Sex and Stroke in Thrombolyzed Patients and Controls

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Background and Purpose—We hypothesized that any sex-related difference in outcome poststroke is explained by other prognostic factors and that the response to intravenous recombinant tissue-type plasminogen activator (r-tPA) is equal in males and females after adjustment for such factors.

Methods—We accessed an independent collection of randomized clinical trials—the VISTA (Virtual International Stroke Trials Archive). Data were preprocessed by selecting complete cases (n=8028) and matching females to males (coarsened exact matching, n=4575, 24.3% r-tPA). Outcome was assessed by the 7-point modified Rankin Scale (mRS) measured at 90 days after ischemic stroke. Relationship among variables was estimated by adjusted regression analysis.

Results—In nonthrombolyzed patients, ordinal analysis of mRS adjusting for stroke- and sex-related prognostic factors suggested comparable outcomes for females and males (odds ratio, 0.96; 95% confidence interval, 0.85–1.06). Females responded comparably to r-tPA as did males, irrespective of the outcome definition of mRS (ordinal: $P_{\text{Interaction}}$=0.46, relative excess risk because of interaction=0). The number needed to treat was 6.8 and 11.2 for 1 female to achieve mRS score of 0 to 2 and 0 to 1, which was highly congruent with males. Analysis for a nonlinear variation of age-by-sex revealed a good outcome for females <45 years with significant disadvantage thereafter (mRS score of 0–2: $P_{\text{Interaction}}$=0.004). No relationship between sex, r-tPA, and bleeding complications was evident.

Conclusions—Functional outcome (mRS) without r-tPA was overall similar between the sexes, as was the response to r-tPA. Nonlinear sex-by-age interaction improved estimates of functional independence; this should be considered in sex-related studies in stroke. **(*Stroke*. 2017;48:367-374. DOI: 10.1161/STROKEAHA.116.014323.)**

Key Words: confidence intervals ■ regression analysis ■ risk ■ sex ■ stroke

See related article, p 250.

Human physiology differs significantly between females and males. In particular, the 2 sexes vary in regulating thrombosis, coagulation, and fibrinolysis.[1–8] Endogenous estrogen influences the activity of plasminogen activator inhibitor-1, the key player in fibrinolytic activity in both healthy[5] and pathological conditions.[6–8] It is unknown whether females respond differently to pharmacological agents that interact with coagulation and fibrinolysis.

Few stroke studies have investigated any sex-related effect on intravenous recombinant tissue-type plasminogen activator (r-tPA) in comparison to untreated controls.[9–11] An analysis of 5 clinical trials in acute ischemic stroke found that females experience a greater treatment effect, measured by differential attainment of modified Rankin Scale (mRS) score of 0 to 1 versus placebo and compared with males at 90 days.[9] In contrast, pooling those data with all other randomized controlled trial data presently available in an individual patient data meta-analysis, no sex-by-r-tPA treatment interaction was evident (*P* for heterogeneity <3 hours=0.95 and 3–4.5 hours=0.53).[11]

As for the natural course of an acute ischemic stroke, surprisingly few studies investigated outcome differences between the sexes. Among those, a minority used the widely accepted mRS at day 90 or later as outcome measure[12–18] emphasizing the need for data on functional outcome on this scale analyzed by sex. However, several aspects should be considered because they may lead to sex-specific differences in observational studies and clinical trials.

Some important risk factors in stroke are more prevalent in females than males—for example, diabetes mellitus,[19,20] atrial fibrillation,[21–23] and arterial hypertension.[24] All of these are known to influence the pathophysiology and functional outcome of stroke.[25] In addition, age-dependent and life-phase–dependent stroke risk for females differs considerably from males.[26] Below the age of 85 years, ischemic stroke strikes

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fewer females than males, but this tendency is reversed at higher age.[27–29] Specific hormonal states (eg, postmenopausal[30,31]) also lead to elevated stroke risk in females. Age—a proven risk factor for stroke—is the best surrogate marker to take into account these risk differences because they are likely to impact outcome; nonconsideration might lead to bias.

