The International Stroke Trial (IST) was a prospective, randomized control trial conducted between 1991 and 1996 and was primarily sponsored by the UK Medical Research Council, the UK Stroke Association, and the European Union BIOMED-1 program with some support from other collaborators. Its main goal was to determine whether early administration of aspirin, heparin, or both impacted the clinical course of acute ischemic stroke. The clinical trial used a 2×3 factorial design with 6 treatment allocations:

1. aspirin 300 mg & heparin 12500 IU
2. aspirin 300 mg & heparin 5000 IU
3. aspirin 300 mg & no heparin
4. no aspirin & heparin 12500 IU
5. no aspirin & & heparin 5000 IU
6. no aspirin & no heparin

Patients in the active treatment group (aspirin, heparin, or both) were given their first treatment dose immediately after the randomization process was done. Treatment was to be continued for 14 days until discharge. The trial had two study visits, one at 14 days after first treatment dose and one at six months after first treatment dose. Patients were monitored for intracranial hemorrhage, ischemic stroke, or major extracranial hemorrhage that required transfusion or caused death during the 14-day follow-up after the first treatment dose. The end of follow-up was six months after the first treatment dose.

A computer program was used to randomly allocate the study treatments. Minimization was used to reduce imbalance in prognostic features between treatment groups. At the six-month follow-up, the assessors were blinded to the treatment allocation. The pilot study showed that most of the patients could not remember their treatment, so the patients could be considered blinded.

Potential biases included patients remembering their initial diagnosis at 6 months, and clinicians being more likely to arrange repeat CT scanning for patients that worsened clinically, thus detecting more intracranial hemorrhage.

IST study data will be analyzed to detect an association between aspirin consumption and hemorrhagic stroke. The aspirin 300 & no heparin group will be compared to the control group (no aspirin and no heparin). The outcome variable of interest is hemorrhagic stroke after 14 days of follow-up, which is a binary yes/no outcome.

H_0: There is no difference in the proportion of hemorrhagic stroke between the two treatment groups after 14 days of follow-up.

H_A: There is a difference in the proportion of hemorrhagic stroke between the two treatment groups after 14 days of follow-up.

This study included 9,718 participants who were randomly assigned to receive either the intended treatment (aspirin 300 mg & no heparin) or the control treatment (no aspirin & no heparin). The intended treatment group contained 4,858 participants, and the control group contained 4,860 participants. Seven participants were lost to follow-up during the 14-day follow-up period. Of these seven participants, 3 were in the intended treatment group and 4 were in the control group. A total of 6 participants' outcomes were recorded as unknown, 2 in the intended treatment group and 4 in the control group. Those 6 unknowns plus the 7 subjects with missing data result in 13 participants being excluded. The analysis only included 9,705 participants, 4,853 in the intended treatment group and 4,852 in the control group.

Table 1: Baseline demographics and clinical characteristics by treatment group.

Baseline Characteristics			
Treatment	**Aspirin 300 mg & no heparin** (n=4,858)	**Placebo** (no aspirin & no heparin) (n=4,860)	**Total** (n=9,718)
Sex (n,%)			
Female	2,309 (47.53%)	2,250 (46.30%)	4,559 (46.91%)
Male	2,549 (52.47%)	2,610 (53.70%)	5,159 (53.09%)
Age (mean, SD)	71.77 (11.63)	71.61 (11.72)	71.69 (11.68)
Conscious State (n,%)			
Alert	3,681 (75.77%)	3,781 (77.80%)	7,462 (76.79%)
Drowsy	1,109 (22.83%)	1,019 (20.97%)	2,128 (21.90%)
Unconscious	68 (1.40%)	60 (1.23%)	128 (1.32%)
Visible Infarct (n,%)			
Yes	1,611 (33.16%)	1,631 (33.56%)	3,242 (33.36%)
No	3,247 (66.84%)	3,229 (66.44%)	6476 (66.64%)
SBP (mean, SD)	159.47 (27.52)	160.08 (27.90)	159.91 (27.71)
Face Deficit (n,%)			
Yes	3,537 (72.81%)	3,517 (72.37%)	7,054 (72.59%)

