



Etiology Unknown: Are Less Recognized Respiratory Pathogens Associated with a Milder Course of Illness?

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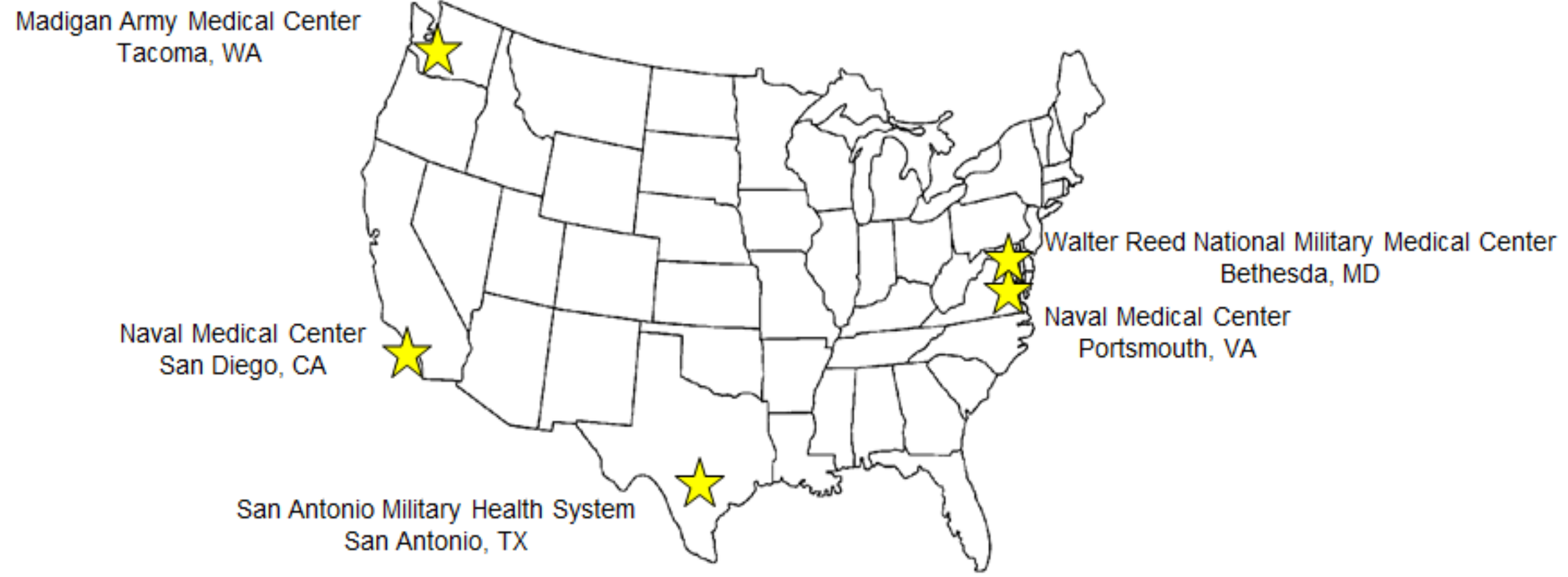
Background

- The use of multiplex diagnostic platforms has greatly improved rates of etiologic determination among patients with influenza-like illness (ILI).
- A viral respiratory pathogen was identified in >60% of otherwise healthy individuals enrolled in our observational study of ILI. Herein we describe the characteristics and clinical severity of illness among those for whom a viral pathogen was not identified.

Methods

- Since 2009, we enrolled otherwise healthy military personnel and beneficiaries into an observational, longitudinal study of influenza-like illness (ILI) at five military treatment facilities across the continental United States (Figure 1).