We aimed to investigate sex-specific differences in poststroke outcome in individual patient-level data pooled from randomized controlled trials. Using novel matching techniques, we approximated a randomized experiment accounting for covariates that differ between sexes. We evaluated 2 hypotheses allowing for potential effects in different age groups to emerge. First, we investigated whether the natural course of stroke is different between males and females without r-tPA treatment, after adjustment for relevant prognostic factors. Second, using a similar adjustment for relevant prognostic factors, we investigated whether the response to r-tPA was different between males and females.

Methods

Data Source and Selection

Data were extracted from the VISTA (Virtual International Stroke Trials Archive). We collected demographics, clinical data, and outcome measures from trials in acute ischemic stroke conducted from 1998 to 2008. Within these, many patients had received intravenous thrombolysis as standard of care.[32] Our analysis did not require new ethical approval. Trials that are pooled within VISTA follow the Declaration of Helsinki and were approved by local authorities.

Preprocessing

To achieve multivariate balance in the distribution of covariates between the 2 sexes (female and male) and to simulate a randomized trial, coarsened exact matching was performed independent of outcome. Importantly, unobserved variables are not accounted for, which clearly differentiate this method from a prospective randomization process. Coarsened exact matching differs conceptually from other matching methods such as propensity score matching.[33,34] Coarsened exact matching aims at reducing the degree of model dependence. It also attempts to reduce bias in the estimation of the outcome.[35] The method has been described in detail elsewhere,[33,34] but a short summary can be found in the Methods in the online-only Data Supplement.

Variable Selection

We based our variable selection for the matching process on sex-specific and general pathophysiological considerations of acute ischemic stroke. We used age,[26,36] baseline National Institutes of Health Stroke Scale (NIHSS),[22] hemisphere indicating the localization of the stroke lesion,[37] and history of diabetes mellitus,[19,20,38] atrial fibrillation,[22,23] myocardial infarction,[39] and hypertension.[40] Age and baseline NIHSS strata were determined based on equality of frequencies in each stratum. We analyzed patients with a time from onset of stroke symptoms to randomization of <7 hours.

Statistical Analysis

Continuous variables are described as median and interquartile range, and categorical variables as count and percentages. For univariate group comparisons, the Student *t* Test, the Mann–Whitney *U* Test, or the Fisher exact test was used as appropriate.

The primary outcome measure was the mRS at 90 days analyzed using ordinal logistic regression.[41] Dichotomized outcomes (mRS score of 0–1 for favorable outcome and mRS score of 0–2 for good outcome) were considered secondary outcomes and calculated by logistic regression analysis. Model fits were examined by inspecting residual plots, calibration measures (Akaike information criterion), and the Hosmer and Lemeshow goodness of fit test. We report common odds ratios (OR) and 95% confidence intervals (CI). The number needed to treat (NNT) was calculated by the adjusted risk difference method.[42] For survival analysis, a cox proportional hazard model was estimated. We report hazard ratio (HR) and 95% CIs in addition to the visualized Kaplan–Meier estimate. About bleeding complications, the following definitions were analyzed: (1) any hemorrhage, (2) any serious or fatal hemorrhage, (3) symptomatic intracranial hemorrhage after NINDS (National Institute of Neurological Disorders and Stroke) and ECASS (European Cooperative Acute Stroke Study)-II definition (for more details please visit the online-only Data Supplement).

For nonlinearity of most influential variables of age and NIHSS, restricted cubic splines were used if the model including splines showed benefit over the old model by means of a likelihood ratio test. We allowed for additive (4 factor variable,[43] relative excess risk because of interaction [RERI][44]) and multiplicative interactions (likelihood ratio test) between sex and r-tPA status and between age and sex. RERI is a metric of additivity of effects on a relative risk scale indicating the public health importance of interactions. RERI 95% CIs that cross zero indicate nonsignificance of additive interaction effects.