No	1,256 (25.85%)	1,269 (26.11%)	2,525 (25.98%)
Can't Assess	65 (1.34%)	74 (1.52%)	139 (1.43%)

Arm/Hand Deficit (n,%)

Yes	4,155 (85.53%)	4,157 (85.53%)	8,312 (85.53%)
No	675 (13.89%)	666 (13.70%)	1,341 (13.80%)
Can't Assess	28 (0.58%)	37 (0.76%)	65 (0.67%)

Leg/Foot Deficit (n,%)

Yes	3,639 (74.91%)	3,665 (75.41%)	7,304 (75.16%)
No	1,150 (23.67%)	1,133 (23.31%)	2,283 (23.49%)
Can't Assess	69 (1.42%)	62 (1.28%)	131 (1.35%)

Dysphasia (n,%)

Yes	2,156 (44.38%)	2,124 (43.70%)	4,280 (44.04%)
No	2,561 (52.72%)	2,577 (53.02%)	5,138 (52.87%)
Can't Assess	141 (2.90%)	159 (3.27%)	300 (3.09%)

Hemianopia (n,%)

Yes	792 (16.30%)	747 (15.37%)	1,539 (15.84%)
No	3,048 (62.74%)	3,120 (64.20%)	6,168 (63.47%)
Can't Assess	1,018 (20.96%)	993 (20.43%)	2,011 (20.69%)

Visuospatial Disorder (n,%)

Yes	836 (17.21%)	764 (15.72%)	1,600 (16.46%)
No	3,161 (65.07%)	3,214 (66.13%)	6,375 (65.60%)
Can't Assess	861 (17.72%)	882 (18.15%)	1,743 (17.94%)

Brainstem/Cerebellar Signs (n,%)

Yes	506 (10.42%)	546 (11.23%)	1,052 (10.83%)
No	3,944 (81.19%)	3,936 (80.99%)	7,880 (81.09%)
Can't Assess	408 (8.40%)	378 (7.78%)	786 (8.09%)

The IST study did not have randomization stratification factors and the baseline characteristics were found to be balanced between treatment groups so there was no need to adjust for any baseline covariates. However, for the purpose of this class, sex and age were chosen as covariates to adjust for.

An unadjusted logistic regression was used to test the null hypothesis that the parameter estimate for the association between treatment group and recurrent hemorrhagic stroke within 14 days of follow-up is equal to zero. The Wald chi-square statistic was 1.2681 with 1 degree of freedom and the resulting p-value was 0.2601. With a p-value greater than the α=0.05 significance level, the null hypothesis was not rejected. There is insufficient evidence to conclude that aspirin 300 mg and no heparin was more effective at reducing recurrent hemorrhagic stroke than the placebo. The parameter estimate for this association was 0.4364 (SE=0.3875, 95% CI: -0.3231, 1.1958). The odds ratio was 1.5471 (95% CI: 0.724, 3.306), meaning that someone who received aspirin 300 mg & no heparin had 1.5471 times the odds of a recurring hemorrhagic stroke than someone who received no aspirin & no heparin. The confidence interval for the log odds contains 0 and the confidence interval for the odds ratio contains 1, which indicates that the calculated effect sizes were not statistically significant.

Next, an adjusted logistic regression was used to test the null hypothesis that the parameter estimate for the association between treatment group and recurrent hemorrhagic stroke within 14 days of follow-up is equal to zero, adjusting for age and sex (using female as the reference group). The Wald chi-square statistic was 1.3097 with 1 degree of freedom and the resulting p-value was 0.2525. With a p-value greater than the α=0.05 significance level, the null hypothesis was not rejected. There is insufficient evidence to conclude that aspirin 300 mg & no heparin was more effective at reducing recurrent hemorrhagic stroke than the placebo, adjusting for age and sex.

Table 2: Adjusted Logistic Regression Results.

