EVALUATING THE SEVERITY OF ACUTE RESPIRATORY INFECTIONS:

Advantage of meditation over exercise; Viral and bacterial co-infection; and Reduction of Wisconsin Upper Respiratory Symptom Survey

By

Chidi N. Obasi

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The dissertation is approved by the following members of the Final Oral Committee:

Bruce Barrett, MD, PhD, Associate Professor, Family Medicine

Roger Brown, PhD, Professor, Nursing/Research Design and Statistics Unit

Christopher Coe, PhD, Professor, Psychology

Karen Cruickshanks, PhD, Professor, Population Health Sciences

James Gern, MD, Professor, Pediatrics

ABSTRACT

Acute respiratory infection (ARI) exerts a heavy financial burden on the US health care system and afflicts persons of all ages. Using the Wisconsin Upper Respiratory Symptom Survey (WURSS), a study on "Meditation or Exercise for Preventing Acute Respiratory Infection" (MEPARI) showed trainings in meditation or exercise can reduce illness outcomes compared to control, however, meditation fostered better reduction of global severity of illness than exercise. This thesis expands on this finding by evaluating the advantage of meditation over exercise and identifying specific WURSS items responsible for the difference. This work also broadens research on severity of ARI illness by evaluating frequency of bacterial co-infection and also demonstrating a shorter version of the WURSS used in assessing ARI unwellness.

Using data from n=149 adults who completed the MEPARI trial between 2009 and 2010, this dissertation showed meditators had improved quality of life compared to exercisers. Compared to WURSS items representing specific symptoms, items symbolizing function and quality of life were responsible for greater reduction in global severity of illness. Using data from n=194 randomly selected nasal wash specimens which were obtained from participants with ARI illness between 2004 and 2008, this thesis showed presence of pathogenic bacteria may be associated with increased inflammatory biomarkers. Compared to virus-positive illness, virus-negative illness may be associated with more pathogenic bacteria. Finally, this work combined WURSS-21 data from 4 separate trials including MEPARI and demonstrated a shorter and reliable WURSS-11 using factor

analysis. This new WURSS-11 showed similar dimensional structure as the older WURSS-21.

The results of this dissertation have significant public health implications. They support the use of meditation or exercise which may help reduce the burden of ARI illness. It also extends scientific knowledge on bacterial association during illness. Future research is not only needed to validate the WURSS-11 but to evaluate the frequency of nasal pathogenic bacteria in asymptomatic adults.

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DEDICATION

This work is dedicated to my ever loving family:

To the memory of my late father, Dr. N.K. Obasi who laid the foundation of love and education in his family;

To my mother, Elder (Dr) Mrs. M.N. Obasi, who sustained these values and instilled the principle of responsibility. She is my role model;

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CHAPTER 1. INTRODUCTION, SPECIFIC AIMS AND SIGNIFICANCE

1.1 Introduction

Acute respiratory infection (ARI) is a common malady that causes major morbidity and mortality in the US and elsewhere.[1, 2] It includes influenza and common cold, and has lower incidence in adults (2-4 episodes/year) compared to children (6-8 episodes/year).[3, 4] ARI exerts a heavy financial burden on the US health care system and accounts for significant work absenteeism and missed school days.[5, 6] A study on "Meditation or Exercise for Preventing Acute Respiratory Infection" (MEPARI study) has suggested that meditation may result in better reduction of global severity of illness compared to exercise.[7] Given the increased public health interest in these 2 behaviors and the burden of ARI illness on the society, evaluating the rationale for better reduction of global severity of illness from meditation compared to exercise may help improve individual health choices. Although the frequency of bacterial co-infection in adults with ARI illness is not clear[8], there are significant numbers of antibiotic prescriptions for uncomplicated illness by clinicians.[9] Examining the frequency of bacterial co-infection in ARI sufferers may contribute to scientific knowledge. This study proposed not only to use the Wisconsin Upper Respiratory Symptom Survey (WURSS)[10] in achieving the above objectives but also to develop a smaller version of WURSS.

1.2 Specific Aims

The aims of this study are:

Aim 1: Identify specific WURSS items responsible for the differences in global severity of illness between exercise and meditation in the MEPARI study.

The null hypothesis is, "there is no difference when comparing specific WURSS item estimates between the exercise and meditation groups".

Aim 2: Evaluate the frequency of detectable pathogenic bacteria in the nasopharynx of participants with ARI illness, and examine the relationship between these identified biomarkers and severity of the illness.

The null hypothesis is, "compared to viral only or no pathogen detection, bacterial detection is not associated with greater illness severity".

Aim 3: Develop a newer WURSS version with fewer items.

1.3 Background and significance

1.3.1 Comparison of illness severity between meditation and exercise: Studies have shown that there are health benefits from meditation and exercise. [11-16] Exercise may reduce ARI severity and days of illness.[11] Meditation has been shown to improve quality of life, and reduce stress.[14] However, a study on "Meditation or Exercise for Preventing Acute Respiratory Infection" (MEPARI study) is the only randomized controlled trial (RCT) that has examined the results of both behaviors in one study.[7] The study reported

potential benefits on the illness outcomes and showed different degrees of reductions for global severity of illness between meditation and exercise.

The MEPARI study is an RCT that was conducted by Dr Barrett and colleagues at the University of Wisconsin –Madison. The primary outcomes of the study were the incidence, duration and global severity of ARI illness. Duration was defined as "episode duration" and estimated as total days of illness during monitoring. Global severity of illness was estimated using area under the curve (AUC) trapezoidal approximations from severity scores, which were self-reported on the Wisconsin Upper Respiratory Symptom Survey-24 (WURSS-24) each day of illness. The WURSS-24 was generated by adding items assessing headache, bodyache and fever to the validated WURSS-21.[10, 17] The additional items accounted for influenza-like symptoms and had been tested in a previous version, the WURSS-44. The clinical trial took place in University of Wisconsin facilities in 2009-2010. Participants (n=149) were randomized into meditation, exercise or usual care control groups. Participants in the intervention groups, received 8 weeks of either mindfulness meditation or moderate intensity exercise. Bi-weekly telephone and bi-monthly in person interviews were used in monitoring the study.

Following the RCT, 55 % (n=28) of the control group reported a total of 40 illness episodes while 36 % (n=17) of the exercise group, and 41 % (n=21) of the meditation group reported 26 and 27 illness episodes respectively. The control group also reported the worst global severity of illness (average score, 358) and the highest duration of illness (mean days=8.9). Although exercise and meditation groups showed similar

illness durations (average 5.1 and 5.0 days respectively), their reduced global illness severities differed somewhat, with the meditation group (mean score, 144) reporting greater reduction in severity compared to the exercise group (mean score, 248). Based on these results, it was useful to examine the individual WURSS items to better understand whether individual items explained the apparent advantage of meditation over exercise.

The objective of the study was to evaluate the rationale for differential effects of meditation and exercise on severity of ARI illness. The specific aim was to identify specific WURSS items or domain levels which accounted for the differences in symptom severity or quality of life estimates between moderate intensity exercise and mindfulness meditation. The primary outcome evaluated within-group severity estimates of each WURSS item while the secondary outcome examined between-group individual and pooled items effect-sizes. To compare effects from exercise and meditation, I calculated the effect sizes compared to control, and then subtracted one intervention effect from the other. The WURSS scores were used to compute these item level estimates. The null hypothesis between the exercise and meditation groups of "no difference" in illness severity estimates or zero effect-size was rejected at $p \leq 0.05$.

1.3.2 Viral and bacterial co-infection and relationship with severity of illness:

Microorganisms such as bacteria [18, 19] and viruses [20, 21] may inhabit the nasal-pharyngeal cavity and may cause symptomatic respiratory infections.[22] Viruses are the major causes of ARI illness in both adults and children.[23] Various experimental[24] and epidemiological[25, 26] studies have identified viruses as the etiologic pathogens for most

illnesses. A significant number of illness episodes have unknown etiologies despite improved diagnostic procedures.[8] Bacteria are implicated during complications such as streptococcal pharyngitis, pneumonia, otitis media, and acute bacteria sinusitis.[27] Such secondary infections may require management with antibiotics. However, uncomplicated illness has been reported as the 2nd most common reason for antibiotic prescription by clinicians.[9] This may contribute significantly to the emergence of antibiotic resistance, which is a major public health problem.[28]

The viral etiologies belong to different classifications and families. Deoxyribonucleic acid -DNA classification may include; the Adenoviridae (adenovirus) and the Parvoviridae (bocavirus). The ribonucleic acid -RNA classification include; Picornaviridae (human rhinovirus, enterovirus), Coronaviridae (coronavirus), Paramyxoviridae (metapneumovirus, parainfluenza virus, respiratory syncythia virus-RSV), Orthomyxoviridae (influenza virus), etc. The influenza, paramyxovirus and coronavirus are more common in winter periods unlike the others which are common in the fall and spring.[20] Most viral illnesses may be indistinguishable including infection with rhinovirus (the most common); however, infection with influenza (the most serious) is characterized by fever and aches. Virally induced symptoms have been linked to 2 different mechanisms; direct epithelial damage (cytopathic effect) or immunological response. [29, 30] Rhinovirus may cause respiratory symptoms during illness episodes via the immunological mechanism with increased interleukin-8 (IL-8)[24, 26] and recruitment of neutrophils.[31] On the other hand, viruses like influenza and RSV may cause severe

respiratory infections via the direct damage of the epithelia cells lining the respiratory mucosa .[26] Studies have shown viral symptoms to be positively correlated with inflammatory cytokines such as IL-8[26], kinins [32] and neutrophils. [31]

Bacteria such as Streptococcus pneumonia (Gram-positive cocci), Haemophilus influenzae (Gram-negative facultatively anaerobic rods) and Moraxella catarrhalis (Gram-negative aerobic rods and cocci) may inhabit the respiratory airway in adults. The degree of bacteria colonization of the nasopharynx may vary between healthy and infected patients.[18] Although there have been suggestions of bacteria involvement in such illness, few studies on bacteria pathogen and ARI illness have been reported.[8] In their study involving 507 ARI sufferers, Heald et al (1993) reported 56% positive bacteria cultures from nasopharyngeal secretions. They also reported significant association between Haemophilus influenza, Moraxella catarrhalis and Streptococcus pneumonia with leucocytes from nasopharyngeal secretions.[8] However, none of these bacteria etiologies were found among their healthy controls. In studies of asymptomatic participants, the percentage of positive bacteria from nasopharyngeal cultures has been rare, with reported estimates varying between 4-9% for Haemophilus influenza and 1-13% for Streptococcus pneumonia.[18, 33]

This study examined the frequency of bacterial pathogens and relationship of such biomarkers with illness severity. The results may add to pre-existing knowledge from the very few investigations of bacteria and ARI in adult participants. It may also provide a preliminary foundation for a future pilot study evaluating the comparison of

nasopharyngeal bacteria between ill participants and matched asymptomatic adults recruited as controls.

1.3.3 Wisconsin Upper Respiratory Symptom Survey (WURSS): Unlike polymerase chain detection of virus[34], serological markers[35, 36], rhinomanometry[37], mucus weight[38] and the widely used Jackson scale[39], the WURSS reflects the symptom severity and functional impairments of individuals afflicted with ARI illness. The development of the original instrument began in the spring of 1999 in an RCT comparing the effect of Echinacea against placebo (n=142, 853 total person days of illness).[40] This initial instrument had a total of 20 items: 15 symptoms (9 point Likert-scale); 4 functional outcomes (yes/no scale); and a global severity of illness item.

In order to include direct terminology and input from ARI sufferers and to capture the measures most important to them, a subsequent qualitative study was conducted (n=74 adults and 56 total person days of illness).[41] This study used interviews (n=56) and 3 focus groups (n=20), with 2 participants being in both interview and focus groups. The results of this study led to development of a modified WURSS with 32 symptom items, 10 functional impairment items,1 global illness severity item and 1 item which assessed change (within 24-hour) in severity. The 9- point Likert-scale was also reduced to a 7- point Likert-scale: 1(very mild), 3(mild), 5 (moderate), and 7(severe).[41]

A 2002 to 2003 prospective study (n=149, 1681 total person days of illness) conducted by Barrett and colleagues[17] validated the WURSS-44 and showed significant

Pearson correlations (R) with SF-8 (-0. 60 to -0.84; *P*<0.001) and the Jackson scale (0. 73 to 0.93; *P*<0.001). These correlations were greater when compared to those between SF-8 and Jackson scale (-0.55 to -0.78; *P*<0.001). Factor analysis techniques of WURSS-44 resulted in good indices of fit for 36 items into 10 dimensions.[17] Using responsiveness and importance to patient criteria, the WURSS-21[17] was generated from the WURSS-44 and contains 21 items (1= global illness severity, 10= specific symptoms, 9 = function and quality of life, and 1= daily change). Study results showed general symptoms, and function and quality of life were more important to patients than specific symptoms. The WURSS-21 showed slightly better Guyatt's responsiveness (0.80) compared to WURSS-44 (0.71), SF-8(0.54) and Jackson scale (0.61)[17]. Guyatt's responsiveness was estimated by dividing the minimal important difference (MID) by the square root of twice the mean squared error (MSE) of participants who reported stable response ("almost same") to the WURSS question assessing global change.[42] MID can be considered as the minimal amount of positive change, a patient rates as beneficial.

[Responsiveness =MID/ $(2 \times MSE)^{1/2}$]

Barrett et al[10] (2009) also validated WURSS-21 in a 2003 to 2007 prospective study (n=230, 2457 total person days of illness). The WURSS-21 showed significant Pearson correlations (95% confidence intervals) when compared against WURSS-44: R= 0.93(0.90, 0.94), Jackson scale: R=0.85(0.81, 0.88), SF-8 mental: R=-0.55(-0.45,-0.63) and SF-8 physical: R= -0.79(-0.74,-0.84). The WURSS-44 also showed similar correlations with Jackson scale and SF-8 (mental and physical). Using factor analysis

techniques and methods employed by Kroonenberg and Lewis, [43] 3 dimensions were identified for WURSS-21 while 8 dimensions were identified for WURSS-44.[10] These dimensions showed good indices of fit, after assessing models allowing for 2 to 20 and 3 to 43 dimensions for the WURSS-21 and WURSS-44 respectively. The results from this study also identified function and quality of life measures to be more important than symptom measures to ARI sufferers.[10]

The WURSS instrument continues to be used by numerous local, national and international academics in their various researches (http://www.fammed.wisc.edu/wurss). Yang et al (2011) recently assessed and reported the reliability and validity of a Korean version (WURSS-K) using successive forward and backward approach.[44] In their prospective study which enrolled 107 ARI sufferers, Yang et al validated the WURSS-K against the SF-8.