We present the matched analysis in the main part of the article, and the unmatched and sensitivity analysis in the online-only Data Supplement of the article. For sensitivity analysis, we investigated subgroups that are known to differ between sexes, namely atrial fibrillation and diabetes mellitus. VISTA includes data from the Glycine Antagonist in Neuroprotection trials,[45,46] which excluded patients with early neurological recovery. Acknowledging this as a possible source of bias, we also reran the analysis excluding those data.

Statistical analysis was performed using R[47–49] and the statistical package for the social sciences (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, NY).

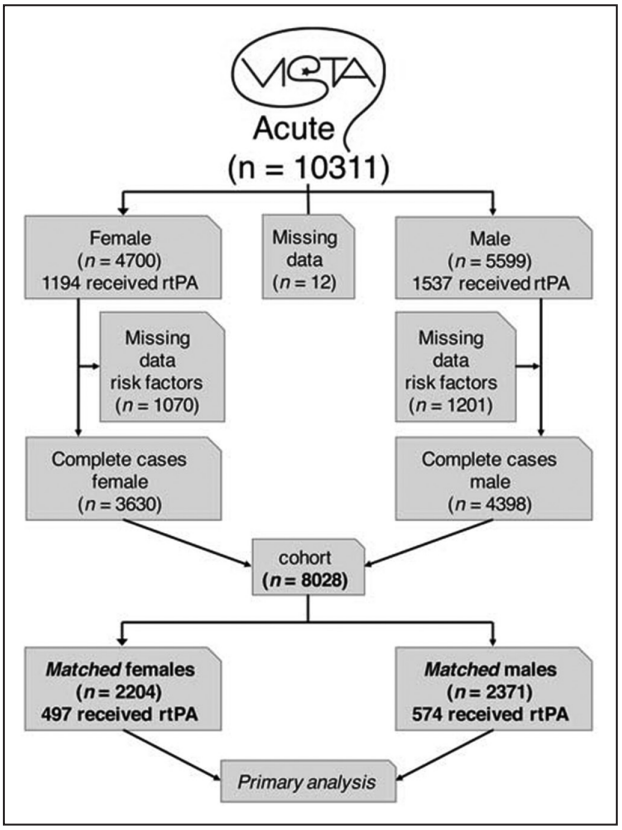

Figure 1. Study flow chart. r-tPA indicates recombinant tissue-type plasminogen activator; and VISTA, Virtual International Stroke Trials Archive.

Table 1. Baseline Characteristics Before and After Matching

	Entire Cohort			Matched		
	Female, n=3630	Male, n=4398	*P* Value	Female, n=2204	Male, n=2371	*P* Value
Covariates, median (interquartile range)						
Age, y	74 (65–80)	70 (60–77)	<0.001	70 (59–77)	70 (59–77)	0.696
Onset to time of randomization, h	4 (3.4–5)	4 (3.3–5)	0.044	4.1 (3.4–5)	4 (3.3–5.1)	0.676
Stroke severity at baseline, NIHSS	13 (8–18)	12 (8–17)	<0.001	11 (8–16)	11 (8–16)	0.874
Body mass index	26.1 (23.3–29.4)	26.1 (24–29.1)	0.757	26.4 (23.4–29.6)	26.1 (24–29)	0.276
Risk factors, n (%)						
History of hypertension	2772 (76.4)	2980 (67.8)	<0.001	1672 (75.9)	1799 (75.9)	>0.999
History of diabetes mellitus	815 (22.5)	1027 (23.4)	0.354	323 (14.7)	347 (14.6)	>0.999
History of atrial fibrillation	1149 (31.7)	1038 (23.6)	<0.001	414 (18.8)	445 (18.8)	>0.999
History of myocardial infarction	395 (10.9)	818 (18.6)	<0.001	113 (5.1)	122 (5.1)	>0.999
Treatment status, n (%)						
Recombinant tissue-type plasminogen activator	1035 (28.5)	1368 (31.1)	0.012	534 (24.2)	574 (24.2)	>0.999

NIHSS indicates National Institutes of Health stroke scale.

A study flow chart gives the reader an overview of the study work flow of data selection of complete cases, quasirandomization, and analysis (Figure 1; detailed methods of statistical analysis are available in the online-only Data Supplement).