Effect	Parameter Estimate	Odds Ratio	95% Confidence Interval		p-value
Treatment	0.4436	1.5583	0.729	3.331	0.2525
Sex	0.1809	1.1983	0.554	2.590	0.6455
Age	-0.0282	0.9722	0.945	1.001	0.0549

For those of the same sex and age, subjects in the treatment group had 1.5583 (95% CI: 0.729, 3.331) times the odds of a recurring hemorrhagic stroke than those in the placebo group. For those who received the same treatment and were the same age, male subjects had 1.1983 (95% CI: 0.554, 2.590) times the odds of a recurring hemorrhagic stroke than female subjects. For those of the same sex and received the same treatment, subjects had 0.9722 (95% CI: 0.9445,

1.001) times the odds of a recurring hemorrhagic stroke than those who were 1 year younger. However, none of these associations were found to be statistically significant.

Lastly, an inverse probability weighted analysis was performed to remove the hypothetical confounding of age and sex. The z-score was 1.14 and the resulting p-value was 0.2538. With a p-value greater than the α=0.05 significance level, the null hypothesis was not rejected. There is insufficient evidence to conclude that aspirin 300 mg & no heparin was more effective at reducing recurrent hemorrhagic stroke than the placebo, adjusting for age and sex. The parameter estimate for this association was 0.4323 (SE=0.3875, 95% CI: -0.3173, 1.2018). The odds ratio was 1.5562 (95% CI: 0.7281, 3.3260), meaning that someone who received aspirin 300 mg & no heparin had 1.5562 times the odds of a recurring hemorrhagic stroke than someone who received no aspirin & no heparin. The confidence interval for the log odds contains 0 and the confidence interval for the odds ratio contains 1, which indicates that the calculated effect sizes were not statistically significant.

H_0: There is no difference in the proportion of hemorrhagic stroke between the intended treatment group (aspirin 300 mg and no heparin) and the placebo group (no aspirin & no heparin) after 14 days of follow-up, adjusting for age and sex.

H_A: There is no difference in the proportion of hemorrhagic stroke between the intended treatment group (aspirin 300 mg and no heparin) and the placebo group (no aspirin & no heparin) after 14 days of follow-up, adjusting for age and sex.

The three analyses performed gave very similar odds ratios comparing subjects in the treatment group to those in the placebo group. The unadjusted logistic regression analysis calculated an odds ratio of 1.5471 (95% CI: 0.724, 3.306), the adjusted logistic regression analysis calculated on odds ratio of 1.5583 (95% CI: 0.729, 3.331). The inverse probability weighted analysis gave an odds ratio of 1.5562 (95% CI: 0.7281, 3.3260). However, none of these associations were statistically significant. There is insufficient evidence to conclude that aspirin 300 mg & no heparin was more effective at reducing recurrent hemorrhagic stroke than the placebo treatment.

Since only 7 out of 9,718 participants were lost to follow-up and the missing subjects were approximately evenly divided between the two treatment groups, there was not much concern for bias due to missing data. The study period of interest was only the first 14 days after randomization and treatment was administered by healthcare professionals in a hospital setting, so adherence to the intended treatment was most likely extremely high.

Final diagnosis of initial hemorrhagic stroke, a binary yes/no variable, was suspected to be a potential effect modifier between the association of treatment type and recurrent hemorrhagic stroke.

H_0: $OR_{\text{no initial hemorrhagic stroke}} = OR_{\text{initial hemorrhagic stroke}}$

There is no interaction between treatment type and initial hemorrhagic stroke on recurrent hemorrhagic stroke. The OR for recurrent hemorrhagic stroke comparing treatment and placebo groups is **the same** for those with no initial hemorrhagic stroke and those with initial hemorrhagic stroke.

H_A: $OR_{\text{no initial hemorrhagic stroke}} \neq OR_{\text{initial hemorrhagic stroke}}$

There is interaction between treatment type and initial hemorrhagic stroke on recurrent hemorrhagic stroke. The OR for recurrent hemorrhagic stroke comparing treatment and placebo groups is **NOT the same** for those with no initial hemorrhagic stroke and those with initial hemorrhagic stroke.

