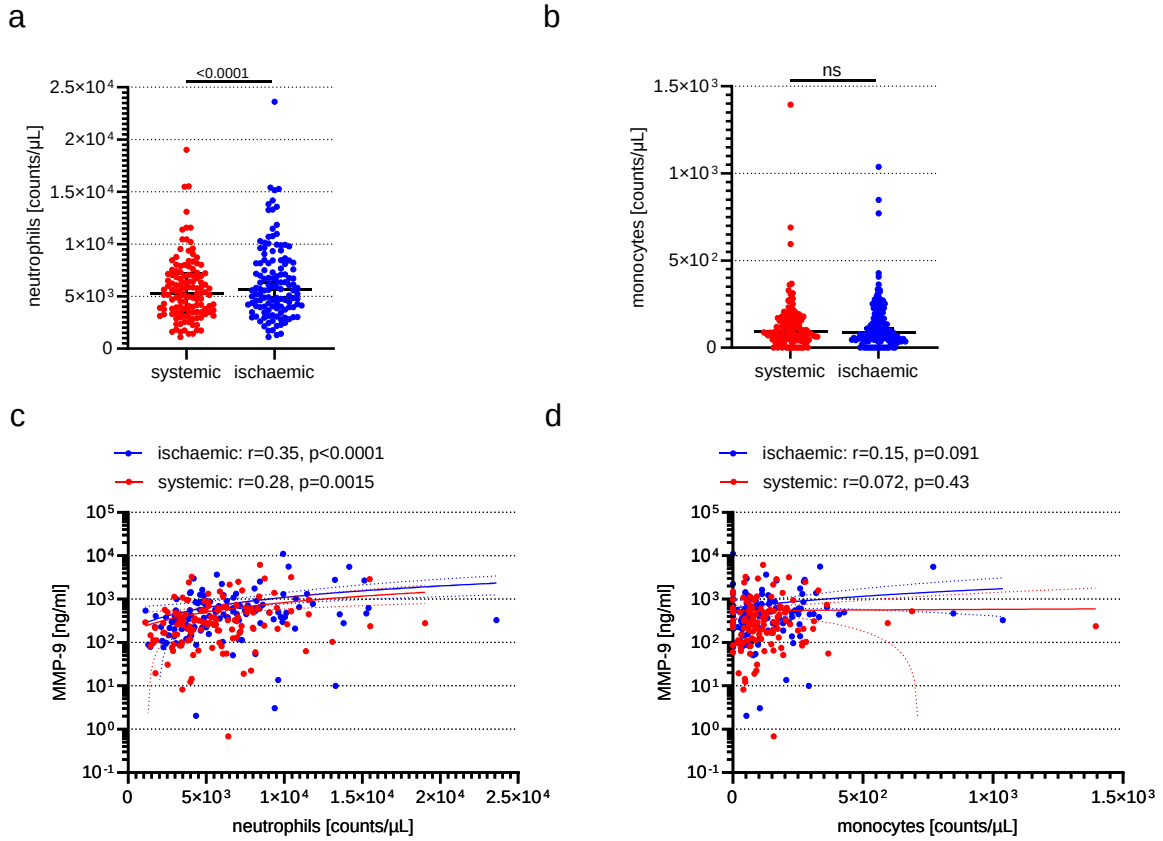
Figure S2: Regional MMP-2 plasma concentrations and neutrophil MMP-2 expression with plasma concentrations stratified by type of intracranial bleeding and functional outcome category



Semi-log plots of MMP-2 plasma concentrations (a, c, and d) and fluorescence intensity of neutrophils stained for MMP-2 (b) in large-vessel ischaemic stroke. Labelling according to the sampling location (systemic control at cervical level of the internal carotid artery vs ischaemic sample from collateral blood vessels).