With the increasing interest and use of WURSS, reducing items in the current version of WURSS-21 would lead to reduction in the time it takes research participants to complete the survey. To achieve this, factor analysis was used to evaluate the underlying dimensional structure of WURSS-21. This new information identified fewer items for the new version of WURSS, and also was compared to older WURSS identified dimensions.

1.4 Contributions and intended audience

This dissertation describes an abbreviated Wisconsin Upper Respiratory Symptom Survey (WURSS- a self-report survey) which may be useful in decreasing survey completion time. A reduced WURSS not only retains direct inputs from patients but also maintains the underlying structure of the parent WURSS survey. The results of this thesis demonstrate that improved quality of life reduces disease burden and may advance public health. Use of easy and pleasurable activities can improve health and quality of life. This thesis also underlines the implication of pathogenic bacteria in acute respiratory infection. Thorough evaluation of bacteria co-infection contributes to scientific knowledge which may be useful in raising awareness related to antibiotics therapy and the danger of prescription driven antibiotic resistance.

Finally, this dissertation acknowledges the economic and public health implications of acute respiratory infections. It also provides a rationale to evaluate the frequency of pathogenic nasopharyngeal bacteria in healthy adults. The outcomes from this work are intended for the general populace, healthcare providers, public health institutions and personnel.

1.5 Thesis Statement

Self-reported assessment of acute respiratory infection may enhance interpretability of illness severity and reduce disease burden by focusing on details of greater importance to patients.

1.6 Overview of chapters

Chapter One contains the introduction and specific aims of this study. This chapter also contains the background and significance supporting the study objectives.

Chapter Two, the first manuscript examines whether the apparent advantage following training in mindfulness meditation over exercise can be attributed to specific symptoms or quality of life items on the WURSS survey.

Chapter Three, the second manuscript, evaluates the frequency of detectable pathogenic bacteria in the nasopharynx of participants with the ARI illness. It also examines the relationship between identified biomarkers and severity of the illness.

Chapter Four, the third manuscript demonstrates a smaller and easier to use version of the WURSS instrument using factor analysis technique.

Chapter Five discusses the study results from the manuscripts. It also highlights limitations and implications of this thesis

CHAPTER 2. ADVANTAGE OF MEDITATION OVER EXERCISE IN REDUCING COLD AND FLU ILLNESS IS RELATED TO IMPROVED FUNCTION AND QUALITY OF LIFE.

(Accepted for publication in Influenza and Other Respiratory Viruses)

(Chidi N.Obasi, MD, Roger Brown, Ph.D, Tola Ewers, Shari Barlow, Michele Gassman, Aleksandra Zgierska, MD Ph.D, Christopher L. Coe, Ph.D, and Bruce Barrett, MD Ph.D)

Introduction

Improved immune vigor attributable to exercise or reduced stress [13, 45] may prevent or reduce the burden of acute respiratory infection (ARI illness).[7] ARI illness includes both the common cold and influenza, and is a major cause of morbidity and mortality in the United States and elsewhere.[1, 2] It affects people of all ages with average incidence of 6-8 occurrences/year in children and 2-3 occurrences/year in adults.[3, 4, 46] The burden of non-influenza ARI illness in the United States has been estimated to average $40 billion per year with corresponding 189 million missed school days and 126 million missed work days.[5, 6] With no current effective therapy, health-enhancing preventive strategies such as meditation and exercise may not only lessen disease burden, but may lead to substantial quality-of-life benefits and health care cost reductions (Unpublished observation: U.W. Madison, Rakel et al 2012 *submitted*).[47]

Mindfulness meditation has been reported to improve quality of life and psychological well-being.[48] Previous research has shown that exercise may play a role

in the prevention of ARI illness, reducing both infection and duration of symptoms.[11, 49] However, prior to ours, no study had evaluated potential differential effect between training in meditation and exercise. Our randomized controlled trial (RCT) entitled Meditation or Exercise for Preventing Acute Respiratory Illness (MEPARI), examined the benefits of these two interventions for reducing ARI illness using the validated Wisconsin Upper Respiratory Symptom Survey (WURSS).[7] MEPARI findings not only showed that regular meditation and exercise can influence ARI outcomes but suggested an advantage of meditation compared to exercise. The duration (mean days) and global severity of illness (mean area-under-the curve) were least for meditation (5.0, 144) compared to exercise (5.1, 248) and control (8.9, 358).

Methods used to develop and validate the WURSS instrument have been described previously.[10, 17] Briefly, the WURSS instrument is a self reporting survey that not only measures both ARI symptoms and impact on daily life activities, but also reflects overall changes in illness severity with time. It was designed and validated as a patient-oriented self-report evaluative outcome instrument for use in clinical trials. The instrument was developed using iterative mixed methods, semi-structured interviews and focus groups.[17] Utilizing importance-to-patients and responsiveness as major standards, the WURSS-21 was derived from the WURSS-44, and then independently validated.[10] The WURSS-21 contains 1 item rating global illness severity ("how sick do you feel today?"), 10 items rating specific symptoms (including runny nose and sneezing), 9 items rating function and quality of life (including interference with ability to think clearly and

interact with others), and 1 item rating daily change ("compared to yesterday, I think my cold is…"). Items assessing influenza-like symptoms (fever, headache and muscle aches) taken from the WURSS-44 were added to the WURSS-21 to create the WURSS-24, which was used in the MEPARI study.

The objective of the present analysis was to investigate the differences in ARI global severity ratings following training in exercise versus meditation, as compared to results for matched control participants. Our main results primary outcome paper looked at global severity based on summing scores of WURSS items each day and then calculating area-under-the-curve over the entire illness. The specific aim of this current paper was to identify specific WURSS items or domain levels that accounted for the differences in symptom severity or function and quality of life estimates between moderate intensity exercise and mindfulness meditation. This would not only help describe the apparent advantage of meditation over exercise but may help identify WURSS items with the greatest impact during ARI illness.

Methods

The MEPARI trial took place in University of Wisconsin facilities from September 2009 to May 2010, randomizing 154 participants into meditation, exercise or wait-list observational control groups. Recruitment targeted healthy community residents in Madison, WI and the surrounding area who were ≥50 years old and reported at least 1

cold per year. Previous meditation training, engagement in moderate exercise (more than twice per week), histories of autoimmune or immunodeficiency diseases were reasons for exclusion. Eligible participants were screened by telephone interviews, met personally for informed consent and enrolled in a 2-week run-in trial to assess adherence. The run-in trial involved providing contact information and completion of baseline and data-collection questionnaires.

Participants were randomized on successful completion of the run-in-trial in 2 cohorts (n=94 in Cohort 1 and n=60 in Cohort 2). Cohort 1 began the interventions in September 2009 while Cohort 2 began in January 2010. Both cohorts were monitored until May 2010. Five participants dropped out before any study data could be gathered. Reasons for withdrawal were: health needs following unrelated diagnosis of uterine cancer, displeasure at control group assignment, need to take care of elderly parents, refusal to get the protocol-required influenza vaccine, and conflict with changed work schedule. Participants in the intervention groups received 8 weeks of training in either mindfulness meditation or moderate intensity sustained exercise. Meditation training followed the Mindfulness Based Stress Reduction (MBSR) program developed by Jon Kabat-Zinn et al at the University of Massachusetts Medical Center. The exercise program was matched to the MBSR program in terms of attention, location, and duration of class time and home practice. Exercise intensity was assessed using Borg's Rating of Perceived Exertion (RPE)[50] and included jogging and walking. Both meditation and exercise were matched based on weekly 2-1/2-hour group sessions and daily 45-minute

home practices and assessed using Mindfulness Attention Awareness Scale (MAAS)[51] and International Physical Activity Questionnaire (IPAQ)[52] respectively .

Bi-weekly telephone and bi-monthly in-person interviews were used for monitoring study participants including control group. Participants who reported a new ARI were encouraged to complete the WURSS instrument once daily from the first symptom until at least a day or two after the illness had resolved. The start and finish of a cold were marked respectively, following 2 consecutive daily answers of "Yes" to either "Do you think you are coming down with a cold?" or "Do you think you have a cold?", and "No" to "Do you think that you are still sick with this infection?" Each illness required a score of ≥ 2 on the Jackson scale, including sneezing, sore throat, nasal discharge or obstruction. WURSS-24 was used for self-reporting daily symptoms including fever on a scale ranging from "0" – Do not have this symptom to "7" – Severe. The protocol did not include thermometer measurement of body temperature. Additionally, multiplex PCR was used to identify specific viruses. MEPARI was funded by the National Center for Complementary and Alternative Medicine (NCCAM), and was approved and monitored by the U.W. Institutional Review Board (IRB).

The primary outcomes of the MEPARI study were incidence, duration and global severity of ARI illness. Duration was assessed as total days of ARI illness. Date and time were assessed at beginning and end of illness. Global severity was calculated using area-under-the-curve (AUC) trapezoidal approximations, with WURSS-24 severity as the y-

axis and duration as the x-axis, such that low AUC indicates lesser overall (global) severity.

A total of 149 participants completed the trial (82% female, 94% white, mean age 59.3 ± Standard deviation 6.6 years) (Table 2.1). Fifty-five percent (n=28) of the control group reported a total of 40 ARI episodes while 36% (n=17) of the exercise and 41% (n=21) of the meditation groups reported 26 and 27 ARI episodes, respectively. The control group also reported the worst global illness severity (average score, 358) and a longer duration of illness (mean days=8.9). Although exercise and meditation groups reported similar illness durations (average 5.1 and 5.0 days, respectively), their global illness severities differed with exercise reporting more severe colds (Mean = 248) than meditation group (Mean = 144) (Figure 2.1).

Based on these differential effects, we thought it of value to analyze the individual WURSS items in order to better understand which items accounted for the apparent advantage of training in meditation over exercise.

Statistical analysis

For this study, global severity AUC estimates were calculated by first summing severity scores over time from 22 WURSS items, and then applying trapezoidal approximation. These estimates also formed the primary outcome of the MEPARI trial. The first and last WURSS-24 items were not included in the daily summary scores because they referred to different time frames, and were analyzed separately. Following

intention-to-treat principles, we averaged ARI severity scores across all randomized participants for whom we had follow-up data.

To explore the potential differences in global illness severities, we conducted an item-level analysis. The WURSS items were grouped into 2 domains: 1) Symptom Severity and 2) Impact on Function and Quality of Life. Cohen's "*d"* statistics [53] were used for all individual item and domain effect-size estimations. SAS® Version 9.2 and NCSS® 2007 statistical programs[54, 55] were used for analyses.

Results

For 21 of the 22 WURSS items, mean AUC global severity scores were lowest for the meditation condition, next lowest for exercise, and highest for control (Table 2.2 and Figure 2.2). Among the 22 items, only Fever did not follow this pattern, as the Fever reports were slightly higher in exercisers, as compared to control. Mean Fever scores were lowest among all items, while the highest severity estimates were Feeling tired (for exercise and control) and Plugged nose (for meditation).

Using Cohen's *d* statistic to estimate overall effect size differences in illness severity between meditation and control groups (based on AUC scores), meditation appeared to significantly reduce severity by 0.4 standard deviation units ($d = -0.4$, $p<0.001$). Exercise showed a lower magnitude of reduction in severity by 0.2 standard

deviation units, although still statistically significant (d =0.2, p<0.001) when compared to control (Table 2.3).

Grouping the WURSS items into Symptom Severity versus Impact on Function and Quality of Life suggested a disparity between these 2 domains. Compared to controls, meditation significantly reduced severity of ARI illness by 0.3 standard deviation units (d = -0.3, p<0.001) within the Symptom Severity domain, and showed improved reduction by 0.6 standard deviation units (d = -0.6, p<0.001) in the Function and Quality of Life domain. Within the Function and Quality of Life domain, exercise reduced the severity of illness relative to controls by 0.2 standard deviation units (d = -0.2, p<0.001). This trend was also reflected in the Symptom Severity domain, but the magnitude was more marginal (d = -0.1, p= 0.04).

Comparing the effects of the interventions with all items included, meditation elicited significantly better overall reduction in illness severity (d = -0.3, p<0.001), as compared to exercise. Similar trends were also observed among the other clustered domains. The Function and Quality of Life impairment domain (d = -0.3, p<0.001) was responsible for more of the benefit from the interventions when compared to the Symptom Severity domain (d = -0.2, p<0.001).

Discussion

The results from the MEPARI trial indicate that training in meditation evoked a larger reduction in global ARI illness severity as compared to exercise or the wait-list control participants. This significant impact was found among individual, sub-grouped and the overall pooled WURSS items. Compared to exercise or control, subjects who were meditating reported the lowest severity estimates on 21 of 22 items of the WURSS. Because these trends consistently favor meditation over exercise, and exercise over control, it is unlikely that the findings are due to chance only. Fever displayed the least sensitivity to the intervention of all WURSS items, but was lowest in meditation, and highest in control. The finding of low reporting of fever-inducing infections is consistent with the low prevalence of flu during the 2009-2010 influenza season.

Although both interventions appeared effective, exercise appeared to confer less protection from ARI illness severity in this study than did meditation. Our study shows ARI severity was reduced in the exercise group compared to the control group, but MBSR reduced it even more. Nevertheless, this finding agrees with prior literature which show moderate intensity exercise not only improves the immune system but also reduces incidence of ARI illness.[56, 57] Our study and others also support the general health benefits of exercise for preventing ARI illness.[49] In a recent 12-week study involving 1002 adults, Nieman et al (2010), reported reductions in duration and severity of ARIs with exercise.[11]

Quality of Life and Function during ARIs appeared to be improved with both exercise and meditation, consistent with the known and hypothesized health benefits of these behaviors. The additional impact of meditation on our measures of Function and Quality of Life beyond Symptom Severity reduction may be an important finding. This effect may reflect the psychological and perceptual benefits of mindfulness meditation, which help people to be more aware of bodily sensations without being distressed by them. [58]

These findings demonstrate the clinical value of assessing daily and functional activities of individuals during an illness episode.[41] Adequate assessments of Quality of Life and Function as well as Symptom Severity are essential if we are to understand the full impact of ARIs in the course of investigating the value of preventive and therapeutic interventions.

