Sex Differences in Influenza: The Challenge Study Experience

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BACKGROUND

- Sex-linked differences in influenza disease are not well understood but have been observed in animal and human studies.
- Female mice demonstrate more symptoms than male mice during influenza infection.[1,2]
- Female humans of reproductive age have higher rates of influenza and influenza-associated hospitalizations than male humans.[3,4]
- Retrospective studies in humans suffer from confounding factors.
- Influenza challenge studies provide an opportunity to study sex differences in a homogenous group of participants under controlled conditions.

OBJECTIVES

- To determine if there are any differences between female and male participants in influenza challenge studies with respect to symptoms and shedding.
- To correlate any observed differences in symptoms and shedding with hemagglutination inhibiting (HAI) and neuraminidase inhibiting (NAI) antibody levels as well as estradiol and testosterone levels.

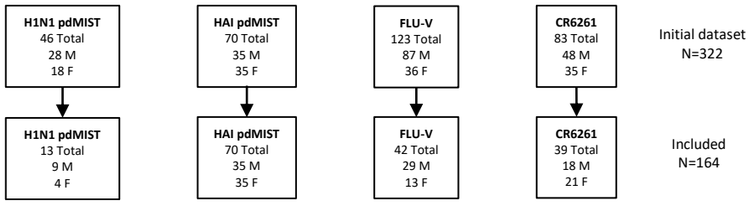
METHODS

- Data from 4 previous H1N1 influenza challenge studies (H1N1 pdMIST,[5] HAI pdMIST,[6] FLU-V,[7] CR6261[8]) were aggregated.
- Participants were included for analysis if they received a dose of challenge virus of 10^7 tissue culture infectious dose 50 ($TCID_{50}$).
- Participants were excluded if they received any experimental treatment or vaccine or if they were found to be coinfected with another respiratory virus.

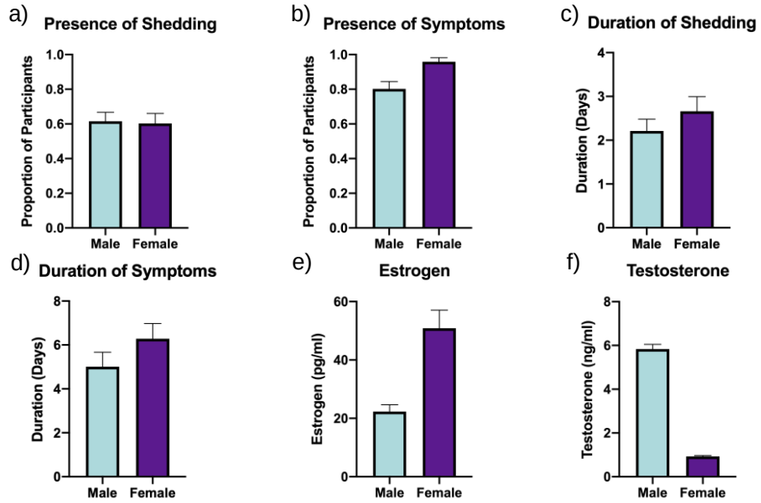
Table 1: Challenge study datasets and selection criteria applied

Study	Study Description	Data Selection
H1N1 pdMIST	Influenza dose escalation challenge study	Only participants who received influenza challenge at 10^7 $TCID_{50}$ included
HAI pdMIST	Influenza challenge comparing baseline low and high HAI titers	All participants were included
FLU-V	Phase 2 placebo-controlled study assessing efficacy of a novel vaccine through influenza challenge	Only participants who received placebo were included
CR6261	Phase 2 placebo-controlled study assessing efficacy of a monoclonal antibody through influenza challenge	Only participants who received placebo were included