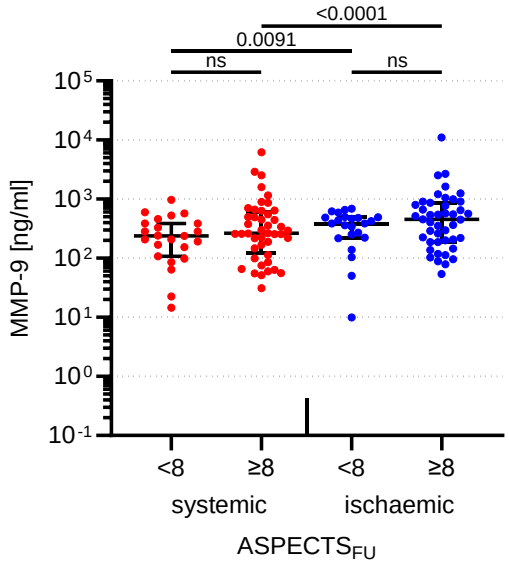
Intracranial bleedings (c) were anatomically categorized according to the Heidelberg Bleeding Classification (HBC, categorisation corresponding to Table S1), and grouped with respect to the type of bleeding (HBC classes 1a+b, minor [petechial] intracerebral haemorrhage; HBC classes 1c–3a, major intracerebral haemorrhage [parenchymal haematoma]; HBC classes 3b–d, intracranial-extracerebral haemorrhage). Functional outcome (d) was assessed by the modified Rankin Scale (mRS: mRS≥5, severe disability or death at hospital discharge).
Data are given as median with interquartile range and analysed using Wilcoxon matched-pairs signed rank test (a-d), Kruskal-Wallis test with Benjamini, Krieger and Yekutieli post hoc analysis (c), and Mann-Whitney test (d). The threshold for significance was $p<0.05$ (two-sided).
CTCF, corrected total cellular fluorescence; HBC, Heidelberg Bleeding Classification; MMP-2, Matrix Metalloproteinase-2; mRS, modified Rankin Scale; ng/ml, nanograms per millilitre; ns, not significant.

Figure S3: Neutrophil and monocyte cell counts, and correlation with MMP-9 plasma concentrations



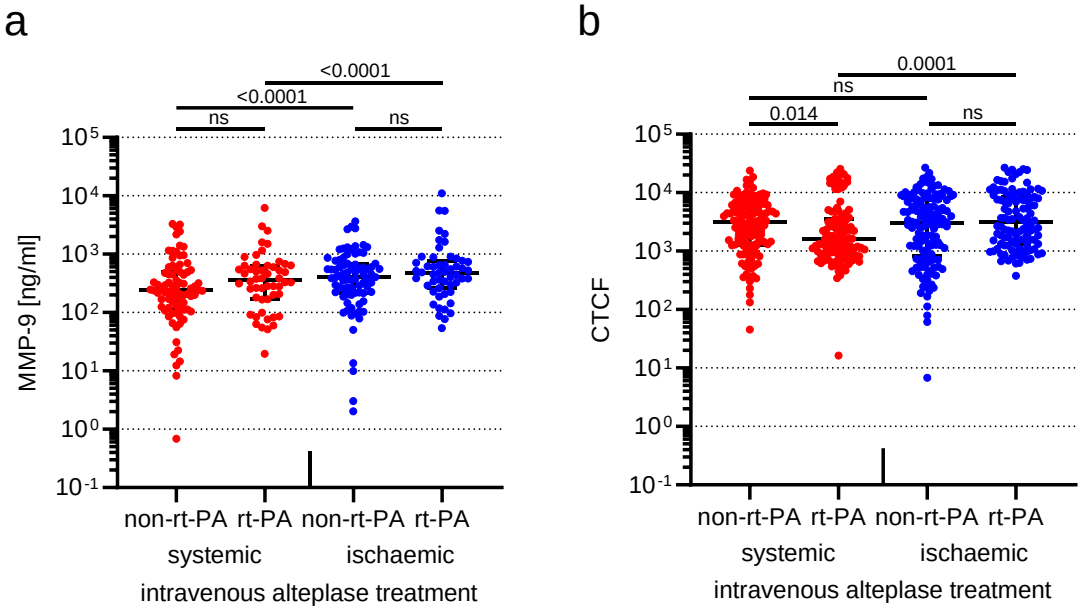
Neutrophil and monocyte cell counts (a and b), and correlation with MMP-9 plasma concentrations (c and d) by location of arterial sampling in large-vessel ischaemic stroke. Labelling according to the sampling location. The long horizontal bar shows the median value, and the short error bar shows the interquartile range. Data were analysed using Wilcoxon matched-pairs signed rank test (a and b) and Spearman's rank order correlation (c and d). Short-dotted lines represent the 95% CI of the correlation line (c and d). The threshold for significance was $p<0.05$ (two-sided).
MMP-9, Matrix Metalloproteinase-9; ng/ml, nanograms per millilitre; ns, not significant.