The conclusions from this study may be limited by the following:1) potential biases resulting from self report, perhaps related to expectancy, placebo or Hawthorne effects, 2) relatively small number of participants who had an ARI during the MEPARI trial, or 3) possibility of less symptom reporting during the late phase of illness, which may be associated with resolution of natural colds.[59]

Nevertheless, the findings were strengthened by rigorous methodology of a randomized clinical trial, high retention of participants, and the use of a validated instrument for capturing both symptom and quality of life measures. The analyses were

further substantiated by the use of item-level effect size estimations. Although many researchers have tended to pay more attention to statistical significance rather than effect size, the use and understanding of effect size has gained a growing following among those interested in clinical and translational research, evidence-based medicine, and comparative effectiveness.[53, 60, 61]

In conclusion, previous research has suggested that behavioral interventions, such as mindfulness meditation or regular, moderate intensity exercise, can enhance health by improving immune [62, 63] and psychosocial functions.[48] Exercise has previously been reported to reduce the risk, severity and duration of ARIs.[11] The MEPARI trial suggests that both moderate intensity exercise and mindfulness meditation may prevent ARIs and reduce the impact, and that mindfulness meditation may be the more potent of the two interventions. This report extends knowledge gained from the MEPARI trial by examining the impact at an item-level analysis of symptoms, function, and quality of life. The analyses indicate that training in mindfulness meditation may provide even greater benefit than exercise in reducing overall illness severity, attributable more to improved function and quality of life than to the severity of specific symptoms.

Table 2.1: Demographic characteristics of MEPARI study population

Randomized Groups / Demographics	Exercise (n=47)	Meditation (n=51)	Control (n=51)
Age (years) mean (SD)	59.0 (6.6)	60.0 (6.5)	58.8 (6.8)
Female n (%)	39 (83.0)	42 (82.4)	41 (80.4)
Non-smokers' n (%)	43 (91.5)	48 (94.1)	48 (94.1)
Race White n (%)	43 (91.5)	49 (92.5)	48 (94.1)
Ethnicity Non-Hispanic, n (%)	47 (100)	51 (100)	49 (96.1)
BMI mean (SD)	29.0 (6.9)	29.0 (6.0)	29.8 (6.8)
College graduate or higher, n (%)	27 (57.4)	36 (70.6)	35 (68.6)
Income > $50,000, n (%)[#]	25 (53.2)	31 (60.8)	29 (56.9)

BMI=Body Mass Index (weight/height^2); [#] missing information on income from meditation group (n=2)

Table 2.2: WURSS[†] item-level mean severity estimates (within groups).

WURSS items	Meditation(n=51) Mean AUC[‡] (95%CI[§])	Exercise(n=47) Mean AUC[‡] (95%CI[§])	Control(n=51) Mean-AUC[‡] (95%CI[§])
Runny nose	9.029(3.14,14.92)	11.19(2.87,19.52)	20.75(10.32,31.19)
Plugged nose	10.16(4.60,15.71)	11.87(2.57,21.18)	18.24(10.50,25.97)
Sneezing	7.24(2.47,12.00)	9.54(1.42,17.66)	10.84(5.91,15.77)
Sore throat	5.16(1.065,9.25)	9.60(2.081,17.11)	10.43(5.54,15.32)
Scratchy throat	4.90(0.95,8.85)	9.14(1.79,16.48)	12.79(6.79,18.80)
Cough	7.65(2.39,12.90)	15.65(6.18,25.12)	20.10(11.46,28.73)
Hoarseness	8.41(2.56,14.26)	9.36(2.072,16.65)	13.29(5.94,20.65)
Head congestion	9.25(4.059,14.45)	11.96(2.62,21.29)	19.34(11.023,27.66)
Chest congestion	6.23(1.76,10.69)	11.50(2.27,20.73)	13.50(6.24,20.76)
Headache	5.54(1.97,9.11)	8.73(1.84,15.63)	12.22(7.13,17.30)
Body ache	4.59(1.55,7.63)	10.16(2.22,18.10)	11.36(6.39,16.34)
Fever	1.84(-0.090,3.78)	3.87(-0.15,7.89)	2.48(0.67,4.29)
Feeling tired	9.059(4.090,14.03)	17.19(6.54,27.84)	24.99(15.69,34.29)
Think clearly	4.80(2.053,7.55)	9.41(2.46,16.37)	15.44(8.52,22.37)
Sleep well	6.049(2.47,9.63)	14.00(5.39,22.61)	20.12(11.73,28.51)
Breathe easily	8.059(3.75,12.37)	13.22(4.60,21.85)	21.25(12.88,29.61)
Exercise, walk or climb stairs	6.90(2.94,10.87)	14.11(4.74,23.47)	19.51(10.54,28.48)
Accomplish daily activities	6.49(2.59,10.39)	12.87(4.31,21.44)	19.60(11.25,27.94)
Work outside the home	4.77(1.48,8.067)	10.91(3.00,18.83)	14.86(7.90,21.83)
Work inside the home	6.21(2.35,10.064)	12.49(4.049,20.93)	19.020(10.74,27.30)
Interact with others	5.52(2.20,8.84)	10.38(2.51,18.25)	18.68(10.32,27.034)
Live your personal life	5.76(2.22,9.31)	11.22(3.35,19.10)	19.078(10.62,27.53)

[‡] Area under the Curve [§]95% Confidence Interval

[†]Wisconsin Upper Respiratory Symptom Survey data from participants with ARI illness.

Table 2.3: WURSS† item-level effect size estimates (between groups).

WURSS items	Exercise (n=47) vs. Control (n=51) "*d*"(95%CI)	Meditation(n=51) vs. Control (n=51) "*d*"(95%CI)
Symptom severity domain		
Runny nose	-0.29(-0.68,0.11)	-0.39(-0.78,0.0054)
Plugged nose	-0.21(-0.61,0.18)	-0.33(-0.73,0.056)
Sneezing	-0.056(-0.45,0.34)	-0.21(-0.60,0.18)
Sore throat	-0.038(-0.43,0.36)	-0.33(-0.72,0.064)
Scratchy throat	-0.16(-0.55,0.24)	-0.43(-0.83,-0.041)
Cough*	-0.14(-0.54,0.26)	-0.49(-0.88,-0.092)
Hoarseness	-0.15(-0.55,0.24)	-0.21(-0.59,0.18)
Head congestion	-0.24(-0.64,0.16)	-0.41(-0.80,-0.032)
Chest congestion	-0.069(-0.47,0.33)	-0.34(-0.73,0.054)
Headache	-0.17(-0.56,0.23)	-0.42(-0.82,-0.032)
Body ache	-0.053(-0.45,0.34)	-0.46(-0.85,0.066)
Fever	0.13(-0.27,0.53)	-0.095(-0.48,0.29)
Pooled effect size	*-0.12(-023,-00051)*	*-0.34(-0.45,-0.23)*
*Difference between intervention effects***	*-0.22(-0.32,-0.12)*	
Impact on Function and Quality of life domain		
Feeling tired*	-0.22(-0.62,0.17)	-0.60(-0.99,-0.20)
Think clearly	-0.25(-0.65,0.15)	-0.56(-0.96,-0.17)
Sleep well*	-0.21(-0.60,0.19)	-0.61(-1.010,-0.21)
Breathe easily	-0.27(-0.67,0.13)	-0.55(-0.95,-0.16)
Exercise walk or climb stairs*	-0.17(-0.57,0.23)	-0.51(-0.90,-0.11)
Accomplish daily activities	-0.23(-0.62,0.17)	-0.56(-0.96,-0.17)
Work outside the home	-0.15(-0.55,0.25)	-0.52(-0.91,-0.12)
Work inside the home	-0.22(-0.62,0.17)	-0.55(-0.95,-0.16)
Interact with others	-0.29(-0.69,0.11)	-0.58(-0.97,-0.18)
Live your personal life	-0.27(-0.67,0.13)	-0.57(-0.97,-0.18)
Pooled effect size	*-0.23(-0.35,-0.10)*	*-0.56(-0.69,-0.44)*
*Difference between intervention effects***	*-0.33(-0.37,-0.30)*	

Continuation of Table 2.3: WURSS† item-level effect size estimates (between groups).

Combined WURSS items		
Overall Pooled effect size	*-0.17 (-0.25,-0.084)*	*-0.44(-0.52,-0.36)*
*Difference between intervention effects***	*-0.27(-0.35,-0.20)*	

"*d*"= Cohen's standardized effect size (computed using mean, standard deviation and sample size).

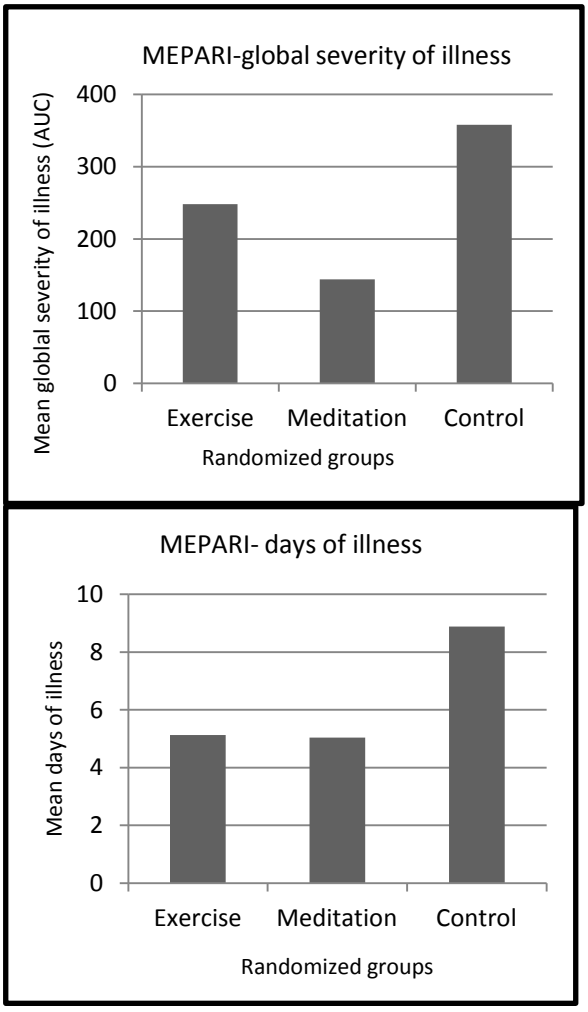
CI-Confidence Interval

*Items significant on probability analysis (2-sided $p<0.05$).

**2- sample t-test used to compare intervention effects between exercise and meditation.

† Wisconsin Upper Respiratory Symptom Survey data from participants with ARI illness.

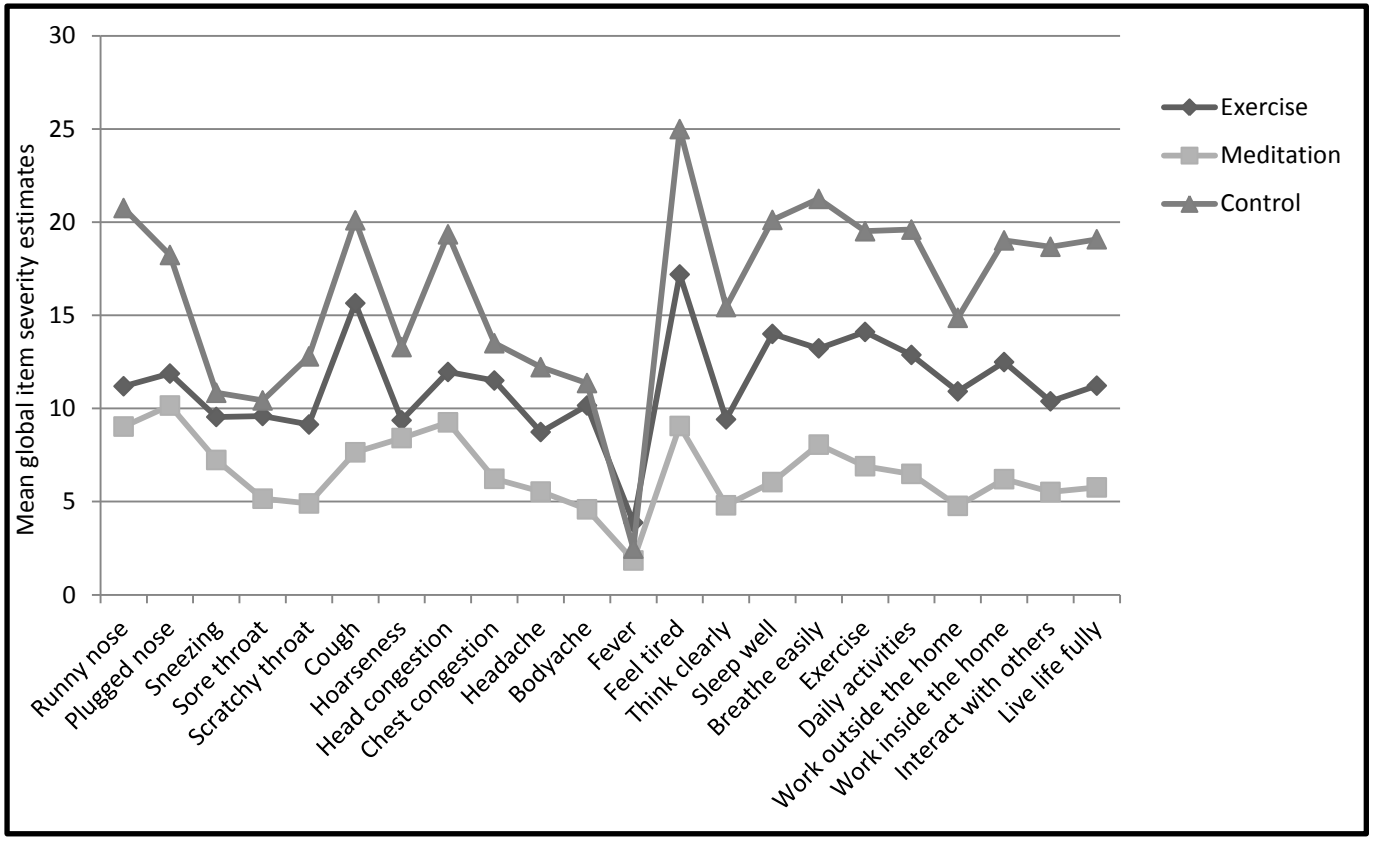
Figure 2.1: Mean global severity and duration of illness from MEPARI study.



