ASSOCIATIONS BETWEEN PERCEIVED STRESS LEVELS AND POST-ACUTE FATIGUE SEQUELAE OF COVID-19:

RESULTS FROM A NESTED CASE CONTROL STUDY

by

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Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.

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We respectfully acknowledge the University of Arizona is on the land and territories of Indigenous peoples. Today, Arizona is home to 22 federally recognized tribes, with Tucson being home to the O'odham and the Yaqui. Committed to diversity and inclusion, the University strives to build sustainable relationships with sovereign Native Nations and Indigenous communities through education offerings, partnerships, and community service.

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ABSTRACT

Background: Continued COVID-19 symptoms past the typical illness period (≥30 days) are post-acute sequelae of COVID-19, or PASC. The most common PASC symptom is fatigue, which follow the post-viral symptoms reported by survivors of other infections. Stress has been shown to increase susceptibility to viruses and risk for chronic infectious outcomes. This study examined the relationship between perceived stress during illness onset and chronic PASC fatigue, as well as how gender, illness severity, and sleep duration affect this association.

Methods: Arizona CoVHORT participants completed baseline and follow-up questionnaires about demographics, perceived stress, and sleep patterns. Cases had PASC and fatigue lasting for at least three months, and controls had PASC cases and no fatigue at three months or later. Multiple logistic regression estimated the odds ratios (OR) and 95% confidence intervals (CIs) of the relationship between perceived stress and chronic PASC fatigue. Models adjusted for age and pre-existing conditions and were fit with interaction terms for gender and illness severity to assess effect modification. A bootstrap mediation analysis determined the proportion mediated by sleep duration in this relationship.

Results: Chronic PASC fatigue prevalence within this sample was 33%, with an average perceived stress score of 17.5±6.3. Odds of chronic PASC fatigue were 7% higher for every one unit increase in perceived stress after adjustment (95% CI: 0.99, 1.15). Women had 4% lower odds for chronic fatigue compared to men (adjOR: 0.96; 95% CI: 0.78, 1.18). Odds in those with moderate illness severity decreased compared to low illness severity (adjOR: 0.96; 95% CI: 0.78, 1.18), while high illness severity had 5% greater odds (adjOR: 1.05; 95% CI: 0.82, 1.36). 11.8% of stress's effect on fatigue was attributed to sleep.

Conclusions: Our results indicate a positive relationship between chronic PASC fatigue and perceived stress during COVID-19 onset, with marginal statistical significance after adjustment. Illness severity and gender did not significantly modify the effect of stress on fatigue, while sleep duration partially mediated this association. Further investigation of the relationship between chronic fatigue, perceived stress, and sleep is needed as PASC continues in COVID-19 survivors.

INTRODUCTION

When the COVID-19 pandemic began at the end of 2019, establishing safety protocols and vaccine measures quickly became the main priorities of the research world. However, after over 500 million confirmed cases and two million deaths worldwide in the last two years of combatting the SARS-CoV-2 virus, it has become abundantly clear that surviving this infectious disease can have unexpected long-term consequences. Symptoms that persist past the typical recovery periods for both mild and severe COVID-19 have been observed by many studies following patients' experiences post-infection.[1] This phenomenon has been officially labelled as post-acute sequelae of COVID-19 (PASC) but may also be referred to as long-COVID or long-haul COVID.[2] While waiting for an official case definition for PASC to be released by the Center for Disease Control (CDC), many researchers has used the presence of symptoms past the acute phase of illness (≥30 days) as the timeframe criteria for this condition.[3–5]

The most commonly reported symptoms among PASC cases are fatigue, difficulty breathing, myalgia, and neurological changes such as altered sense of taste and smell or post-traumatic stress disorder.[6,7] The prevalence of chronic symptoms experienced by recovered COVID-19 cases has varied considerably, with the estimated number of survivors who later develop PASC ranging from 20-70% in some studies.[8,9] This variability in results may be due to lack of clear reporting guidelines and diagnostic criteria, as well as the use of differently effected samples of the population. Despite these inconsistencies, it is clear that PASC is a growing problem in the wake of widespread COVID-19 infections, and further research on the occurrence of prolonged symptoms and possible risk factors for this condition is necessary.