Figure S4: MMP-9 plasma concentrations by location of sampling and ASPECTS category at follow-up imaging



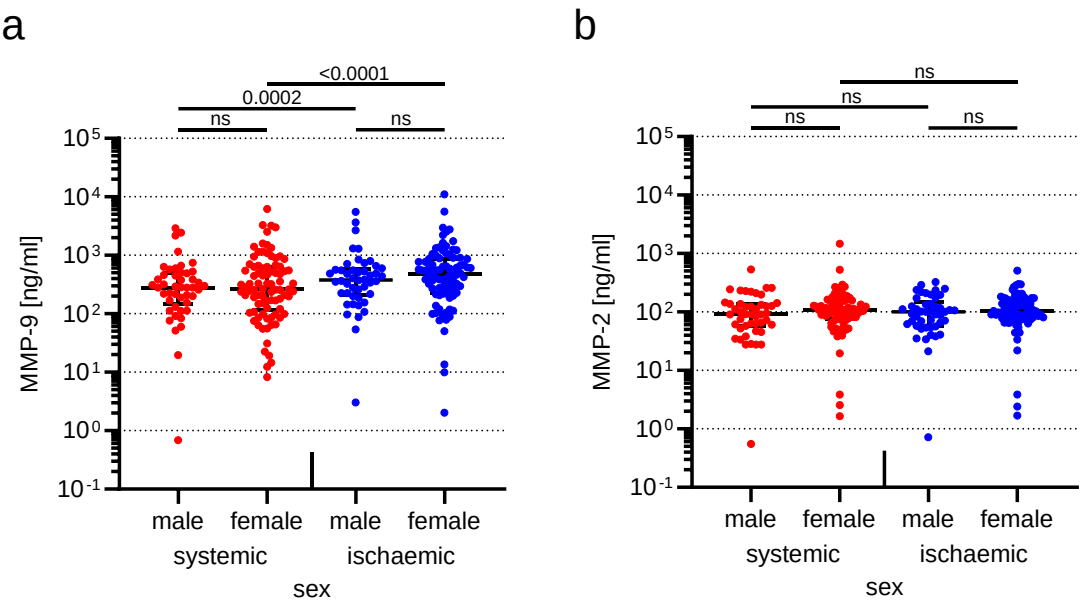
Semi-log plot of MMP-9 plasma concentrations by ASPECTS category at follow-up imaging in large-vessel ischaemic stroke, labelling according to the sampling location. Data are given as median with interquartile range and analysed using Wilcoxon matched-pairs signed rank test and Mann-Whitney test. Individual p values are given for $p<0.05$ (two-sided).
$ASPECTS_{FU}$, Alberta Stroke Program Early CT score at follow-up imaging; MMP-9, Matrix Metalloproteinase-9; ng/ml, nanograms per millilitre; ns, not significant.

Figure S5: Regional MMP-9 plasma concentrations and fluorescence intensity at single-neutrophil level according to intravenous alteplase treatment



Semi-log plots of MMP-9 plasma concentrations (a) and fluorescence intensity of neutrophils stained for MMP-9 (b) in large-vessel ischaemic stroke, labelling according to the sampling location. Data are given as median with interquartile range and subjected to statistical analysis by Mann-Whitney test and Wilcoxon matched-pairs signed rank test. Individual p values are given for $p<0.05$ (two-sided).
CTCF, corrected total cellular fluorescence, MMP-9, Matrix Metalloproteinase-9; ng/ml, nanograms per millilitre; ns, not significant, rt-PA, recombinant tissue plasminogen activator (alteplase).

Figure S6: Regional MMP-9 and MMP-2 plasma concentrations by sex



Semi-log plots of MMP-9 (a) and MMP-2 (b) plasma concentrations in large-vessel ischaemic stroke, labelling according to the sampling location. Data are given as median with interquartile range and analysed using Mann-Whitney test and Wilcoxon matched-pairs signed rank test. Individual p values are given for $p<0.05$ (two-sided). MMP-2, Matrix Metalloproteinase-2; MMP-9, Matrix Metalloproteinase-9; ng/ml, nanograms per millilitre; ns, not significant.