AUC=Area under the curve severity estimates calculated as trapezoidal approximations using daily scores on Wisconsin Respiratory Symptom Survey and duration of illness.

Duration of illness calculated in hours and minutes, and then converted to decimalized days of reported illness.

Figure 2.2: Graphical display of mean WURSS† item severity estimates.



Global item severity estimates calculated using area-under-the-curve trapezoidal approximation of the WURSS scores and duration of illness.

† Wisconsin Upper Respiratory Symptom Survey data from participants with ARI illness.

CHAPTER 3. VIRAL AND BACTERIAL CO-INFECTION IN ACUTE RESPIRATORY INFECTIONS.

Introduction

Bacteria [18, 19] and viruses [20, 21] may inhabit the nasal-pharyngeal cavity and cause acute respiratory infection (ARI).[22] ARI includes both influenza and common cold, and has a worldwide pervasiveness.[1] ARI afflicts persons of all ages with greater annual incidence in children (6-8 episodes) compared to adults (2-3 episodes).[3, 4] Without good treatment, common cold accounts for significant loss of productivity and enormous burden on the healthcare system. Its annual costs include $40 billion and over 200 million lost work and school days in the US.[5, 64]

Although the frequency of bacterial co-infection in adults with ARI illness is not clear, [8] there are significant numbers of antibiotic prescriptions by clinicians for uncomplicated ARI illness.[9] Widespread inappropriate use of antibiotics contributes to the emergence of antibiotic resistance and therefore to increased health care costs.[28] Examining the frequency of bacterial co-infection in ARI sufferers may not only improve understanding of the correlation between bacterial co-infection and ARI severity but will also contribute to scientific knowledge.

Viruses are the major causes of ARI in both adults and children.[65] Various experimental[24] and epidemiological[25, 26] studies have identified viruses as the pathogens for most ARI illness and have shown significant correlations with inflammatory

cytokines such as Interleukin-8 (IL-8)[26], various kinins[32] and neutrophils.[31] A significant number of ARI episodes have unknown etiologies despite improved diagnostic procedures.[22, 66, 67] Bacteria such as *Streptococcus pneumonia* (Gram-positive cocci), *Haemophilus influenzae* (Gram-negative facultatively anaerobic rods) and *Moraxella catarrhalis* (Gram-negative aerobic rods and cocci) have been implicated during complications such as streptococcal pharyngitis, pneumonia, otitis media, sinusitis, and acute bronchitis.[27] These bacteria may inhabit the respiratory airway and also vary based on age and health conditions.

In studies of asymptomatic children[33] and adults[18] the percentage of positive bacteria from nasopharyngeal cultures has been reported as 4-9% for *Haemophilus influenza* and 1-13% for *Streptococcus pneumonia*.[18, 33] Compared to children, adults may have lower bacteria prevalence in their respiratory tracts.[68] This trend may be due to improved immune system in adults.[69]

There have been suggestions of bacteria involvement with ARI illness. However, results from relevant studies have been inconsistent.[8, 70] Studies have shown significantly increased bacteria frequency in either symptomatic children[68] or adults [8] compared to healthy controls.[71, 72] In a study involving 507 ARI sufferers[8] Heald et al (1993) reported 56% positive bacteria cultures from nasopharyngeal secretions of adults with ARI illness, but found no bacteria in healthy controls. However, Winther et al (1984) found no changes in bacterial nasal flora between healthy and ARI ill

conditions.[70] Their study examined both nasal and nasopharyngeal samples during and after cold illness in adults and found few pathogenic bacteria.

For our study, we examined the frequency of pathogenic bacteria among adult participants with ARI illness. We used nasal wash specimens which were evenly selected based on the presence and absence of viral pathogens.

Design and Method

Overview

The presence of virus and bacteria were assayed utilizing nasal wash specimens obtained from the randomized controlled trial titled "Placebo: Physician or Pill? RCT in a Common Cold Model (PEP)". The PEP trial spanned from 1/2004 to 8/2008 and enrolled 719 participants.[73] The study rationale and methods have been described previously.[74] Briefly, using a 2-way factorial design, the PEP trial examined placebo effects in relation to drugs and physician-patient encounter. The physician-patient encounter axis consisted of: 1) no encounter, 2) standard (usual clinical interaction), and 3) enhanced (including attributes such as empathy and empowerment), while the medication axis consisted of: 1) no medication, 2) concealed placebo, 3) concealed Echinacea, and 4) unconcealed Echinacea. PEP was funded by National Center for Complementary and Alternative Medicine at the National Institutes of Health (1-RO1-AT-1428) and was approved by the U.W. Madison Institutional Review Board.

PEP-Participant enrollment and biomarkers

Using U.W, Department of Family Medicine enrollment sites at St Mary's Hospital Madison and Verona Health clinic, participants with ARI illness aged 12 and above were recruited from Dane County Wisconsin. N=719 participants were enrolled and n=713 completed the trial. They were mostly Caucasian (87.9%), female (64.1%), and college graduates (84.0%) with an average age of 33.7 years (standard deviation 14.4). Participants were eligible if they acknowledged having a cold or coming down with one. They also had to have ≥2 points on the Jackson symptom scale[75] including ≥1 of these symptoms: nasal discharge, obstruction, sneezing or sore-throat, with onset of first symptom ≤ 36 hours from enrollment. Histories of allergy and asthma were reasons for exclusion if active symptoms were observed at enrollment. Use of antibiotics or immune related medication was also reason for exclusion.

Each participant submitted nasal wash specimens on day-1 (enrollment day) and day-3 for interleukin-8 (IL-8) and neutrophill assessments. Methods used in quantifying these biomarkers have been described previously.[74, 76] Briefly, of 5ml sterile saline instilled and recovered from each nostril, 2ml were used to assay for neutrophil counts, while 3ml were frozen (-80^0C) in aliquots of 300ul. Subsequently, enzyme-linked immunoassay techniques were used to assay for IL-8 from the frozen samples.[76] Viral pathogens from nasal secretions collected on day-1 were identified using Respiratory MultiCode-PLx Assay (EraGen Biosciences, Madison WI). This assayed combined polymerase chain reaction (PCR) and high-throughput microsphere flow cytometry

(Luminex). It is a multi-target, rapid and sensitive technique for detection of respiratory viruses.[77]

PEP-Outcomes

ARI severity and duration of illness were outcomes for the PEP trial. Duration was defined as time from symptom onset until participant responded with "No" to the question "Do you think you still have a cold"? ARI severity was assessed using area-under-the-curve trapezoidal approximations with duration on the x-axis and symptom scores on the y-axis. Symptom scores were self reported on the Wisconsin Upper Respiratory Symptom Survey (WURSS-21).[10] The WURSS-21 consists of 10 symptom and 9-quality of life items used in severity estimations. (See appendix 1) Two remaining items assessing magnitude "(How sick do you feel today?") and daily change of illness ("Compared to yesterday, I think my cold is…") were assessed separately. PEP findings indicated no significant between-group differences in severity and duration of illness.

Present bacteria study

For this analysis, n=194 nasal wash specimens were randomly selected (www.randomizer.org) from PEP day-1 samples (n=713) and analyzed for H. influenza, M. catarrhalis and S. pneumonia. The sample size was based on 2-sided α=0.05, β=0.8 and 20% effect size difference. They were evenly picked among specimens previously identified with viral (n=97) and no viral (n=97) nucleic acid.

The study sample was analyzed for bacteria in a 2-part procedure. The 1st procedure involved DNA extraction from 300µl of nasal wash specimens using protocol described

by BiOstic Bacteremia DNA isolation kit (MO BIO Laboratories Carlsbad, CA). To prevent potential degradation of the bacteria DNA, the sample was modified with addition of 0.1mM of EDTA.

The 2nd procedure involved quantitative PCR detection for *H. influenza, M. catarrhalis* and *S. pneumonia*. The detection range for each bacterium was from 10 to 1 million CFU using a McFarland Standard. Multiplex q-PCR employing P6 and copB genes were used for the detection of *H. influenza*[78] and for *M. catarrhalis*[79] respectively. Quantitative lytA PCR[80] was exclusively used for detection of *S. pneumonia*. (See table 3.1) All primers and probes were purchased from Applied Biosystems, Foster City, CA.

Statistical analysis

The general linear model (GLM) was used to examine the relationship between pathogens, severity of illness on day-1, IL-8 and neutrophils markers also collected on day-1. Box-Cox transformations were used to normalize the skewed distributions of the biomarkers. Square-root transformation was employed to normalize the distribution of WURSS severity scores on day-1 (LAMBDA=0.36), while natural log (LN) transformations were used to normalize distributions of the biomarkers (Lambda: IL-8 =0.04; neutrophils = 0.02). Covariates include: age, gender and seasons of the year. Median and non-parametric analysis (Kruskal-Wallis test) were calculated based on the original skewed distribution of these biomarkers. SAS® Version 9.2 and NCSS® 2007 statistical programs were used for analyses.[54, 55]

This study on bacteria frequency was funded by the U.W. Department of Family Medicine small grant program and approved by U.W. Madison Health Sciences Institutional Review Board.

Results

Of the total 194 nasal wash samples assayed for pathogenic bacteria nucleic acid, n=97 were virus negative while n=97 were virus positive. Ninety-five percent of the virus positive samples contained only 1 type of virus while the remaining 5% contained 2 different viruses. Types of viruses varied within the virus positive samples including Human Rhinovirus (72%), Coronavirus (10%), Influenza virus (2%) and Bocavirus (1%). The majority of the samples were collected from symptomatic participants during fall (35%) and winter (39%) compared to spring (17%) and summer (9%). The overall degree of bacteria detection followed similar trends with most occurring during winter (39%) while summer (7%) showed the least. However, the presence or absence of virus appeared to influence the seasonality and bacteria trends. Among the virus negative-group, fall season (49%) showed the highest bacteria frequency, while winter season (46%) resulted in most for the virus-positive group.

Main results

Among the n=194 nasal specimens, 37% (n=71) were positive for pathogenic bacteria nucleic acid compared to 63% (n=123) which did not show any bacteria. Significantly

more bacteria were detected from the virus-negative samples 47% (n=45), compared to the virus-positive 27 % (n=26) (Chi-square, P<0.01). (See figure 3.1).

Types of bacteria

Twelve percent (n=23) of the nasal specimens had 2 co-existing bacteria (predominantly H.influenza and M. catarrhalis) while no specimen contained all 3 bacteria. Overall, H.influenza was identified in 28% (n=54) of the nasal specimens, compared to M. catarrhalis 14% (n=27) and S. pneumonia 7% (n=13). Virus-negative specimens had more H.influenza (35%), M. catarrhalis (22%) and S. pneumonia (9%), compared to virus-positive nasal specimens: H.influenza (21%), M. catarrhalis (6%) and S. pneumonia (4%). (See figure 3.2)

ARI severity of illness

There was a trend towards increase in illness severity with the pathogenic bacteria. Severity of illness (mean; 95% confidence interval) was slightly increased with bacteria only (6.5; 5 to 7.1) or combination of bacteria and virus (6.5; 5.7 to 7.2) compared to virus only (6.3; 5.8 to 6.7) or no pathogen (6.1; 5.5 to 6.6). Adjusting for age, gender and season of year did not significantly alter these trends. Median estimates showed similar trends and were also not statistically significant using Kruskal-Wallis test. (See table 3.2)

IL-8 and neutrophil biomarkers

Compared to virus, presence of bacteria was associated with higher levels of IL-8 and neutrophils. GLM analysis on the transformed data showed combination of virus and

bacteria had the highest levels (mean; 95% CI) of IL-8 (6.4: 5.8 to 6.9) and neutrophil (3.8; 3.2 to 4.4). Median estimates of IL-8 and neutrophil were higher for combination of bacteria and virus (550.3; 42) compared to bacteria only (229.7; 14), virus only (229.5; 10.5) or no pathogen (162.7; 10). Kruskal-Wallis test showed significant difference among all 4 groups. (See table 3.2).

Discussion

Viruses are thought to be the most common cause of common cold and flu illnesses.[39, 81, 82] Despite advanced laboratory techniques, a significant number of illness episodes have unknown etiologies.[66, 67] Using nasal wash specimens with and without detectable virus, our study evaluated the frequency of bacteria co-infection. We found 37% (n=71) of our sample had bacteria, with the majority (n=45) of these coming from the virus-negative group. *H. Influenza* (28%) was most common compared to *M. Catarrhalis* (14%) and *S. Pneumonia* (7%).

Although previous reports of bacteria frequency have varied, most are consistent with our findings.[8, 18] Heald et al (1993) reported 56% bacteria in adults with upper respiratory tract infection.[8] However they also reported lower culture rates for *H.influenza* (9%) and *M. catarrhalis*(7%) than we found. Although, Jouismes-Somer et al (1989) reported much higher bacteria among military participants with sinusitis (*H.influenza* (61%), *S. pneumonia* (25%)), it is possible the use of sinusitis may have influenced their finding.[18]

The bulk of our sample contained only virus pathogens (n=71), supporting the general knowledge that virus is the most common cause of cold and flu illnesses. The possibility of yet-to-be identified respiratory pathogens may account for our findings of some nasal wash specimens with no viral pathogens. Majority of our samples were obtained during winter (39.4%), compared to fall (32.4%), spring (21.1%) and summer (7%). This also supports known seasonal variability of the common cold with most occurring during fall school season.

Our findings demonstrate similar estimations of illness severity between having no pathogen or at least one pathogen present. This may suggest ongoing alternate sources of inflammation among the no pathogen group. These unrecognized sources may include yet-to-be identified pathogens or non-infectious inflammatory processes in the nasal cavity. Nonetheless, we found relatively greater estimations of IL-8 and neutrophils among the bacteria-virus combinations. While previous studies have shown positive correlations between viral or bacterial pathogens with immune biomarkers, [8, 26] future research could attempt to further explore for possible differential relationships between the biomarkers and pathogens.