In general, PASC appears to affect more women than men, and those who experienced a more severe disease course, were hospitalized, or needed supplemental oxygen via cannula or intubation have increased risk for chronic symptoms post-recovery.[4] Diagnosis with at least one pre-existing condition and increasing age have also been found to be associated risk factors for PASC.[3,10] Biomarker analyses show that those with higher serum levels of tumor necrosis factor-alpha (TNF-α) and interferon-γ-induced

protein 10 during their acute infection phase have a higher risk for developing PASC later on, and those who report persisting symptoms can have elevated levels of interleukin-6 (IL-6), peroxiredoxin 3 (PRDX3), and carbamoyl phosphate synthase (CPSI) up to six months or more during their post-recovery period.[11,12] While elevated levels of inflammation are to be expected after an infection, especially for those who experienced cytokine storms or more severe illness, this trend in association with prolonged symptoms in recovered COVID-19 cases indicates that the acute infection may leave lasting damage within the body.

As with inflammation, some level of fatigue is expected after an illness as the body recuperates, but long-term fatigue like that being reported by PASC cases is often a sign of a persisting issue within the body and can become a debilitating problem. Fatigue operates on a physical, psychological, and social level, where one's ability to function normally in society and complete their usual tasks is drastically reduced as a result of extreme exhaustion or lack of energy.[13] Disrupted or disordered sleep has been proposed as a possible cause of fatigue in the past, but those who consistently experience fatigue tend to sleep for longer periods of time than those without fatigue, and their sleep is not restful or restorative even if they sleep without disruptions.[14] When patients report prolonged symptoms of fatigue that cannot be attributed to another illness or over-exercise, they may be diagnosed with myalgic encephalomyelitis, which is also known as chronic fatigue syndrome (ME/CFS).[15]

ME/CFS is a largely undiagnosed health condition that effects approximately eight million Americans according to the CDC.[16] The diagnostic criteria for this chronic fatigue disorder established by the Institute of Medicine in 2015 includes the presence of fatigue for over six months, unrestful sleep, abnormal post-exertion malaise, and some degree of cognitive or orthostatic dysfunction, while juvenile diagnoses and the National Health Service's 2021 criteria only require three months of symptoms.[17,18] In order to be diagnosed with ME/CFS, cases must first be screened for conditions such as Hashimoto's thyroiditis, rheumatoid arthritis, or multiple sclerosis, and it is extremely comorbid with other fatigue related disorders such as fibromyalgia, irritable bowel syndrome, and mood disorders.[19] The etiology

behind ME/CFS has yet to be established, but genetic patterns, mitochondrial dysfunction, and environmental factors have all been explored as possible causes.[20] When comparing similarities between ME/CFS and PASC symptoms, another exposure of interest is viral infections, as increased chronic fatigue symptoms are associated with higher levels of viral particles in the cerebrospinal fluid (CSF).[21]

Post-viral fatigue is a common symptom in survivors of viral infections, and many of those who recovered from Epstein Barr virus (EBV), severe acute respiratory syndrome (SARS), or Middle Eastern respiratory syndrome (MERS) have also reported ME/CFS-like.[15,22] In one study on adolescents recovering from infectious mononucleosis, 14% of cases were diagnosed with ME/CFS after 6 months, while 7% continued to have ongoing chronic fatigue one year after their initial infection.[23] Recovered SARS cases have reported high levels of lingering fatigue and generalized body pain, as well as depression and anxiety.[24] Poor sleep and decreased lung function for up to a year or more after their primary illness has also been found to be common in both SARS and COVID-19 patients.[25,26] Similarly, those who survived MERS have reported reduced quality of life and increased fatigue levels after twelve months, with approximately 30% of patients still struggling with PTSD and sleep disturbances at eighteen months post-infection.[27,28] Viral particles have also been identified in the CSF of recovered COVID-19 cases, which is likely due to the increased ability of the SARS-CoV-2 virus to permeate the blood brain barrier.[2]

When studying the relationship between viral infections and these post-acute fatigue symptoms, there are multiple external factors that should be considered, especially as research is needed to understand why some people develop PASC fatigue and some do not. One possible aggravating factor of interest is stress. Stress is commonly described as both a physical and a psychological state of high arousal due to some triggering event.[29] It varies from person to person, and some individuals are more resilient when it comes to coping with high stress events or environments based on their lived experience. The body releases counter-regulatory hormones such as glucagon, epinephrine, norepinephrine, and cortisol in response to stress, and this change sends the body into “fight or flight mode” resulting in

changes in heart rate, breathing patterns, and metabolic processes.[30] In general, a small amount of stress can be beneficial due to its ability to strengthen threat appraisals, social connections, and specific adaptive and innate immune cell functions, but heightened or chronic levels of stress can have damaging effects on the body's immune system and regulatory processes.[31]