Figure 1. Clinical sites participating in the ARIC Natural History Study



- Eligibility.** Patients presenting for care <72h after the onset of ILI, defined as fever (temperature of 100.4° F or greater at the time of evaluation, or by self-report) and sore throat or one of the following respiratory symptoms: cough, sputum production, shortness of breath, or chest pain. Patients with underlying medical conditions were excluded.
- Clinical and demographic information,** and a nasopharyngeal swab was collected at baseline (day 0). Participants returned on days 3±1, 7±2 and 28±7; a daily symptom diary was completed for the first seven days following ILI onset. Symptom presence and severity was recorded either by self-report (diary) or interview as: 0 (none); 1 (mild: not changing activity or requiring treatment); 2 (moderate: requiring some modification in activity and/or medication); and 3 (severe: incapacitating, unable to perform normal activities, requiring bed rest and/or medication). Participants were trained by research personnel on the definitions of each score. Swabs were tested for influenza by real-time reverse transcription polymerase chain reaction (rtRT-PCR) at the Naval Health Research Center (San Diego, CA).
- A target-enriched multiplex PCR (TEM-PCR) panel for 13 bacterial and 10 viral respiratory pathogens** was developed by Diatherix Laboratories, LLC. (Huntsville, AL). The platform relies upon nested multiplex PCR to provide the initial target enrichment and super primers to amplify and label the PCR products:
 - Viral pathogens:** Adenovirus, coxsackievirus/echovirus, bocavirus, coronavirus, human metapneumovirus, rhinovirus, influenza A/B, parainfluenza and respiratory syncytial virus
 - Bacterial pathogens:** *Acinetobacter baumannii*, *Bordetella pertussis*, *Chlamydomphila pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, MRSA, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* (Group A)
- We measured presence of a symptom at any severity level (mild, moderate and severe); further stratified comparisons were based on moderate/severe versus none/mild, and severe versus mild/moderate/none. Composite measures were the sum of individual symptom scores in the following categories: (1) lower respiratory symptoms: cough, breathing difficulty, hoarseness and chest pain, (2) upper respiratory symptoms: earache, runny nose, sore throat and sneezing; (3) systemic symptoms: chills, muscle ache, headache and fatigue; (4) total symptoms: sum of the above three categories.
- Statistical analyses were performed using SAS (Version 9.3; SAS Institute, Cary, NC). The study was approved by the Infectious Disease Institutional Review Board of the Uniformed Services University of the Health Sciences (IDCRP-045).

Results

- From 2010-2014, a sample of 898 cases with ILI were tested for viral and bacterial respiratory pathogens using Diatherix TEM-PCR.
- A third of these cases [35.1% (315/898)] were negative for any of the targeted viral pathogens, while the remaining cases were positive for at least one viral etiological agent.
- Older age, year of enrollment and site was associated with negative detection of viral pathogens in the multivariable logistic regression model (Figure 2 and Table 1)

Figure 2. Distribution of number of viral pathogens detected among 898 ARIC patients using Diatherix TEM-PCR Respiratory Panel, by age