Results

The complete cases data set contained 8028 patients (Figure 1), 2403 (29.9%) of whom received intravenous thrombolysis treatment (r-tPA). After preprocessing, the matched cohort comprised 4575 patients with 2204 (48.2%) females and equal proportion of thrombolyzed patients among females and males (24.2% r-tPA, respectively).

Age distribution (median 70 years; interquartile range 60–78) between the sexes was characteristically shifted as seen in population-based samples, with more younger males between the age of 45 and 70 years and more older females between the age of 75 and 95 years (Figure I in the online-only Data Supplement). Matched subjects showed substantial improvement in age distribution overlap (Figure I in the online-only Data Supplement). Median NIHSS score was 12 (interquartile range 8–17) in the whole cohort and 11 (interquartile range 8–16) in the matched cohort. Baseline characteristics by groups of sex before and after matching are presented (Table 1).

The outcome distribution of mRS at day 90 for the entire cohort (Figure 2A) and for the matched cohort (Table 2) with strata of sex and r-tPA status is shown.

Females and Males Without r-tPA (Natural Course)

In patients who did not receive intravenous thrombolysis (n=3504), ordinal analysis of mRS adjusting for stroke- and sex-related prognostic factors (age, NIHSS, onset of stroke to time of randomization, body mass index, and risk factors [hypertension, diabetes mellitus, smoking, atrial fibrillation, and myocardial infarction]) suggested comparable results for females (n=1707) versus males (n=1797; OR, 0.93; 95% CI, 0.83–1.06). Dichotomized measures were also comparable between the sexes (favorable outcome: OR, 1.03; 95% CI, 0.88–1.22 and good outcome: OR, 0.93; 95% CI, 0.79–1.09).

Effect Measure of r-tPA by Sex

Ordinal Measure

Cochran–Mantel–Haenszel *P* value for the crude analysis (*P*=0.0268) suggested a sex-specific difference in favor of males in the response to r-tPA (OR, 0.895; 95% CI, 0.813–0.986). However, after preprocessing (matching) and adjusting for confounders of age, NIHSS, onset of stroke to time of randomization, body mass index, stroke localization, and risk factors (hypertension, diabetes, smoking, atrial fibrillation, and myocardial infarction) in regression analysis, no significant effect modification of sex on r-tPA was found ($P_{Interaction}$=0.46, RERI=0; Table 2; Table I in the online-only Data Supplement). Various levels of adjustment did not alter this finding (data not shown). Predicted probabilities by each response category of the mRS for treatment groups within sex strata are shown (Figure 2B).

Favorable and Good Functional Stroke Outcome

The adjusted NNT for 1 female to achieve favorable and good functional outcome at 90 days after stroke were 11.2 and 6.8, respectively; these were similar to the NNTs for males (Table 2). Consequently, no significant sex-by-r-tPA interaction was found—neither for favorable ($P_{Interaction}$=0.185, RERI=–0.06) nor for good functional outcome ($P_{Interaction}$=0.792, RERI=–0.13; Table 2).

Mortality Within 90 Days After Stroke

In the matched cox regression, mortality in females was significantly lower than in males (female: HR, 0.82; 95% CI, 0.71–0.95; Figure 3). Adjusting for r-tPA (HR, 0.88; 95% CI, 0.74–1.05), age (HR, 1.05; 95% CI, 1.04–1.06), and baseline NIHSS (HR, 1.13; 95% CI, 1.12–1.14) did not noticeably change the influence of sex (female: HR, 0.81; 95% CI, 0.7–0.94). Further adjustment with all risk factors