No Initial Hemorrhagic Stroke	No Recurrent Hemorrhagic Stroke	Recurrent Hemorrhagic Stroke	Total
Treatment (Aspirin 300 mg & no heparin)	4,698 (99.83%)	8 (0.17%)	4,706
Placebo (no Aspirin 300 mg & no heparin)	4,693 (99.77%)	11 (0.23%)	4,704
Total	9,391	19	9,410

Initial Hemorrhagic Stroke	No Recurrent Hemorrhagic Stroke	Recurrent Hemorrhagic Stroke	Total
Treatment (Aspirin 300 mg & no heparin)	141 (97.92%)	3 (2.08%)	144
Placebo (no Aspirin 300 mg & no heparin)	139 (95.86%)	6 (4.14%)	145
Total	280	9	289

Two adjusted logistic regressions were performed for both strata of the potential effect modifier, final diagnosis of initial hemorrhagic stroke, adjusting for age and sex. The control treatment group (no aspirin & no heparin) was used as the reference group for treatment and females were used as the reference group for sex.

For the population with a final diagnosis of initial hemorrhagic stroke, the likelihood ratio test found that the overall model was not significant at the α=0.05 significance level (N = 289, χ^2 = 2.7398, df = 3, p = 0.4335). The parameter estimate for treatment was 0.6798 (SE = 0.7197, 95% CI: -0.7305, 2.0901). This association is not statistically significant, as the null value of 0 lies within the 95% confidence interval (p = 0.3448). The odds ratio was 1.9735 (95% CI: 0.482, 8.086), meaning that among those with a final diagnosis of initial hemorrhagic stroke, someone who received aspirin 300 mg & no heparin had 1.9735 times the odds of a recurring hemorrhagic stroke than someone who received no aspirin & no heparin. The confidence interval for the odds ratio contains 1, which indicates that the calculated effect size was not statistically significant.

For the population with a final diagnosis of no initial hemorrhagic stroke, the likelihood ratio test found that the overall model was not significant at the α=0.05 significance level (N = 9,410, χ^2 = 3.3886, df = 3, p = 0.2723). The parameter estimate for treatment was 0.3281 (SE = 0.4653, 95% CI: -0.5838, 1.2400). This association is not statistically significant, as the null value of 0 lies within the 95% confidence interval (p = 0.4807). The odds ratio was 1.3883 (95% CI: 0.482, 8.086), meaning that among those with a final diagnosis of no initial hemorrhagic stroke, someone who received aspirin 300 mg & no heparin had 1.3883 times the odds of a recurring hemorrhagic stroke than someone who received no aspirin & no heparin. The confidence interval for the odds ratio contains 1, which indicates that the calculated effect size was not statistically significant.

From the stratified logistic regressions, both adjusted odds ratios showed that subjects in the treatment group had a higher odd of a recurrent hemorrhagic stroke than those who received the placebo. There seems to be consistence in the effect size across both strata of final diagnosis of initial hemorrhagic stroke.

A multiple logistic regression analysis was used to test whether recurrent hemorrhagic stroke was associated with the interaction between treatment group and initial hemorrhagic stroke, adjusting for age and sex. The likelihood ratio test found that the overall model was significant at the α=0.05 significance level (N = 9,699, χ^2 = 35.8534, df = 5, p < 0.0001). From the Wald test for the interaction term, the F-statistic was 0.1767 and resulting p-value was 0.6742. With a p-value greater than the α=0.15 significance level, the null hypothesis of there being no association between treatment group and initial hemorrhagic stroke was not rejected. There is insufficient evidence to conclude that the effect of treatment on recurrent hemorrhagic stroke differs between those with and without an initial hemorrhagic stroke, adjusting for age and sex.

Final diagnosis of initial hemorrhagic stroke was not found to have an interaction effect between treatment group and recurrent hemorrhagic stroke. Further analysis should proceed using an adjusted multiple logistic regression model with no interaction term.

If effect modification was present, it would be quantitative because the direction of the effect size was same over both strata. The results for the outcome of interest do not have to be presented separately by stratum; an overall summary measure would be sufficient.

A potential mediator is pulmonary embolism (DPE), which is a categorical predictor coded as yes, no, or unknown. Pulmonary embolism was chosen as a potential mediator because it can eventually lead to a stroke of any kind. The hypothesis we will explore is whether aspirin 300 mg & no heparin has an effect on recurrent hemorrhagic stroke *through pulmonary embolism*. Perhaps aspirin 300 mg & no heparin reduces the risk of pulmonary embolism, thus reducing the risk of recurrent hemorrhagic stroke.