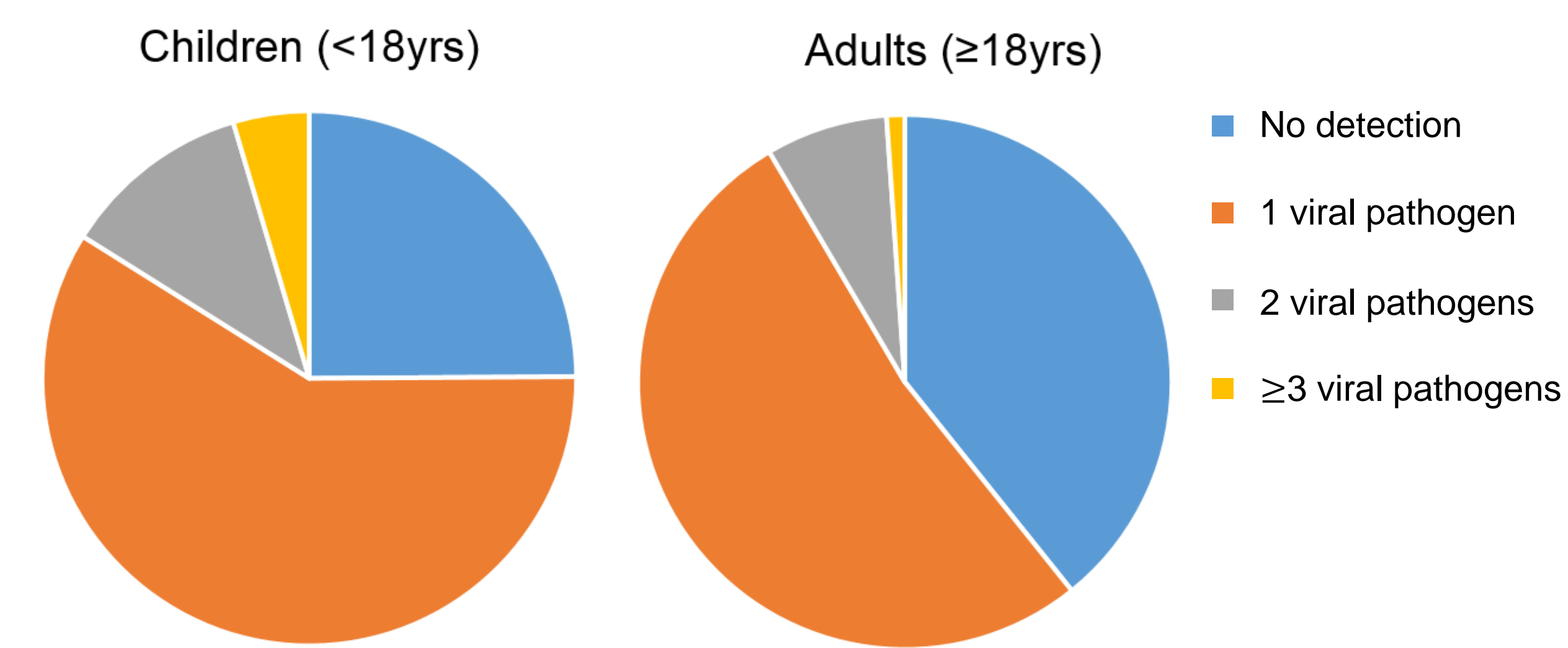
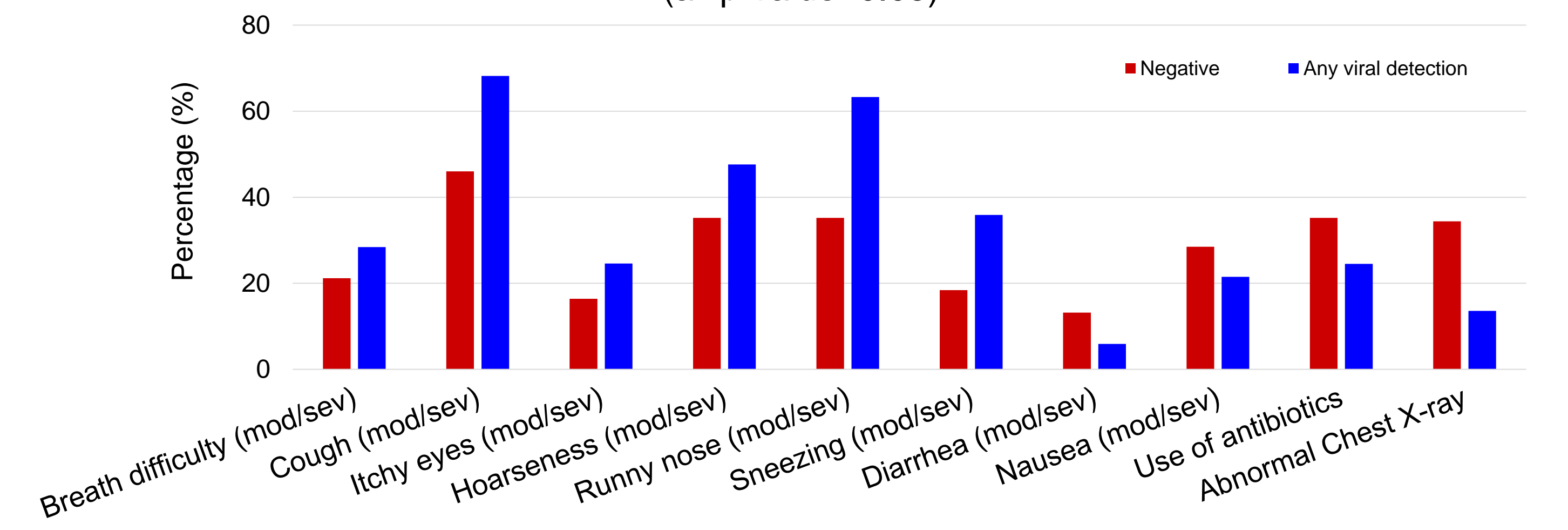


Table 1. Characteristics of 898 ILI patients tested by Diatherix TEM-PCR Respiratory Panel, by viral detection status