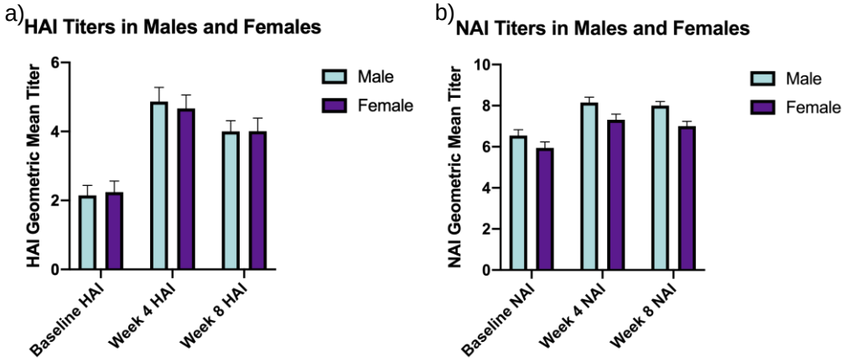
Figure 1: Flow diagram of datasets used for analysis



RESULTS



Figures 2: a,b) Females shed virus at similar frequency to males, but were more likely to have symptoms. c,d) Duration of shedding and symptoms were not statistically different in females compared to males. e,f) Estrogen levels were higher in females and testosterone levels were higher in males. Bar graphs represent proportions in a,b and means in c,d,e,f. Error bars represent standard errors.



Figures 3: a) HAI titers increased from baseline after influenza challenge and declined from week 4 to week 8. HAI titers were not different between sexes at baseline, week 4, or week 8. b) NAI titers increased from baseline after influenza challenge and plateaued from week 4 to week 8. NAI titers were higher in males than females at week 4 and week 8 after influenza challenge but were not different between sexes at baseline. Bar graphs represent mean geometric titers and error bars represent standard errors.

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Table 2: Statistical analysis demonstrating difference in testosterone, estradiol, week 4/8 NAI titers, presence of symptoms, days of symptoms, and number of symptoms between male and female participants

	Male (n=91)	Female (n=73)	p-value
Age (years)	29.3	29.8	0.50
Testosterone (ng/ml)	5.84	0.93	**$2.2*10^{-16}$**
Estradiol (pg/ml)	22.3	50.9	**$6.3*10^{-6}$**
Pre-Challenge HAI Titer	2.22	2.27	0.86
Week 4 HAI Titer	4.86	4.66	0.83
Week 8 HAI Titer	3.93	4.03	0.73
Pre-Challenge NAI Titer	6.55	5.94	0.13
Week 4 NAI Titer	8.15	7.32	**0.047**
Week 8 NAI Titer	8.03	7.39	**0.044**
Presence of Symptoms	0.80	0.96	**0.0028**
Presence of Shedding	0.62	0.60	0.87
Duration of Symptoms (days)	5.18	6.37	0.064
Number of symptoms	3.45	4.95	**0.011**
Duration of Shedding (days)	2.29	2.70	0.47

Mean values shown for each variable by sex. P-values calculated using Wilcoxon rank-sum test and test of proportions. Antibody titers reflect geometric mean titers.

Table 3: Outcomes of interest and significant predictor variables obtained using linear and logistic regression models

Outcome of Interest	Significant Predictor Variables	p-value
Presence of Symptoms	Pre-Challenge NAI Titer	**0.0415**
Presence of Shedding	Pre-Challenge NAI Titer	**$9.51*10^{-5}$**
Days of Symptoms	Pre-Challenge NAI Titer	**0.0132**
Days of Shedding	Pre-Challenge NAI Titer	**$9.66*10^{-10}$**
Number of symptoms	Pre-Challenge NAI Titer	**$7.48*10^{-7}$**

CONCLUSIONS

- Females in our challenge studies were more likely to have symptoms and had a higher number of symptoms compared to males while their NAI titers were lower at 4 and 8 weeks after influenza challenge.
- Pre-challenge HAI titer, testosterone level, and estradiol level were not predictive of outcomes of interest.
- The differences between sexes in this study were related to differences in pre-challenge NAI titers, which predicted multiple outcomes of interest: presence of shedding, presence of symptoms, days of shedding, days of symptoms, and number of symptoms.

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