Figure I. DAG to display the relationship between treatment, mediator, and outcome.
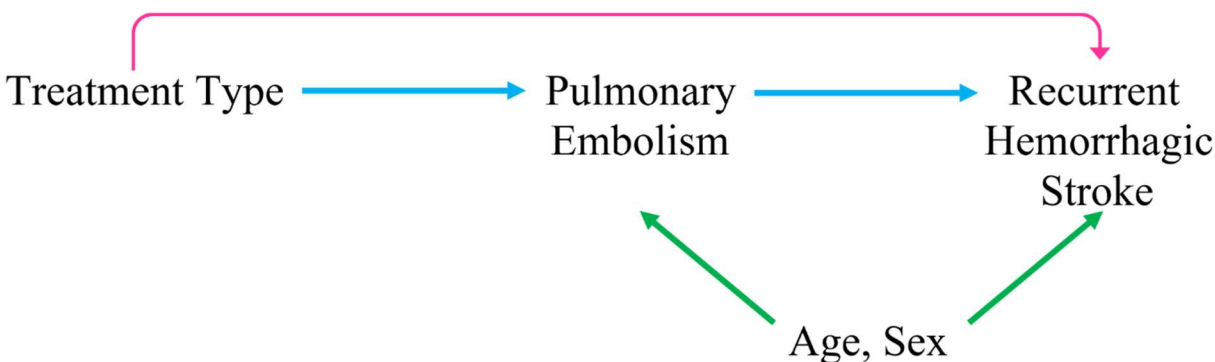
Due to a balance in baseline characteristics between the two treatment groups from randomization, there are no known potential treatment-mediator confounders. Potential mediator-outcome confounders include age and sex. A retrospective cohort study of medical records from 1966 to 1990 found incidence of pulmonary embolism increased with age for both sexes. Among patients younger than 55 years, females had a higher incidence of pulmonary embolism, while males had higher rates of pulmonary embolism among patients older than 55.[1]

The odds ratio for the association between treatment and recurrent hemorrhagic stroke is 1.5471, indicating that a subject assigned to receive the treatment (aspirin 300 mg & no heparin) had 1.5471 times the odds of a recurring hemorrhagic stroke compared to someone who received the control treatment (no aspirin & no heparin). However, as 95% confidence limits for this odds ratio (95% CI: 0.724, 3.306) contains 1, this association is not statistically significant.

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[1] Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998 Mar 23;158(6):585-93. doi: 10.1001/archinte.158.6.585. PMID: 9521222.

Table I. Causal mediation analysis results.

	Estimate	95% CI Lower	95% CI Upper	p-value
ADE (treated)	0.0013633	-0.0007973	0.00	0.23
ACME (treated)	-0.0000781	-0.0004260	0.00	0.25
Total Effect	0.0013131	-0.0009073	0.00	0.25
Prop. Mediated (treated)	-0.0211428	-0.6576451	0.23	0.45

The total effect of treatment on recurrent hemorrhagic stroke is 0.0013131 (95% CI: -0.0009073, 0.00).

The natural different effect was calculated to be 0.0013633 (95% CI: -0.0007973, 0.00). This is interpreted as the risk difference of recurrent hemorrhagic stroke between those treated with aspirin 300 mg & no heparin versus those treated with no aspirin & no heparin, with pulmonary embolism set to what it would have been under no heparin is 0.0013633 (95% CI: -0.0007973, 0.00).

The natural indirect effect was calculated to be -0.0000781 (95% CI: -0.0004260, 0.00). This can be interpreted as the excess risk of hemorrhagic stroke for those receiving aspirin 300 mg & no heparin due to varying pulmonary embolism from what it would have been under aspirin 300 mg & no heparin versus under no aspirin & no heparin is -0.0000781 (95% CI: -0.0004260, 0.00).

The proportion mediated is -0.0211428 (95% CI: -0.6576451, 0.23), but this is not interpretable as the indirect effect and total effect have different signs. Based on these findings, it cannot be concluded that the effect of aspirin 300 mg & no heparin on recurrent hemorrhagic stroke is mediated by pulmonary embolism.