When stress becomes chronic, the body enters into a state of overarousal according to the Yerkes-Dodson Law, where the body can experience increased levels of fatigue, anxiety, and breakdown.[32] Prolonged exposure to stress is associated with risk for health conditions such as diabetes, heart disease, and ulcerative colitis, and it is also linked to heightened susceptibility to viral infections because of the long-term suppressive effect of stress on the immune system.[33,34] Overproduction for cortisol can lead to adrenal dysfunction and neuroinflammation, which structurally changes in the hypothalamo-pituitary adrenal (HPA) axis and disrupts the release of regulatory hormones needed to return the body to homeostasis.[35] Neuroinflammation due to stress can increase the permeability of the blood brain barrier and allow irregular movement of molecules into the CSF.[36–38] Likewise, it is also alarming when considering the implications for PASC cases because of the previously discussed ability of SARS-CoV-2 virus to pass through the blood brain barrier and because of the widespread stress experienced by individuals throughout the COVID-19 pandemic.[2]

There are many factors that influence the impact of stress on an individual, such as gender and surviving a severe illness, and sleep is another important component when discussing the stress-fatigue relationship.[39] For most individuals, sleep has restorative properties that assist in recovery and recuperation from illnesses and stressful events, which is some people sleep longer during or right after an illness.[40] Shorter sleep duration has been associated with daytime tiredness in people with good and poor sleeping habits, but for cases who have recovered from a viral infection, they tend to sleep for abnormally long periods of time without interruptions and still report fatigue.[41,42] Similarly, ME/CFS diagnoses require lack of restorative sleep in conjunction with chronic fatigue.[43] Interestingly, disrupted sleep patterns or lower sleep quality have been found to impact one's ability to effectively cope with stressful

events which contributes to the state of overarousal and fatigue.[44] High levels of stress can also negatively influence sleep quality and duration, which then aggravates fatigue and exhaustion, especially when the exposure to stress is chronic in nature.[45] Both sleep disturbances and duration of sleep are established mediators in the relationship between general stress and fatigue, so it is crucial to consider the possible mediating effects when studying post-viral fatigue and PASC.[46,47]

Based on the previously established evidence in the literature, there is a strong association between stressful events and the development of chronic fatigue.[13,34] High stress during one's illness has also been associated with the development of post-viral fatigue, so further examination of the relationship between stress and PASC-associated fatigue is necessary due to the high stress levels experienced by many throughout the COVID-19 pandemic.[48–51] Therefore, in this study, we use a nested case control study from a cohort of COVID-19 cases in order to:

1) determine if increased perceived stress levels during month of acute illness onset is associated with chronic PASC fatigue, or post-acute fatigue sequelae of COVID-19 for three months or more;
2) examine if the association between perceived stress and chronic PASC fatigue cases varies by gender and illness severity, with the hypothesis that the effect will be greater for women and those with moderate to high severity in comparison to their respective reference groups; and
3) analyze whether sleep duration has a mediating effect on the relationship between perceived stress and chronic PASC fatigue.

METHODS

Study Design & Sample

Data for the proposed analysis were extracted from the prospective population-based cohort study on COVID-19 survivors known as CoVHORT, which has been conducted in conjunction with the University of Arizona and the Arizona Department of Health Services beginning in May 2020.[52] CoVHORT recruited participants and gathered data using online and paper advertising and over the phone interviews. After baseline enrollment, surveys designed to measure patient well-being were administered every three months subsequently, while surveys to assess patient symptoms were administered one and half months post-baseline and subsequently every three months. CoVHORT participants who reported testing positive for COVID-19 with a positive PCR or antigen test and had persisting symptoms for ≥ 30 days post-positive test were included in the study. These participants were all considered to have PASC. Participants without reported PASC or those with COVID-19 symptom onset dates ≥ 30 days prior to their baseline interview were excluded due to the nature of the stress measurement tool. Those with missing data for all variables of interest were also excluded, as were those who self-identified as a gender other than male or female.

Measures

Outcome

Chronic PASC fatigue was the outcome of interest for this study. The standard definition of chronic fatigue in the United States for adults is fatigue lasting ≥ 6 months.[16] However, given that there is no formally agreed upon definition of PASC and that most definitions are <6 months, we defined PASC chronic fatigue as persisting fatigue symptoms for 3 months or more based on the diagnostic criteria for ME/CFS as recommended by the National Health Service and the CDC's adolescent case definition.[53] Those who reported PASC symptoms for 30 days or longer post-infection and continued to experience

fatigue symptoms at the 3 months well-being follow up and beyond were considered cases, while those with PASC but no fatigue after 3 months were considered controls.