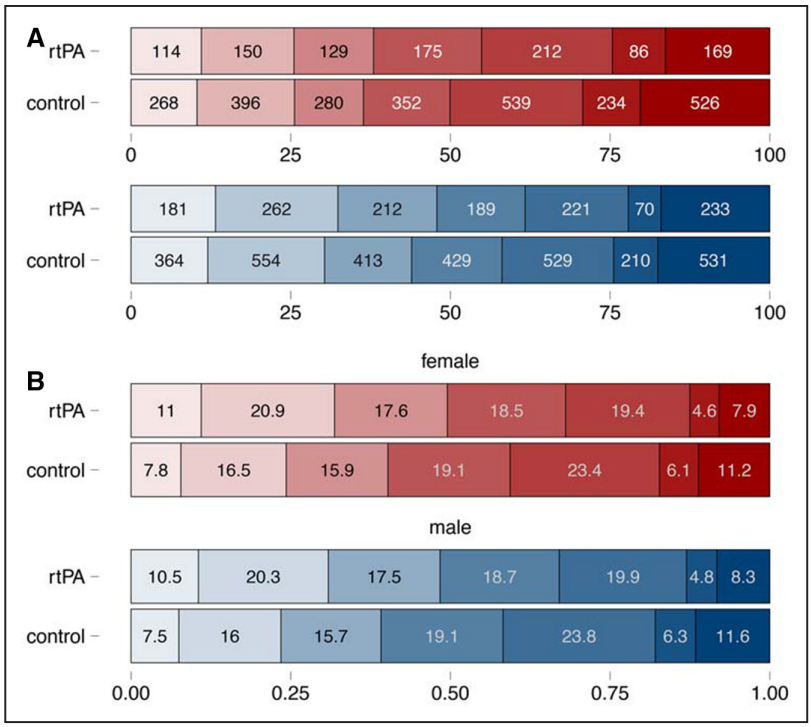

Figure 2. Outcome distributions of 7-point scale of the modified Rankin Scale for females (red) and males (blue) in their respective treatment status are given; (**A**) numbers are absolute numbers of the entire cohort (not matched, n=8028). **B**, Numbers are predicted probabilities from the full proportional odds model of the matched cohort (n=4575). r-tPA indicates recombinant tissue-type plasminogen activator.

did not change the OR (data not shown). We observed no sex-by-r-tPA interaction in the matched adjusted analysis ($P_{\text{Interaction}}$=0.781).

Bleeding Complications

Overall, we found no evidence of a sex-by-r-tPA interaction when analyzing any hemorrhage, any serious or fatal hemorrhage, and symptomatic intracranial hemorrhage following definitions of NINDS and ECASS-II (all P>0.05).

Risk Modification of Age-by-Sex

Estimating good outcome as a function of age using splines with 4 *df* showed significant interaction with sex (Figure 4; age $P_{\text{Interaction}}$=0.0042, RERI=−7.8 [95% bias corrected and accelerated (BCa) CI −44.5, −0.66]; age′ $P_{\text{Interaction}}$=0.0009, RERI=−1.5 [95% BCa CI −9.58, −0.02]; and age″ $P_{\text{Interaction}}$=0.0018, RERI=4.07 [95% BCa CI −0.812, 36.415]). Other outcome definitions showed no effect modification of such kind.

Discussion

Our sex-balanced analysis suggests similar outcomes between the sexes in their natural course of the disease as measured by mRS at day 90 after surviving a stroke. We provide evidence from independent clinical trial data that females respond to recombinant intravenous plasminogen activator similarly to males. Furthermore, we demonstrate relevant outcome variation of age-by-sex emphasizing improved outcome estimates when considering age in a nonlinear manner.

Sex-Specific Natural Course as Measured by mRS at Day 90

In the pooled analysis of 5 randomized controlled ischemic stroke trials,[9] the female control group fared worse than the male control group. Females were under-represented, because of recruitment rates below 43 and as low as 32%. This may be explained by a higher threshold for treating younger females with child-bearing potential, (including the exclusion of these females unless they were on reliable contraception) or by a lower event rate of ischemic stroke in females below the age of 85.[27,29] However, this imbalance weights the analysis of trial data in favor of males.