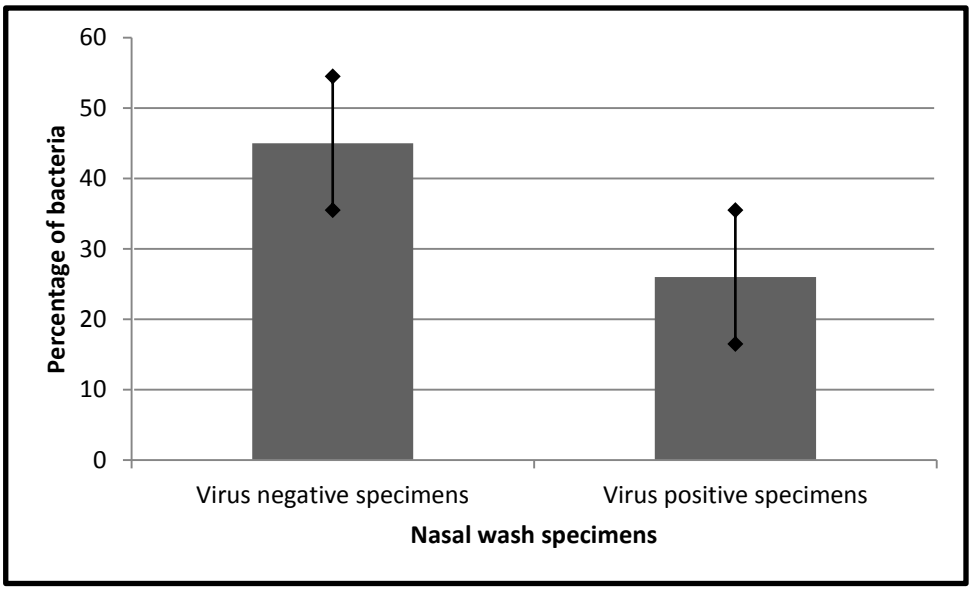
Our findings suggest some bacterial involvement in common cold, nevertheless they do not support the use of antibiotics to treat uncomplicated ARI. The unwarranted utilization of medications may not only increase costs of health care but it may also lead to sustained drug resistance.[28] Furthermore, disproportionate antibiotic administration may lead to alteration of the respiratory tract microbial flora with resulting increase in pathogenic bacteria and decrease in commensals.

This study may be limited by the moderate sample size, lack of asymptomatic control group and its homogenous population (predominantly Caucasians); however it is strengthened by the use of q-PCR which is a sensitive and culture-independent diagnostic technique. PCR is a nucleic acid test with high specificity and is independent of bacteria viability.[83] In as much as degradation of specimens could be a challenge with storage over time, our nasal specimens were effectively preserved at -80^{o}C.[84] The large time span during the PEP trial may also strengthen the generalizabilty of the study.

In conclusion, we have demonstrated the presence of pathogenic bacteria in nasal wash specimens obtained from symptomatic ARI participants. The higher rate of bacteria detection in the absence of identifiable viral etiologies may be secondary to yet-to- be identified viral agents or non- infectious causes of ARI. Presence of bacteria was associated with increased biomarkers levels compared to virus.

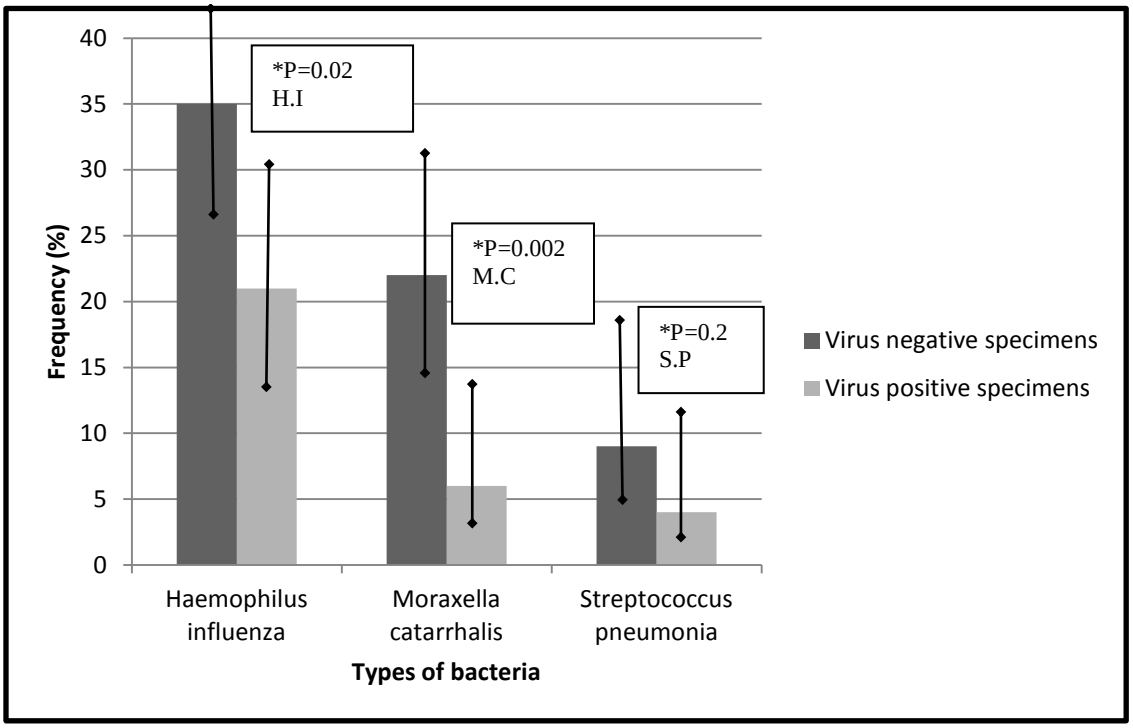
Finally, the result of this study may not only contribute to scientific knowledge but may also shed some light on possible ways of preventing growth of pathogenic bacteria. This possibility includes use of probiotics to increase the presence of competitive nasopharyngeal bacterial commensals. Study finding may also provide a rationale to evaluate the frequency of bacteria in the nasopharnyx of healthy participants.

Figure 3.1: Frequency of bacteria in nasal wash specimens



Virus negative specimens = 46.4%; 95% CI (36.2, 56.8)
Virus positive specimens = 26.8%; 95% CI (18.3, 36.8)
Test of proportional difference (χ^2 test); p=0.0046

Figure 3.2: Type of bacteria in nasal specimens



*Test of proportional difference (χ^2 test)

	Virus negative	Virus positive
(H.I)	Haemophilus = 35.0%; 95% CI (26.4, 44.8)	Haemophilus = 20.6%; 95% CI (14.1, 30.0)
(M.C)	Moraxella = 22.0%; 95% CI (14.9, 31.1)	Moraxella = 6.2%; 95% CI (2.5, 12.7)
(S.P)	Streptococcus = 9.3%; 95% CI (4.6, 16.4)	Streptococcus = 4.1%; 95% CI (1.2, 10.2)

Table 3.1: Primers and Probes for Haemophilus influenza, Moraxella catarrhalis and Streptococcus pneumonia.

	Haemophilus influenza	Moraxella catarrhalis	Streptococcus pneumonia
Forward Primer	Hi P6 F 5'-CCA GCT GCT AAA GTA TTA GTA GAA G-3' (302-326)	Mc copB F 5'-GTG AGT GCC GCT TTT ACA ACC-3' (50-70)	LytA F 5'-ACG CAA TCT AGC AGA TGA AGC A-3'
Reverse Primer	Hi P6 R 5'-TTC ACC GTA AGA TAC TGT GCC-3' (477-457)	Mc copB R 5'-TGT ATC GCC TGC CAA GAC AA-3' (121-102)	LytA R 5'-TCG TGC GTT TTA ATT CCA GCT-3'
Probe	Hi P6 VIC 5'-CAG ATG CAG TTG AAG GTT ATT TAG- MGB-'3	Mc copB NED 5'-TGC TTT TGC AGC TGT TAG CCA GCC TAA-3'-MGB (73-99)	LytA TaqMan probe FAM FAM-5'-TGC CGA AAA CGC TTG ATA CAG GGA G-3' -MGB

Table 3.2: Viral and Bacterial Co-Infection: Primary and secondary outcomes

Primary outcomes				
	No Pathogen (n=52)	Virus only (n=71)	Bacteria only (n=45)	Virus and Bacteria (n=26)
Mean severity of illness Unadjusted* (95% CI)	**6.1(5.5,6.6)**	**6.3(5.8,6.7)**	**6.5(6.0,7.1)**	**6.5(5.7,7.2)**
Severity of illness Median (95% CI) [$]	36.8 (25.0,44.5)	38.5 (31.0,47.0)	39.0 (30.5,52.0)	36.8 (26.5,57.0)
Secondary outcomes				
	No Pathogen (n=50)	Virus only (n=71)	Bacteria only (n=43)	Virus and Bacteria (n=25)
Interleukin-8 (pg/ml) Unadjusted * (95% CI)	**5.3(4.9,5.7)**	**5.5(5.1,5.8)[#]**	**5.7(5.3,6.1)**	**6.4(5.8,6.9)**
Interleukin-8 (pg/ml) Median (95% CI) [$]	162.7 (118.1,281.02)	229.5[#] (164.2,364.1)	229.7 (110.9,438.8)	550.3 (338.9,1900.9)
	No Pathogen (n=49)	Virus only (n=64)	Bacteria only (n=44)	Virus and Bacteria (n=25)
Neutrophil counts Unadjusted * (95% CI)	**2.3(1.8,2.7)**	**2.7 (2.3,3.1)[#]**	**3.0(2.5,3.4)**	**3.8(3.2,4.4)**
Neutrophil counts Median (95% CI) [$]	10.0 (6.0,13.0)	10.5 (8.0,18.0) [#]	14.0 (8.0,26.0)	42.0 (14.0,122.0)

Severity of illness represents self reported WURSS scores on enrollment (illness day-1).
95% CI=Confidence Interval;
Bold=Box-Cox transformation. Severity of illness scores were square root transformed while Interleukin-8 and neutrophils were natural log (LN) transformed
* General Linear Model on transformed variables: adjustment with age, gender and 4 seasons did not change the trend (not shown).
[$] Kruskal-Wallis one way analysis of variance.
[#]2-sided p-value <0.05(comparing virus only to combined virus and bacteria).

CHAPTER 4. FACTOR ANALYSIS OF WISCONSIN UPPER RESPIRATORY SYMPTOM SURVEY (WURSS-21)

Introduction

A shorter and valid version of the Wisconsin Upper Respiratory Symptom Survey (WURSS) is desirable in reducing the time and burden it takes to complete the WURSS. The WURSS is a research tool useful in evaluating the daily severity of acute respiratory illness (ARI).[10] ARI including common cold and influenza, has higher annual incidence among children (6-8 episodes) compared to adults (2-3 episodes).[3, 46] It also accounts for a significant loss of economic productivity and is estimated to be responsible for over 60 million days of combined work and school absences.[5, 64]

The WURSS is reliable, valid and responsive.[17] It is a self- reporting instrument used in assessing ARI symptom severity and impact on quality of life. It is an evaluative instrument that reflects both magnitude and change in ARI severity. The current WURSS-21 contains 1 item grading global illness severity ("*How sick do you feel today?"*); 10 items grading specific symptoms (including "*Sore throat", "Cough"*); 9 items grading function and quality of life (including "*Feeling tired", "Sleep well"*); and 1 item grading daily change ("*Compared to yesterday, I feel that my cold is…"*).[10] All items are scored on 7-point Likert scales including: 1(very mild), 3(mild), 5(moderate) and 7(severe). (See appendix 1)

The development of the original WURSS instrument began in the spring of 1999 with a randomized controlled trial (RCT) comparing the effect of Echinacea against

placebo during ARI illness (n=142, 853 total person days of illness).[40] This initial instrument had a total of 20 items: 15 symptoms (9-point Likert scale); 4 functional outcomes (yes/no scale); and a global severity of illness item. A subsequent qualitative study (n=74 adults, 56 total person days of illness)[41] was conducted to include language and values most important to participants with ARI illness. Study results lead to a modified WURSS-44 with 32 symptom items, 10 functional impairment items, 1 global illness severity item and 1 item which assessed change in severity (within 24-hour). The 9-point Likert scale was also reduced to a 7-point Likert scale: 1(very mild), 3(mild), 5 (moderate), and 7(severe).[41]

The WURSS-44 was validated in a study which spanned from 2002 to 2003 (n=150, 1681 total person days of illness).[17] It not only demonstrated significant correlations with other surveys including Jackson's scale, but also showed good indices of fit with 36 items designated into 10 dimensions. This indicated that general symptoms and quality of life functions were more important to patients than specific symptoms, and led to the development of the WURSS-21.[17]

The WURSS-21 was subsequently compared to the WURSS-44 in a study which spanned from 2003 to 2007 (n=230, 2457 total person days of illness). [10] It showed significant positive correlations (> 0.80) with the WURSS-44 and Jackson scales and negative correlations (> -0.50) with either the physical or mental health questionnaires domains of the short forms (SF-8) general health.

The WURSS instrument continues to be used by numerous local, national and international academic institutions and has been translated into 7 languages including French and Spanish (http://www.fammed.wisc.edu/wurss). Yang et al (2011) recently reported the reliability and validity of a Korean version (WURSS-K) developed using successive forward and backward approach.[44]

With the increasing interest and use of the WURSS, the aim of this study was to derive a shorter version of the WURSS-21. This shortened version could reduce survey completion time and perhaps increase response rates.

Method

Overview

The WURSS is a valid patient oriented-outcome instrument designed to measure everyday severity of ARI illness.[10] The current WURSS-21 is graded on a 7-point Likert scale and consists of 1 item scoring global illness severity, 10 items scoring specific symptoms, 9 items scoring function and quality of life and 1 item scoring daily change.