	Detection of viral respiratory pathogens		Total (n=898)	P-value
	Undetectable (n=315, 35.1%)	Detectable (n=583, 64.9%)		
	N (%)	N (%)		
Age				
Children	65 (24.9)	196 (75.1)	261	<0.01
Adults	250 (39.2)	387 (60.8)	637	
Sex				
Male	175 (35.4)	319 (64.6)	494	0.81
Female	140 (34.7)	264 (65.4)	404	
Site				
WRNMMC	19 (67.9)	9 (32.1)	28	
SAMMC	53 (36.3)	93 (63.7)	146	
NMCSA	99 (27.9)	256 (72.1)	355	
NMCP	108 (45.0)	132 (55.0)	240	<0.01
MAMC	36 (27.9)	93 (72.1)	129	
Influenza season				
2009-10	1 (100.0)	0 (0.0)	1	
2010-11	91 (33.8)	178 (66.2)	269	
2011-12	106 (40.6)	155 (59.4)	261	
2012-13	49 (30.2)	113 (69.8)	162	0.11
2013-14	68 (33.2)	137 (66.8)	205	
Any child under 5y in the household				
No	228 (37.3)	383 (62.7)	611	0.03
Yes	85 (30.0)	198 (70.0)	283	
Index attending daycare? (Children only)				
No	100 (35.8)	179 (64.2)	279	
Yes	17 (16.5)	86 (83.5)	103	<0.01

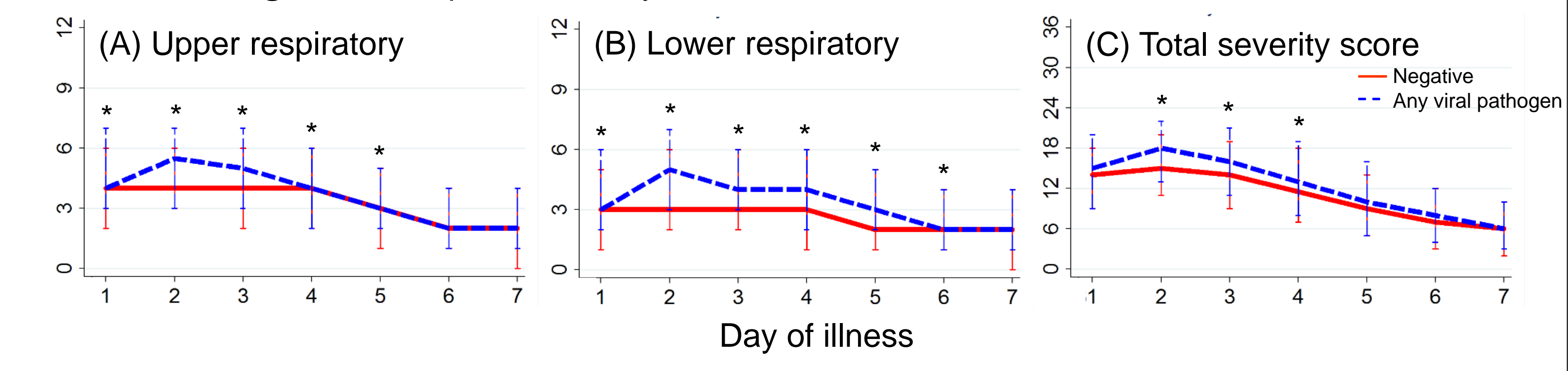
- Among adults, those with any detection of viral pathogens more frequently reported headache, hoarseness, itchy eyes, sneezing, runny nose, and cough, but less frequently diarrhea and nausea. They were less likely to use antibiotics and have abnormal chest X-ray results if chest X-ray was taken.

Figure 3. Clinical severity and outcomes between adults with and without viral detection (all p-value<0.05)



- The composite severity scores reported by adults with viral detection were significantly higher than pan-negative cases.

Figure 4. Composite severity scores between adults with and without viral detection



- Similarly, children with viral pathogen detection had higher severity of upper/lower respiratory symptoms.
- Among adults, those with no viral detection had higher detection of targeted bacterial pathogens compared to those with viral pathogens (p=0.004), while no such difference was observed among children (p=0.21).

Conclusions

- Cases with no detectable viral pathogens (pan-negative) tended to be older and had milder clinical symptoms. It is possible that patients with milder clinical symptoms had lower levels of viral shedding (i.e., below the lower limit of detection).
- Pan-negative adults had higher detection of bacterial respiratory pathogens. No such difference was found in children. The role of bacterial pathogens to ILI warrants further study and may affect management strategies for these patients.

Acknowledgements and Disclaimer

- Support for this work was provided by the Department of Defense Global Emerging Infections Surveillance (GEIS) program and Military Infectious Diseases Research Program (MIDRP). This project has been funded in whole, or in part, with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), under Inter-Agency Agreement [Y1-AI-5072] to Uniformed Services University of the Health Sciences.
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