We reviewed 7 observational studies that reported sex differences using mRS at day 90 or later (for a detailed overview see Table II in the online-only Data Supplement). Investigating predominantly mild strokes, moderate-to-severe strokes were under-represented in those studies.[12–18] Two study cohorts that featured a slightly higher median baseline NIHSS (6[17] and 9.4[13]) reported equipoise between the sexes in functional outcome when adjusting for age and stroke severity. Earlier work investigated sex difference in functional outcome measuring Stroke Impact Scale-16,[50] general dependency,[51] physical disability using modified Katz activities of daily living,[29] quality of life measures,[52] or the Barthel Index.[53] However, these measures may not be directly comparable to the mRS at day 90 or 180 after stroke.[54]

To this body of knowledge, our present study adds a less biased view, being from balanced sex cohorts including a representative stroke severity range and analyzed by means of mRS at day 90. Females and males in ordinal and dichotomized outcomes (excellent outcome mRS score of 0–1 and good outcome mRS score of 0–2) yielded similar estimates. Effect modification of age-by-sex gives us insights into how various female cohorts might represent different outcome risks—showing favor or disfavor when compared with males.

As for mortality, our study complements previously published data.[51,55] For example, results from the Centers for Disease

Table 2. Outcome Measured Using mRS at Day 90 After Stroke in Control Patients and Patients Who Received r-tPA

Outcome Category	Sex	r-tPA	No. of Matched Patients mRS Category							Outcome Measures			Interaction	
			0	1	2	3	4	5	6	Adjusted Odds Ratio (95% CI)	Adjusted Risk Difference r-tPA within sex	Adjusted Number Needed to Treat	RERI*	*P* Value†
Ordinal													0 (−0.149 to 0.128)	0.46
	Male	No	229	353	247	253	306	104	305	1 (Referent)	…	…		
	Male	Yes	82	117	85	77	95	25	93	0.61 (0.51 to 0.73)	…	…		
	Female	No	194	287	195	239	344	151	297	0.93 (0.82 to 1.05)	…	…		
	Female	Yes	62	86	69	76	96	29	79	0.63 (0.53 to 0.75)	…	…		
mRS score of 0–1													−0.06 (−1.018 to 2.404)	0.187
	Male	No	582		1215					1 (Referent)	9% (7.6 to 10.4)	11.1 (9.6 to 13.2)		
	Male	Yes	199		375					1.73 (1.37 to 2.20)				
	Female	No	481		1226					1.04 (0.88 to 1.23)	8.9% (7.5 to 10.3)	11.2 (9.7 to 13.2)		
	Female	Yes	148		349					1.45 (1.13 to 1.86)				
mRS score of 0–2													−0.13 (−0.736 to 0.472)	0.794
	Male	No	829			968				1 (Referent)	14.8% (13.5 to 15.7)	6.7 (6.4 to 7.4)		
	Male	Yes	284			290				1.76 (1.39 to 2.23)				
	Female	No	676			1031				0.97 (0.82 to 1.15)	14.6% (13.3 to 15.6)	6.8 (6.4 to 7.5)		
	Female	Yes	217			280				1.45 (1.12 to 1.88)				

Analyzed are groups of sex (female and male) demonstrating absent effect modification of r-tPA by sex: adjusted for age, baseline National Institutes of Health Stroke Scale, localization of stroke, history of diabetes mellitus, history of atrial fibrillation, history of arterial hypertension, history of myocardial infarction, history of smoking, body mass index, and time from symptom onset to randomization. CI indicates confidence interval; mRS, modified Rankin Scale; and r-tPA, recombinant tissue-type plasminogen activator.

*Measures of interaction on additive scale: relative excess risk because of interaction (RERI)—a value of zero means no interaction.

†Measures of interaction on multiplicative scale: likelihood ratio test.

Control and Prevention WONDER (Wide-ranging Online Data for Epidemiological Research) database highlighted that middle-aged women died less often than men, whereas this finding was reversed in women older than 85.[55] In our study, the median age of women was 70 (interquartile range 59–77), and mortality was significantly lower in women than men supporting the hypothesis of a mean survival benefit in middle-aged women. Noteworthy, our study is the first to report on highly balanced mortality data that take account of differences in age, stroke severity, and sex-specific risk factors for stroke.