Data sources

Study data came from 4 previous studies which generated WURSS-21 information (total n=1167, person-days of illness=9622) conducted by Barrett and colleagues (University of Wisconsin–Madison). These data include #1) WURSS-21 development[17] and #2) WURSS-21 validation[10] which spanned from 2002-2007. Additional data came from 2 RCTs titled #3) “Placebo: Physician or Pill? RCT in a Common Cold Model” (from

2004 - 2008)[74, 85] and #4) "Meditation or Exercise for Preventing Acute Respiratory Infections" (from 2009 - 2010).[7] (See table 4.1). All study protocols including participants' consents were approved and monitored by the UW-Madison Institutional Review Board.

Data-1 (development of WURSS-21)

Residents from Dane county, WI with common cold participated in the study from March 2003 to August, 2003.[17] To be included, participants had to report symptom onset <48 hours prior to enrollment and score ≥ 3 on the Jackson scale. Ongoing symptoms of allergy were reasons for exclusion. Each day, participants completed questionnaires including the 24-hour recall version of general health and quality of life (SF-8) and WURSS-44 until they reported not feeling sick for 2 consecutive days or had exhausted 14 days of illness, which ever was earlier. Study goals included assessment of reliability, responsiveness, and importance to patients, aiming towards reduction of WURSS-44.

A total of 157 participants were enrolled while 150 completed the study (1681 person-days of illness). Study participants were aged 18-80 years (mean=36 years, standard deviation=15), mostly females (70%), Caucasians (88%), non smokers (59%) and had a college diploma (>50%). The WURSS-44 demonstrated significant Pearson correlations (*R*) with the SF-8 (-0.60 to -0.84; $P<0.001$) and the Jackson scale (0.73 to 0.93; $P<0.001$). These correlations were greater than those between the SF-8 and the Jackson scale (-0.55 to -0.78; $P<0.001$).[17]

The WURSS-21 was generated from the WURSS-44 using Guyatt's responsiveness and importance to patient ratings as primary outcomes.[17] In this derivation data set, the WURSS-21 demonstrated slightly better responsiveness (0.80) compared to the WURSS-44 (0.71), the SF-8(0.54) and the Jackson scale (0.61)[17]. Guyatt's responsiveness index was estimated by dividing the minimal important difference (MID) by the square root of twice the mean squared error (MSE). The MID was calculated as the average change in instrument score among those assessing themselves as "a little better" or somewhat better' between two successive days. MID can be considered as the minimal amount of positive change that a patient rates as beneficial.[42]

Data-2 (Validation of WURSS-21)

New onset common cold sufferers were screened and enrolled when they responded to study advertisements including newspapers and posters.[10] Participants were included if their symptoms were < 48 hours in duration, had Jackson scores of ≥ 2 points, including nasal symptoms, and felt they were coming down with a cold. Current symptoms of asthma or allergies were reasons for exclusions. Illness monitoring ended when participants gave a "Not sick" response to the question "How sick do you feel today?" or on the 14th day of illness if symptoms persisted without a "Not sick" response. Study outcomes included comparing the reliability of the WURSS-21 alongside the WURSS-44 or the SF-8 and using factor analysis to identify the dimensional structure of the WURSS-21.

Of 239 enrolled participants, 230 study completers (2457 person-days of illness) were aged between 14 and 83 years (mean=34 years, standard deviation=14), mostly females (67%) and non-smokers (61%), with at least some high school education (99%). Study duration was between August, 2003 and August, 2007 during which participants completed self–report questionnaires twice daily. The WURSS-21 demonstrated significant *R* (95% confidence intervals) when compared against the WURSS-44: 0.93(0.90, 0.94), Jackson scale: 0.85(0.81, 0.88), SF-8 mental: -0.55(-0.45,-0.63) and SF-8 physical: -0.79(-0.74,-0.84).[10] Factor analysis and methods employed by Kroonenberg and Lewis, [43] indicated good indices of fit with 3 dimensions for WURSS-21 and 8 for WURSS-44. These dimensions were selected after appraising several multi-dimensional models.

Data-3 (Placebo: Physician or Pill? RCT in a Common Cold Model" aka PEP)

The PEP trial spanned from January, 2004 to August, 2008 and enrolled 719 participants. [74, 85] N=713 completed the trial (4 early withdrawals, 2 losses to follow-up), filling out the WURSS-21 twice daily for up to 14 days. Study completers accounting for 4810 person-days of illness were mostly Caucasians (88%), females (64%), and college graduates (84%) with an average age of 34 years (standard deviation 14). Participants were eligible if they acknowledged having a cold or coming down with one and also reported ≥2 points on the Jackson scale.[75] Presenting symptoms had to include ≥1 of these: sneezing, sore-throat, nasal discharge or obstruction with onset ≤ 36 hours from enrollment. Histories of allergy and asthma were reasons for exclusion if active

symptoms were observed at enrollment. Use of antibiotics or immune related medication was also reason for exclusion.

PEP employed a 2-way factorial design to evaluate Echinacea pills and physician-patient interaction relative to placebo effects. The pill arm comprised of a) no pill, b) concealed placebo, c) concealed Echinacea and d) unconcealed Echinacea; while the physician-patient arm comprised of a) no interaction, b) usual interaction and c) enhanced interaction (including physician empathy).[74, 85] Primary outcomes were severity and duration of ARI illness. Duration was defined as the time from symptom onset until the participant responded with a "No" to the question "Do you think you still have a cold"? ARI severity was assessed using area-under-the-curve (AUC) trapezoidal approximations with duration on the x-axis and WURSS-21 symptom scores on the y-axis. Findings showed illness duration (mean days) and global severities (mean AUC) were not statistically different between the pill-groups. [85]

Data 4 (Meditation or Exercise to Prevent Acute Respiratory Infection aka MEPARI)

Between September, 2009 and May, 2010, healthy Madison, WI residents were monitored following randomization into meditation, exercise or wait-list control groups. Participants were enrolled if they were ≥ 50 years old and reported a minimum of 1 cold per year.[7] Previous histories of meditation training, moderate exercise practices, autoimmune or immunodeficiency were reasons for exclusion. Eligible participants who completed a 2-week run-in trial were enrolled in the main study. Contact information and baseline data were self-reported on questionnaires during the run-in trial.

Interventions using mindfulness meditation or moderate intensity exercise were given for durations of 8 weeks including weekly 2-1/2 hour group sessions and daily 45 minute home practice. Meditation was assessed using Mindfulness Attention Awareness Scale (MAAS)[51] while exercise including walking or jogging was assessed using International Physical Activity Questionnaire (IPAQ).[52] Borg's Rating of Perceived Exertion (RPE)[50] was used to determine the exercise intensity. Bi-monthly and weekly interviews were used to monitor participants including control.

ARI illness was self-reported on the WURSS-24, with fever, headache and body ache added to WURSS-21 to account for influenza-like illness. For an illness episode to be counted, 2 consecutive daily responses with "Yes" to the question "Do you think you have a cold?" were required. Two days in a row answering "No" to "Do you think you are still sick?" confirmed the end of illness episode. Primary outcomes included duration: - assessed as total days of ARI illness, and global illness severity: -calculated using area-under-the-curve trapezoidal approximations, with WURSS severity ratings as the y-axis and duration as the x-axis.

Of 154 enrolled participants, n=149 (951 person days of illness) completed the trial. Reasons for withdrawal were unrelated to study outcomes. Participants were mostly female (82%) with mean age 59.3years (Standard deviation 6.6). N=66 participants (951 person-days of illness) reported ARI illness during the study. Of the total 951person-days of illness, 674 were accumulated during the 1st illness episodes and will be included in our current analysis, while 277 person-days of illness from the 2nd and 3rd ARI episodes

will not be included. ARI incidence and global severities were worse among the control (n=28, mean AUC =358) compared to meditation (n=21, mean AUC =144) and exercise (n=17, mean AUC =248). Mean duration of illness was also higher among control (9 days) compared to either meditation or exercise (5 days each).[7]

Statistical analysis

The WURSS introductory ("*How sick do you feel today?*") and concluding items ("*Compared to yesterday…*") were excluded from this analysis because they are designed to measure different time frames and are analyzed separately.

Analysis was restricted to the 1st 3 days of illness because ARI illness severity tends to peak within this time.[86] The Kroonenberg and Lewis (KL) approach[43] was adopted. Briefly, the KL-approach depicted splitting the data into 2 subsets with, approach-1 described as exploratory analysis using subset-1 and approach-2 as confirmatory factor analyses on subset-2. Subsequently, the combined subsets were used to obtain final estimates of the reduced WURSS model. The invariance of the reduced model was evaluated using the 1st, 2nd and 3rd days of illness.[87] Stability of measurement parameters including factor loadings were employed in model invariance assessment during these illness days.

Approach-1 used exploratory factor analysis (EFA) to identify the underlying dimensional/factor structure towards a reduced number of WURSS-21 items with significant loadings. An oblique solution was used to account for correlations between the factors. The weighted least squares with mean and variance adjustment (WLSMV

estimator) was used to model the ordered categorical nature of the WURSS items. The feasibility of between 1-10 dimensions was evaluated and the best dimensional model was selected. Model choice was not only based on dimensional cohesion, but also on satisfactory applications of factor analysis. Utilized applications include observed break point on scree plot, number of eigenvalues > 1, cumulative percent of variance explained by model, factor loadings ≥ 0.30, minimal number of cross loading items, and each factor having ≥ 3 WURSS items.[88]

Approach-2 employed confirmatory factor analysis (CFA) on subset-2 to examine the model fit of the selected dimensional structure, including retained WURSS items. Assessing the measurement model validity occurs when the theoretical measurement model is compared with the reality model to see how well the data fits. To check the measurement model validity, several key indicators helps us. For example, Chi-square test and other goodness of fit statistics including RMSEA, CFI, and TLI are some key indicators that help in measuring the model validity.

The chi-square $P>0.05$ (failure to reject the null hypothesis that the specified model is correct) indicates good model fit using expected and observed covariance matrices. Because of the influence of sample size on chi-square statistic, it is useful to include the following indices of fit. [89, 90]Comparative fit index (CFI > 0.95 acceptable fit) or Tucker-Lewis index (TLI > 0.95 acceptable fit) estimates the ratio of sample variance and covariance explained by the model while root mean square error of approximation (RMSEA <0.06 acceptable fit) estimates the model's lack of fit compared to a perfect model. R-square (≥ 0.5 acceptable) measures the proportion of variance

explained by the selected model while residuals and standardized residuals estimate the additional variance not explained.[91] Factor correlations and loadings (standardized coefficients) were also used to examine the association between each dimension and corresponding WURSS items.

NCSS®2007[54] and Mplus® Version 6.12[92] statistical programs were used for analyses.

Results

Among all 4 studies which spanned from 2002 to 2010, total target enrollment was n=1269 ARI sufferers while n=1159 completed the studies. However, n=1167 participants reported ≥ 1 day of illness on the WURSS-21 and were included in this analysis. Of the 1167, most were female (66%), non smokers (65%), and college graduates (49%) with a mean age of 35 years (standard deviation of 15). Participants ranged from 12 to 83 years old while 5% (n=62) of the participants were ≤17years. Randomization (without replacement) of the complete data into 2 subsets yielded n=584 participants in subset-1 and n=583 in subset-2 for the KL-approach. Results showed a decrease in number of observations with each consecutive increase in days of illness. This may have resulted from loss to follow up during the original studies or following early recovery from ARI illness. Therefore data from illness day-1 (n=1167) was used because it contained all observations compared to day-2 (n=1155) or day-3 (n=1152).

The proportion of missing data was less than 1%. Item representing *Head congestion* had the most missing data 0.9% (n=11) while *Cough* had the least 0.2% (n=2). Little's missing completely at random test (MCAR) failed to reject the null hypothesis that no identifiable pattern exists to the missing data[93] The combined random pattern of missing values and estimated low degrees of missingness may reflect ease of WURSS items among participants.

Frequency of rating items as "0"(symptoms or interference with daily function and quality of life) varied among the WURSS items. Among the WURSS-21 items, rating of "0"-"do not have this symptom" ranked least for *Feeling tired* (6%) and most for *Chest congestion* (55%). Items with >30% of their responses having "0" rating were; *Live your personal life* (n=376), *Hoarseness* (n=377), *Interact with others* (n=420), *Work inside the home* (n=467), *Work outside the home* (n=505) and *Chest congestion* (n=640). (See figure 4.1) These 6 items were excluded from further investigation because they demonstrated significant floor effects and therefore may be less able to assess severity over time of ARI illness.

Visual inspection of the correlation matrix (not shown) indicated 3 dimensions/factors for the WURSS items. These include: 1-Head or Nasal; 2-Chest or Throat; and 3-Quality of life (QOL) or Functional impairment. The item-correlations were significant within each dimension with higher correlation among the QOL dimensions (0.5 to 0.8) compared to Nasal (0.3 to 0.6) and Throat (0.2 to 0.7). Six items (*Live your personal life, Interact with others, Work outside, or inside the home,*

Accomplish daily activities, and Walk-climb stairs-exercise) were redundant with higher correlations. Subsequently, only *Accomplish daily activity* was retained to resolve this issue of redundancy.

Of the 19 WURSS items examined, 7 were eliminated based on >30% floor effects or redundancy. Remaining 12 items were subsequently evaluated with factor analysis using the KL-approach.

Approach-1: Exploratory Factor Analysis (EFA) using subset-1

Using breaks in scree plot and eigenvalues >1 criteria, oblique rotation indicated 3 dimensions/factors for the retained WURSS items. (Figure 4.2) The 3-factor model showed strong loadings of the WURSS items. *Cough* showed a loading estimate of <0.3 on the 3 factors. Cross-loading items (*Head congestion, Breathe easily and Sleep well*) with loadings >0.3 were excluded while remaining 9 items were retained for confirmatory analysis. Of the retained WURSS items, 3 items loaded significantly on either Nasal (*Runny-nose*, *Plugged-nose*, *Sneezing)*, Throat (*Sore throat*, *Scratch throat*, *Cough*) or QOL dimension (*Feeling tired, Think clearly, Accomplish daily activities*). (See table 4.2)

Approach-2 Confirmatory Factor Analysis (CFA) using subset-2

Confirmatory analysis of the proposed 3-dimensional model with retained 9 WURSS items showed significant reliability and content validity. Either exclusion or inclusion of *Cough* item during confirmatory analysis resulted in satisfactory indices of model fit with

good estimates. The model excluding the *Cough* item showed better fit (CFI= 0.97, TLI= 0.96, RMSEA= 0.09) compared to model including cough (CFI= 0.93, TLI= 0.89, RMSEA= 0.13). However, *Cough* was retained based on clinical judgment and its validated high frequency of occurrence during ARI episodes.