Table S2: Univariate logistic regression of predictors of major intracerebral haemorrhages and severe disability or death

Predictor variable[a]	HBC Class 1c–3a					mRS ≥ 5				
	Coefficient	SE	OR[b]	95% CI	p value	Coefficient	SE	OR[c]	95% CI	p value
Age	0.009	0.02	1.009	0.96–1.06	0.72	0.009	0.02	1.009	0.98–1.04	0.56
Male sex	-0.08	0.54	0.92	0.32–2.65	0.88	0.11	0.37	1.11	0.54–2.29	0.77
Baseline antiplatelet treatment	1.25	0.52	3.5	1.26–9.7	0.02	0.64	0.38	1.9	0.89–4.03	0.09
Baseline anticoagulant treatment	-0.14	0.56	0.87	0.29–2.62	0.8	-0.08	0.38	0.92	0.43–1.95	0.83
Baseline NIHSS score	0.1	0.04	1.11	1.03–1.19	0.008	0.07	0.03	1.08	1.01–1.14	0.02
Baseline ASPECTS	-0.38	0.15	0.69	0.51–0.93	0.02	-0.35	0.12	0.7	0.56–0.89	0.004
Systolic blood pressure at admission	0.01	0.009	1.01	0.99–1.03	0.16	0.01	0.007	1.01	1.001–1.03	0.03
Diastolic blood pressure at admission	0.02	0.01	1.02	0.99–1.05	0.2	0.01	0.01	1.01	0.99–1.03	0.31
Alteplase administration	0.5	0.51	1.65	0.61–4.48	0.32	0.15	0.36	1.16	0.57–2.34	0.68
Onset-to-puncture	0.0017	0.0015	1.0017	0.1–1.0046	0.26	-0.00034	0.0012	0.1	0.1–1.0021	0.78
MCA–M2 occlusion	-0.49	0.67	0.61	0.17–2.28	0.47	-0.28	0.42	0.76	0.33–1.72	0.5
ICA occlusion	0.42	0.62	1.52	0.45–5.16	0.49	0.74	0.48	2.09	0.82–5.29	0.12
Stent-retrieval manoeuvres	-0.04	0.14	0.96	0.72–1.28	0.79	0.24	0.1	1.27	1.03–1.55	0.02
No reperfusion	0.56	0.63	1.75	0.51–5.99	0.37	1.28	0.52	3.61	1.29–10.09	0.02
Onset-to-final-recanalisation	0.0007	0.0011	1.0007	0.1–1.0029	0.54	0.00074	0.00095	1.0007	0.1–1.0026	0.44
$MMP\text{-}9_{ischaemic}$	0.0003	0.0002	1.0003	1.00–1.0007	0.06	0.0007	0.0003	1.0007	1.0001–1.001	0.02
$MMP\text{-}9_{systemic}$	0.0002	0.0003	1.0002	0.99–1.0007	0.37	0.0004	0.0003	1.0004	0.99–1.0009	0.09

Sampling-to-sampling, min	-0.0024	0.0061	0.99	0.99–1.01	0.69	0.01	0.004	1.01	1.002–1.02	0.02

[a]Odds ratios for continuous variables are reported per unit of increment: age [1 year]; baseline NIHSS score [1-point NIHSS score increase]; baseline ASPECTS [1-point ASPECTS decrease]; systolic/diastolic blood pressure at admission [1 mmHg]; onset-to-puncture time [1 minute]; stent-retrieval manoeuvres [n]; onset-to-final-recanalisation time [1 minute]; MMP-9ischaemic /systemic [1 ng/ml].
[b]Data represent unadjusted odds ratios with 95% CI for major intracerebral haemorrhages equivalent to the combined Heidelberg Bleeding Classification (HBC) classes 1c, 2, and 3a.
[c]Data represent unadjusted odds ratios with 95% CI for severe disability or death as defined by a modified Rankin Scale (mRS) ≥5 at hospital discharge.
ASPECTS, Alberta Stroke Program Early CT Score; CI, confidence interval; ICA, internal carotid artery; MCA–M2, middle cerebral artery M2 segment; MMP-9, Matrix Metalloproteinase-9; mmHg, millimetres of mercury; ng/ml, nanograms per millilitre; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; SE, standard error.