Sex-Specific Response to r-tPA

Our results are in line with the results provided by Emberson et al[11] in the individual patients data meta-analysis reporting no sex-by-r-tPA-treatment interaction in good stroke outcome (mRS score of 0–1, *P* for heterogeneity <3 hours=0.95 and 3–4.5 hours=0.53). An earlier analysis of 5 randomized controlled ischemic stroke trials[9] had found females who received r-tPA to achieve equal outcomes (as defined by mRS score of 0–1) when compared with males who received r-tPA. Because the female control group fared worse in their analysis, the authors inferred that women benefited more than men from intravenous r-tPA treatment. However, in our study, we were not able to reject the null hypothesis of a common treatment effect of r-tPA between the sexes.

Our study has several strengths. Estimates derived from this sex-balanced cohort compare well with results from the pooled analysis.[56] Although matching lowered the

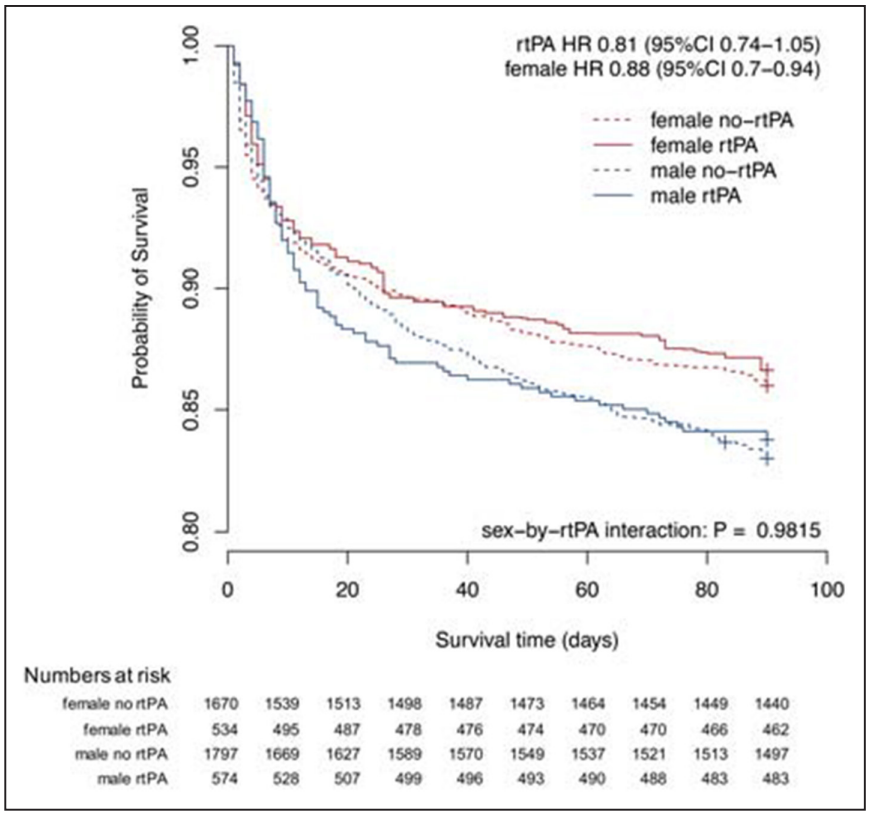

Figure 3. Kaplan–Meier estimates by groups of sex within treatment strata in matched cohort. (Vertical scale was adjusted for visualization and does not extend to zero.) CI indicates confidence interval; HR, hazard ratio; and r-tPA, recombinant tissue-type plasminogen activator.

mean age (68.7±12.9 versus 71±13), stroke severity (12±7 versus 12.7±5.7) and the NNT were largely unchanged (adjusted NNT for mRS score of 0–1: 11 versus NNT 12.6 in the pooled analysis). Our study features sex-specific outcome results measured by mRS at day 90 after the natural course of ischemic stroke disease in a well-balanced cohort adjusted for known prognostic sex-specific and general factors of stroke. Furthermore, it is the first study to evaluate the sex-specific response to r-tPA treatment in an independent, balanced trial cohort. Demonstrating age-by-sex interaction in outcome estimation, it offers valuable insight into how heterogeneous cohorts can be depending on a sex-specific age distribution.