Subsequently, both subsets (-1&-2) were combined and CFA showed high internal consistency with Cronbach's α value of 0.74 for Nasal, 0.71 for Throat and 0.81 for QOL dimensions. Adequate convergent validity was shown with satisfactory composite reliability and average variance extracted. (See table 4.3) Indices of fit estimates were also satisfactory (CFI= 0.95, TLI= 0.92, RMSEA= 0.12). (See table 4.4)

The 3-factor model also demonstrated significant correlations. Factor correlation between QOL and either Nasal (0.52) or Throat (0.49) was higher than correlation between Nasal and Throat (0.27). Standardized factor loadings increased with greater illness severity ($p<0.01$) and varied among the factors: Nasal (0.69 to 0.78), Throat (0.58 to 0.85) and QOL (0.74 to 0.85). (See figure 4.3)

Finally the invariance of the 3-dimensional model was explored over ARI illness days 1, 2 and 3. Result showed an invariant WURSS instrument with stable measurement parameters including factor loadings. Findings also demonstrated satisfactory indices of model fit during these days of illness. (Not shown)

Discussion

This study demonstrates that the WURSS-21 can be reduced to WURSS-11, preserving reliability and domain structure. The introductory ("*How sick do you feel today?*") and concluding ("*Compared to yesterday, I feel that my cold is…*") items which measure different time frames were not included during the analysis. However, these 2 items have been added to the retained 9 WURSS items to form the WURSS-11. The reduced survey can be used for assessing symptom severity and impact on function and quality of daily life during ARI illness. Among the retained items, quality of life measures performed better with higher parameter estimates compared to items representing symptom severity. This supports previous studies which showed that general and quality of life items were more important to patients than specific symptoms.[10, 17]

This study proposed a 3-dimensional model following exploration of multi-dimensional models. The 3 dimensions are Nasal (*Runny nose, Plugged nose, Sneezing*), Throat (*Cough, Sore and Scratchy throat*) and QOL (*Feeling tired, Think clearly, Accomplish daily activities*). This model demonstrates satisfactory indices of fit with good reliability estimates. Most importantly, WURSS-11 retains and reflects similar dimensional construct as the parent WURSS-21. WURSS-11's retention of roughly half of the items from the parent survey may suggest a reduction of WURSS completion time by 50%.

Study results also show stability of WURSS-11 across the 1st 3 days of ARI illness. This invariant property was demonstrated using measurement parameters and

comparable indices of fit.[87] Furthermore, this study suggests WURSS-11 has good reliability and content validity. Construct validity is also satisfactory based on findings of significant convergent and discriminant validities. However, prospective studies are needed to compare the performance of the WURSS-11 to its parent WURSS-21 and perhaps to validated questionnaires such as SF-8 and Jackson scale.

This study may be limited by the lack of external validation against other questionnaires and lack of diversity in the study population because participants were recruited from the Madison, WI community (mostly Caucasians). However, generalizability may be strengthened by diverse participant age groups, large time span (2002-2010), different seasons of ARI illness with incidental viral microbes, and most importantly the large sample size (n=1167). The similarities between the inclusion and exclusion criteria among all 4 WURSS studies are additional strengths.

In conclusion, this study has successfully created the WURSS-11 which is a shorter version of the current WURSS-21. Study findings speculate that the WURSS-11 having similar dimensional structure as WURSS-21 would retain items important to common cold sufferers and at the very least would also be as reliable and responsive as the longer versions of the WURSS survey. This shortened self-reporting survey may reduce WURSS completion time and increase its response rate but certainly needs additional validation in future studies.

Table 4.1: Study data included for Wisconsin Upper Respiratory Survey item reduction

Study data (period)	Number of participant† (% female)	Mean age in years (SD*)	Person days of monitored illness	Inclusion criteria (not all inclusive)	Exclusion criteria (not all inclusive)
All data	1167 (66%)	35 (15)	9622		
Data 1 (3/02 to 8/03)	151 (70%)	35.5 (15)	1681	≥ 18 years old; ≥3 points on Jackson scale**	Any nasal or throat symptom >48 hours in onset; Pregnancy; use of antibiotics, antihistamine or decongestants; active symptoms of allergy or specified chronic diseases
Data 2 (11/03 to 11/07)	232 (67%)	34.1 (14)	2457	≥2 Jackson scores**; Answer "Yes" to "Do you think you have a cold?"	Any nasal or throat symptom >48 hour in onsets; History of asthma, allergy or other non-URI[#]
Data 3 (1/04 to 8/08)	718 (64%)	33.7 (14)	4810	≥12 years old; ≥ 2 points of the Jackson scale**; Answer "Yes" to the question "Do you think you have a cold?" or Do you think you are coming down with a cold?"	Any nasal or throat symptom >36 hours in onset; Pregnancy; History of asthma with ongoing cough, sneezing or shortness of breath, allergic rhinitis, immune system disorders. Use of antibiotics, antihistamine or decongestants.

Continuation of Table 4.1: Study data included for Wisconsin Upper Respiratory Survey item reduction

Data 4 (9/09 to 5/10)	66[##] (82%)	59.3 (7)	674[###]	≥ 50 years old; Must have had at least 2 colds in the last 12 months?" Answered "Yes" to either: "Do you think you are coming down with a cold?" or "Do you think that you have a cold?" ; ≥ 2 points on the Jackson scale**	Previous meditation training or engagement in moderate intensity exercise twice per week; History of immune disease; use of antibiotics or antiviral medications; Any reason for not receiving flu shot during the study.

Legend for Table 4.1: Study data included for Wisconsin Upper Respiratory Survey item reduction

†Number of participants who reported ≥ 1 day of ARI illness on WURSS-21.
*SD=Standard deviation; **Jackson score with 8 symptoms (sneezing, nasal discharge, nasal obstruction, sore throat, cough, headache, malaise, and chilliness) .Symptom severity rated as 0 = absent, 1 = mild, 2 = moderate, or 3 = severe for each of the eight symptoms. Participant must have ≥ 1 nasal or throat symptoms of less than 36-48 hours duration;
[#] URI=upper respiratory illness; [##] 66 participants had been ill from the total of 149 participants monitored during the study; [###] 674 person days of illness were contributed during the 1st cold episode. Combined 2nd and 3rd episodes of ARI episodes generated 277 person days of illness which will not be included in this analysis. A total of 951 person days of illness were monitored during the study.
Acute respiratory illness began on the day participant answered "Yes" to the question "Do you believe that you are coming down with a cold?" and had ≥ 1 nasal or throat symptom from the Jackson scale. Duration was defined as the number of days of reported ARI illness to the last day before the participant answered "Not sick" to the question, "How sick do you feel today?" Or "No" to the question, "Do you think you have a cold?"

Table 4.2: Approach -1(Exploratory Factor Analysis) *

	Dimension/Factor (F)		
WURSS items	F1-loading (Nasal) (Standard error)	F2-loading (Throat) (Standard error)	F3-loading (Quality of Life) (Standard error)
Runny nose	**0.76 (0.025)**	0.030 (0.044)	-0.0090 (0.020)
Plugged nose	**0.71 (0.036)**	-0.040 (0.030)	0.16 (0.054)
Sneezing	**0.70 (0.035)**	0.043 (0.044)	-0.021 (0.038)
Sore throat	-0.030 (0.017)	**0.64 (0.063)**	0.15 (0.055)
Scratchy throat	0.057 (0.072)	**1.035 (0.071)**	-0.003 (0.0040)
Cough	0.18 (0.054)	**0.25 (0.046)**	0.28 (0.055)
Head congestion	0.46 (0.040)	0.040 (0.030)	0.40 (0.046)
Feeling tired	-0.030 (0.041)	0.064 (0.043)	**0.74 (0.037)**
Think clearly	-0.0040 (0.010)	-0.025 (0.027)	**0.83 (0.022)**
Sleep well	0.33 (0.045)	0.048 (0.033)	0.52 (0.043)
Breathe easily	0.52 (0.039)	-0.043 (0.029)	0.45 (0.047)
Accomplish daily activities	0.040 (0.045)	-0.018 (0.024)	**0.85 (0.035)**

WURSS-21excluding 2 items "How sick do you feel today?" and "Compared to yesterday, I feel...?"
Loading estimates in bold indicate domains where WURSS items belong.
Bold borders indicate cross-loaders, to be excluded.
*ARI day-1 data were explored using subset-1.

Table 4.3: Estimates of Reliability and Validity Wisconsin Upper Respiratory Symptom Survey (WURSS-11).

Dimensions / Estimates	Nasal	Throat	Quality of life
Cronbach's α	0.74	0.71	0.81
Composite reliability	0.77	0.78	0.84
Average variance extracted	0.53	0.55	0.64

Adequate convergent validity = Composite reliability> Average variance extracted or Average variance extracted >0.5

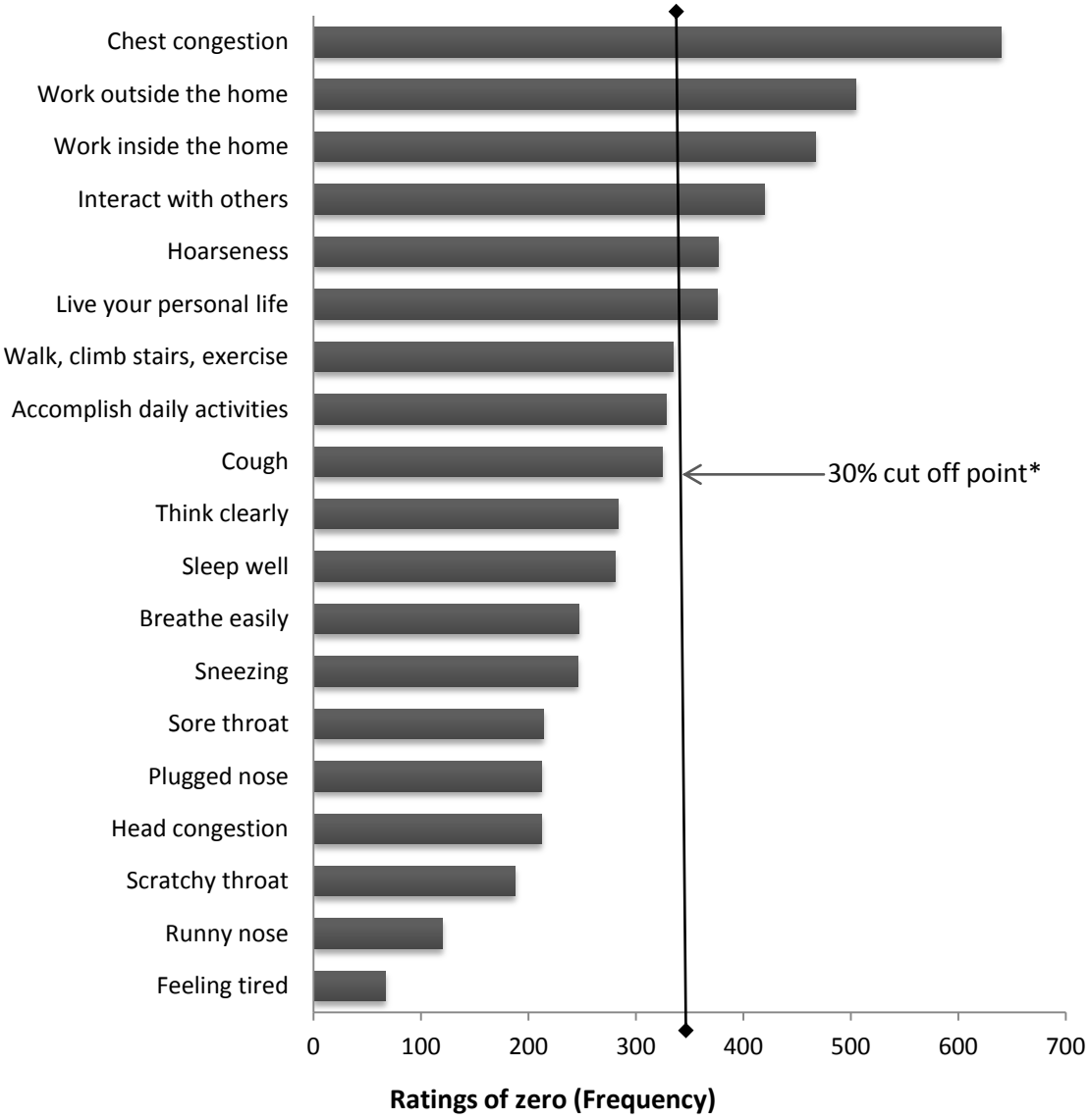
Table 4.4: Approach -2(Confirmatory Factor Analysis)*

Model	χ^2	DF		RMSEA	CFI	TLI	WRMR
Subset-2 of day-1 data	270.91	24	11.29	0.13	0.93	0.89	1.41
Combined day-1 data	443.19	24	18.47	0.12	0.95	0.92	1.76

χ^2=Chi-square; DF=Degree of Freedom; root mean square error of approximation (RMSEA <0.06 acceptable fit); comparative fit index (CFI > 0.95 acceptable fit) or Tucker-Lewis index (TLI > 0.95 acceptable fit); weighted root mean square residual (WRMR).

* ARI day-1 data using subset-2 and combined subsets. Both models include cough.

Figure 4.1: Frequency Ratings of Zero on WURSS-21 during Acute Respiratory Illness



*Any WURSS item with >30% its total count (n=1167) indicating ratings of zero were not retained for further investigation.

Figure 4.2: Scree Plot.