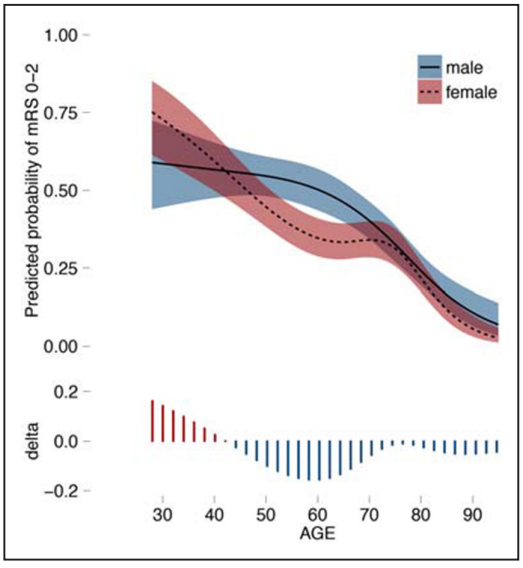

Figure 4. Upper, Probability of reaching good outcome (modified Rankin Scale [mRS] score of 0–2) as a function of age-by-sex strata in the matched cohort. **Lower**, Corresponding delta values.

Our study has limitations. The study sample is not population based, rather it was derived from clinical trial cohorts. However, the study sample shows a typical sex-specific age distribution as seen in population samples.[29] The NIHSS distribution is linked to the selection process in trials, which on the other hand improves comparability to the same. Results should not be generalized before they have been replicated in a population-based sample. No pregnant women were in the trials that are pooled within VISTA—therefore, these results are not generalizable to pregnant women, and there are age-specific changes in selection among females that do not apply to males. In this study, we did not have information about hormonal status of VISTA trial patients. Thus, the study was not intended to investigate for patients who were taking hormone replacement therapy. This may be a potential bias because female stroke patients receiving hormone replacement therapy are at increased risk for stroke.[57]

We sought to remove bias and adjust for potential confounders. Nevertheless, this study is retrospective. Sources of bias may be the unknown number of patients experiencing posterior circulation stroke, the selection of complete cases, and the selection of matching parameters. We did not adjust for potential confounders of admission blood pressure and stroke subtype. Our selection was based on pathophysiological considerations of stroke in general and especially depending on sex in variables that are well-established confounders in stroke outcome estimation.

Conclusions

This study considered sex-specific age and risk factor issues, optimized for comparability between sex cohorts, and compares well to the cohort of the pooled analysis of ischemic stroke trials.

Females in our control group had similar outcomes to males of the control group. As for the sex-specific response to r-tPA, we could not reject the null hypothesis of a common treatment effect of r-tPA between the sexes. We could not find evidence for a meaningful relationship between sex, r-tPA, and bleeding complications. Finally, we found that consideration of a nonlinear sex-by-age interaction significantly improved estimates of outcome—this may be important to be considered in future analyses of sex of data on stroke patients.

Appendix

VISTA-Acute Steering Committee

K.R. Lees (Chair), A. Alexandrov, P.M. Bath, E. Bluhmki, N. Bornstein, C. Chen, L. Claesson, S.M. Davis, G. Donnan, H.C. Diener, M. Fisher, M. Ginsberg, B. Gregson, J. Grotta, W. Hacke, M.G. Hennerici, M. Hommel, M. Kaste, P. Lyden, J. Marler, K. Muir, N. Venketasubramanian, R. Sacco, A. Shuaib, P. Teal, N.G. Wahlgren, S. Warach, C. Weimar.

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Disclosures

Dr Ringleb reports speaker fees and expenses from Boehringer Ingelheim <10 000€/2 years. Dr Lees reports fees and expenses from Boehringer Ingelheim for participation on data monitoring committees and speaker fees. The other authors report no conflicts.

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