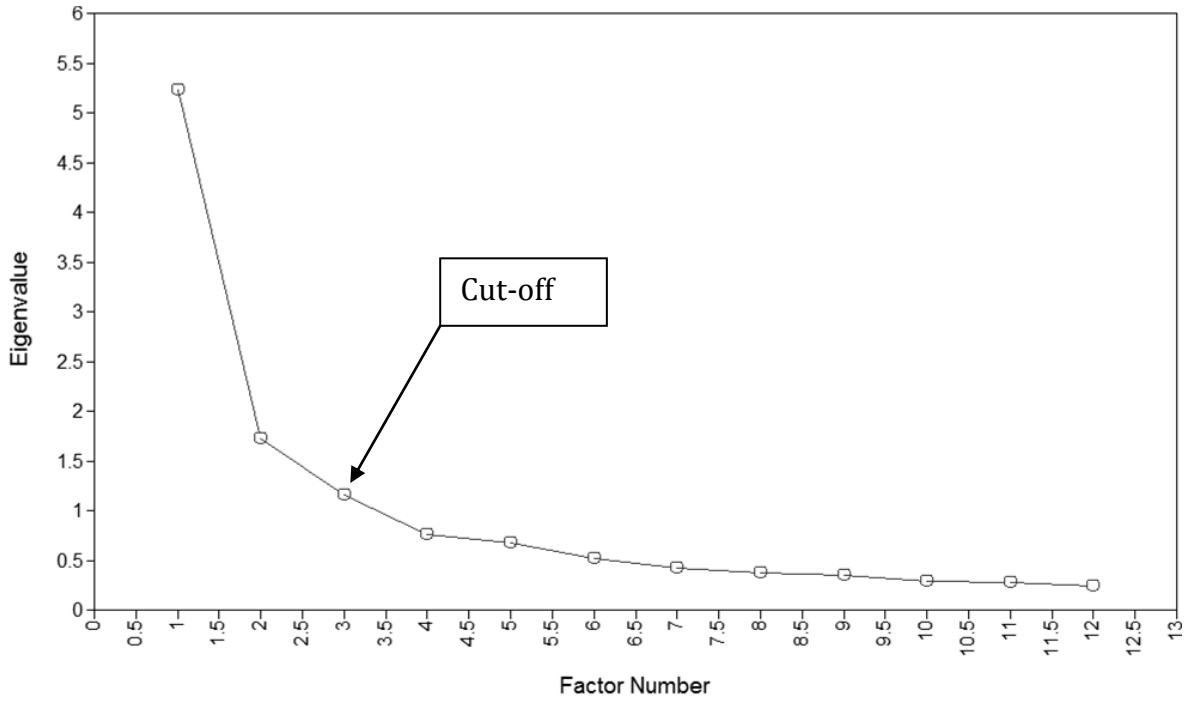
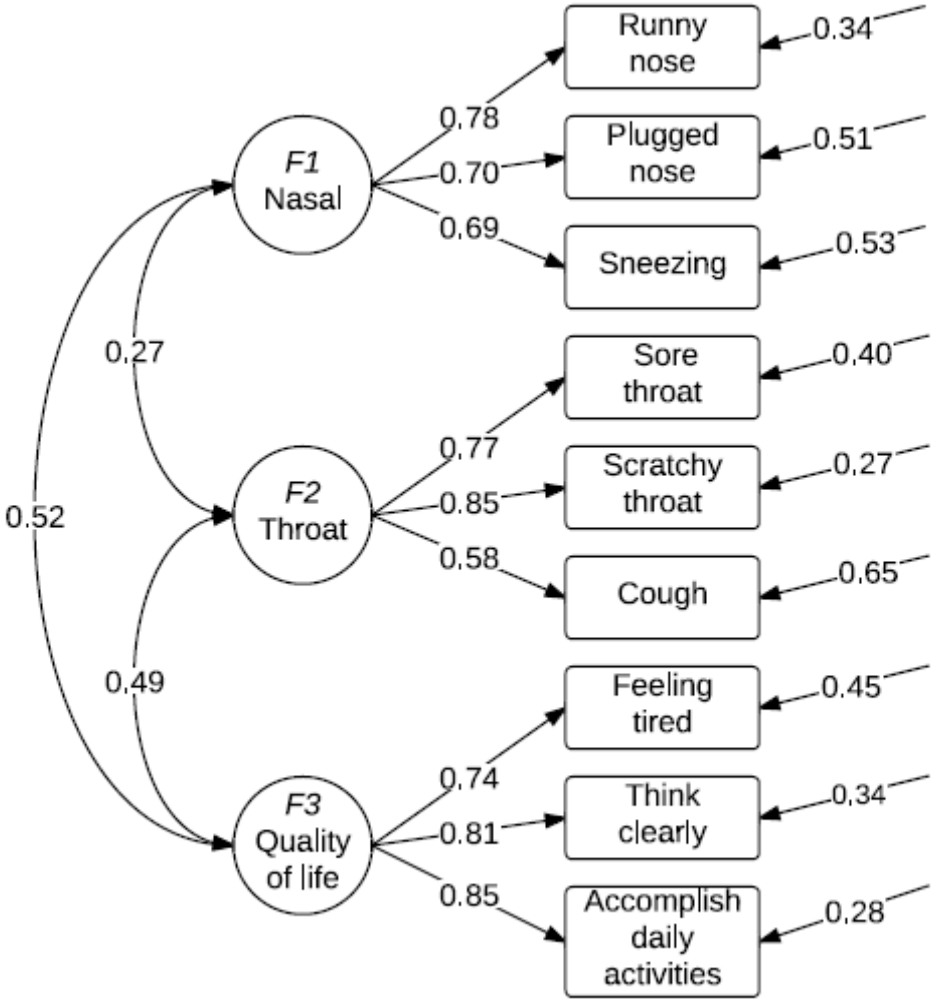


Figure 4.3: 3-dimensional factor model of Wisconsin Upper Respiratory Symptom Survey (WURSS-11)*



*Standardized values
Introductory "How sick do you feel today?" and concluding item "Compared to yesterday, I feel...? are not shown .

CHAPTER 5. DISCUSSION

5.1 Overview

This chapter summaries results from the 3 manuscripts which: 1) evaluated the rationale for better reduction in global severity of illness from meditation, compared to exercise based on results of the MEPARI study, 2) evaluated the frequency of bacterial co-infection in participants with ARI illness and compared the relationship of these biomarkers to illness severity scores, and 3) produced a smaller and easier to use version of the WURSS by reducing the number of items**.**

5.2 Summary of Results

5.2.1 Manuscript 1

This manuscript examined whether apparent advantages following training in meditation over exercise can be attributed to specific symptoms, functional impairments, or quality of life indicators assessed by the WURSS-24. This study hypothesized that there would be differences when comparing specific WURSS items estimates between exercise and meditation. Study data supported this hypothesis.

This study showed that among the 3 groups, meditators showed the lowest severity estimates for 21 of 22 WURSS items. Item-level Cohen's *"d"* indicated most benefit was evident in WURSS items representing function and quality of life. Compared to exercise, meditation fostered larger reductions in illness severity, although due mostly to improved function and the quality of life domain (d = - 0.33, $p<0.001$) compared to

symptom domain (d = -0.22, $p<0.001$). Finally, this study demonstrated that the apparent advantage of training in meditation over exercise for reducing cold and flu illness is explained more by improved function and quality of life than by a reduction in symptom severity.

5.2.2 Manuscript 2

The purpose of this manuscript was: 1) to evaluate the frequency of detectable pathogenic bacteria during ARI illness and 2) to examine the relationship between pathogens and severity of illness. This study hypothesized that compared to virus only or no pathogen detection, bacteria detection would be associated with greater severity of illness. This hypothesis was corroborated with study data.

Study results showed 37% (n=71) of the total nasal wash specimens had bacteria. Virus-negative specimens had 46% (n=45) bacteria compared to virus-positive 27% (n=26). *Haemophilus influenza* occurred in 28% of the total sample compared to *Moraxella catarrhalis* (14%) or *Streptococcus pneumonia* (7%). Compared to virus-only, presence of bacteria was associated with increased biomarker levels. Finally, this study suggested that the presence of pathogenic bacteria may be associated with increased severity of ARI illness. It also demonstrated that compared to virus-positive illness, virus-negative ARI illness may be associated with more pathogenic bacteria.

5.2.3 Manuscript 3

The purpose of this study was to develop a shorter version of the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) – a self reporting instrument for evaluating daily symptoms and functional impairments during ARI illness. This objective was supported by study data.

Study analysis produced an 11-item instrument with similar dimensional structure as the original WURSS-21. EFA indicated a 3-dimensional /factor structure labeled as Nasal (*Runny nose, Plugged nose, and Sneezing*), Throat (*Cough, Sore or Scratchy throat*) and Quality of life (*Feeling tired, Think clearly and Accomplish daily activities*). Cross-loading items (*Sleep well, Breathe easily and Head congestion*) were excluded from the final instrument. Confirmatory indices of fit were satisfactory and were stable during the 1st week of illness. Finally, this study demonstrated a reduced and reliable WURSS-11 with similar dimensional structure as the WURSS-21. This shorter version may reduce the time and burden used in completing the survey, nonetheless requires further validation.

5.3 Potential Limitations

This section highlights limitations that are general to the objectives of this study. However, limitations particular to each specific aim has been described within the corresponding manuscript (chapters 2-4).

5.*3.1 Potential biases resulting from self report*

The WURSS is a self report survey used in documenting the severity of ARI illness and is rated on Likert scales. Such self-reports could be associated with over- or under-estimation of participants' symptoms and functional impairments, which may potentially bias study results. However, the use of Likert scales to rate each WURSS item may provide better estimates of participant's underlying condition than a "yes/no" response, and is standard for these types of outcome instruments.

5.3.2 *Limitations related to use of homogenous population*

Study data were collected from participants who reside within Dane county Wisconsin. The county population is estimated around half a million persons, and mostly white (82%), high school graduates (94%) with median household income around $60,000.[(94)] This homogenous distribution is reflected in the study population (predominantly Caucasians) which may limit the generalizability of the study results to groups including Blacks.

5.4 Implications

5.4.1 Public health

Findings from this study have public health implications. Manuscript-1 supports the use of meditation or exercise to improve daily function and quality of life, and to reduce severity of illness. These common and easy to use health practices may help reduce the

economic burden of ARI illness on the US health care system. Findings from manuscript-2 are intriguing and demonstrate need for further research on reduction of pathogenic nasopharyngeal bacteria, but do not support antibiotics prescription for uncomplicated common colds.

5.4.2 Research participation

Manuscript-3 demonstrates a reliable and reduced WURSS-11, which may increase response rates among participants experiencing survey-burn out. In addition, a reduced WURSS survey may be used in future studies involving children who otherwise would not complete a lengthy questionnaire.

5.4.3 Future research

Manuscript -2 indicated greater bacteria estimates in the absence of detectable ARI viral etiologies, and suggested that presence of bacteria is associated with increased inflammatory biomarkers. This finding may provide rationale for evaluating nasal pathogenic bacteria in asymptomatic participants.

Manuscript -3 demonstrated a reduced WURSS-11, whose performance needs to be compared against the validated WURSS-21, SF-8 and the Jackson scales. The invariance of WURSS-11 needs to be evaluated across different populations.

5.5 Conclusion

ARI illness contributes significantly to morbidity and mortality in the US and exerts a heavy financial burden on the health care system. The MEPARI trial found reduced ARI illness following the use of behavioral trainings, with mindfulness meditation resulting in better reduction of illness severity compared to moderate intensity exercise. This study builds on findings from the MEPARI trial by analyzing estimates from individual WURSS items. Study results show reduced global severity of ARI illness from meditation compared to exercise is mostly due to improved quality of life and daily activities. This study also estimates that during ARI illness, the frequency of nasal pathogenic bacteria may be higher in the absence of detectable viral pathogens and may be associated with increased levels ofinterleukin-8 and neutrophils biomarkers. Additionally, this study demonstrates a reduced WURSS-11 which not only has similar dimensional structure as the WURSS-21, but may reduce survey completion time. However, future studies are needed to validate the new WURSS-11.

APPENDIX 1

Wisconsin Upper Respiratory Symptom Survey – 21 – *Daily Symptom Report*

Date:	*Time:*	*ID:*

Please fill in one circle for each of the following items:

	Not Sick	**Very Mildly**		**Mildly**		**Moderately**		**Severely**
	0	1	2	3	4	5	6	7
How sick do you feel **today**?	❍	❍	❍	❍	❍	❍	❍	❍

Please rate the **average severity** of your cold symptoms over the last 24 hours:

	Do not have this symptom	**Very Mild**		**Mild**		**Moderate**		**Severe**
	0	1	2	3	4	5	6	7
Runny nose	❍	❍	❍	❍	❍	❍	❍	❍
Plugged nose	❍	❍	❍	❍	❍	❍	❍	❍
Sneezing	❍	❍	❍	❍	❍	❍	❍	❍
Sore throat	❍	❍	❍	❍	❍	❍	❍	❍
Scratchy throat	❍	❍	❍	❍	❍	❍	❍	❍
Cough	❍	❍	❍	❍	❍	❍	❍	❍
Hoarseness	❍	❍	❍	❍	❍	❍	❍	❍
Head congestion	❍	❍	❍	❍	❍	❍	❍	❍
Chest congestion	❍	❍	❍	❍	❍	❍	❍	❍
Feeling tired	❍	❍	❍	❍	❍	❍	❍	❍

Wisconsin Upper Respiratory Symptom Survey – 21 – *Daily Symptom Report*

Over the last 24 hours, how much has your cold interfered with your ability to:

	Not at all	**Very Mildly**		**Mildly**		**Moderately**		**Severely**
	0	1	2	3	4	5	6	7
Think clearly	❍	❍	❍	❍	❍	❍	❍	❍
Sleep well	❍	❍	❍	❍	❍	❍	❍	❍
Breathe easily	❍	❍	❍	❍	❍	❍	❍	❍
Walk, climb stairs, exercise	❍	❍	❍	❍	❍	❍	❍	❍
Accomplish daily activities	❍	❍	❍	❍	❍	❍	❍	❍
Work outside the home	❍	❍	❍	❍	❍	❍	❍	❍
Work inside the home	❍	❍	❍	❍	❍	❍	❍	❍
Interact with others	❍	❍	❍	❍	❍	❍	❍	❍
Live your personal life	❍	❍	❍	❍	❍	❍	❍	❍

Compared to yesterday, I feel that my cold is …

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
❍	❍	❍	❍	❍	❍	❍

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