Exposure

We defined perceived stress during month of illness onset as the exposure of interest in this study using the Perceived Stress Scale (PSS-10) that was administered at baseline.[52] The PSS-10 is a 10-item questionnaire that uses a 5-point Likert scale to assess participants' perception of their stress levels within the last month.[54] The total perceived stress score is computed after reverse scoring the positively worded items (Items 4, 5, 7, 8) and can range from 0 to 40. Previous studies using the PSS-10 have identified two main factors within the ten items, where negatively worded items are associated with perceived helplessness, while the positively worded items are associated with perceived self-efficacy.[55] The internal validity of the PSS-10 in those with both English and Spanish languages preferences has been tested using Cronbach's alpha ($_{English}\alpha = .87$; $_{Spanish}\alpha = .78$) and found to be adequate.[56]

Covariates

Ethnicity (Hispanic vs. Non-Hispanic) was examined for descriptive purposes but was not included in the final model. Covariates added to the model were selected *a priori* through a review of the literature on possible confounders in the relationship between stress and fatigue. Based on previously established risk factors for PASC, age (continuous; years) and at least one pre-existing condition with heightened immunological response to COVID-19 (categorical; cardiovascular disease [myocardial infarction, heart disease, congestive heart failure, stroke, and hypertension], diabetes [type I and type II diabetes, pre-diabetes, and gestational diabetes], obesity [Body Mass Index (BMI) ≥ 30.0 kg/m2], respiratory disease [asthma, chronic obstructive pulmonary disease (COPD), coccidiomycosis (Valley Fever), and emphysema/chronic bronchitis], and inflammatory bowel disease [ulcerative colitis, Crohn's disease, and general colitis]) were included in the adjusted model.[3,57]

Interaction terms for gender (Male vs. Female) and illness severity were individually fit to the final model to test for effect modification with stress as suggested by previous literature.[58–61] The illness severity measure in the CoVHORT questionnaire was a scale that ranged from 1 to 10, with 10 being the most severe, but the variable was collapsed into three categories (Low Severity [1-3]; Moderate Severity [4-7]; and High Severity [8-10]) based on the criteria established in the literature on illness severity.[62] Finally, sleep duration after COVID-19 infection (hours) was included as a mediating covariate in the third aim analysis based on previous research on sleep, stress, and fatigue.[46,47,63]

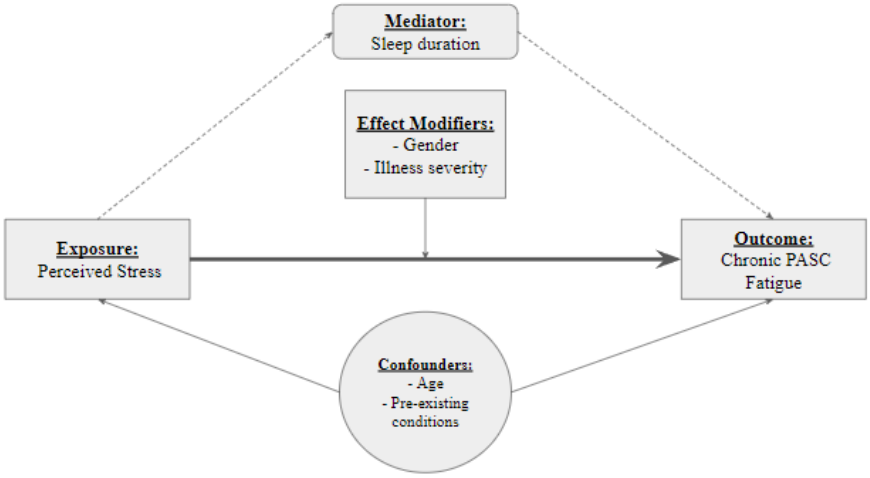

Figure 4. Relationship between perceived stress and chronic PASC fatigue as illustrated by directed acyclic graph.

Statistical Methods

Descriptive summary statistics were estimated for cases (PASC cases with chronic fatigue symptoms) and controls (PASC cases without chronic fatigue symptoms). These results are presented in mean ± standard deviation (SD) format for continuous variables and frequencies and percentages for categorical variables.

To examine the possible association between perceived stress and PASC-associated chronic fatigue, we used unadjusted and adjusted logistic regression models to estimate the odds ratio (OR) and 95% confidence intervals (CI) for this relationship. To increase interpretability, we also calculated the OR for a change of one SD unit for the stress scale. The adjusted model included covariates selected *a priori* to control for confounding by age and the presence of at least one pre-existing condition.

Based on previously established literature on COVID-19 illness severity, a secondary analysis fit an adjusted model with an interaction term for stress and illness with the expectation that the effect of stress on chronic PASC fatigue would differ in cases with moderately to highly severe illness.[3] Another adjusted model was fit with an interaction term for stress and gender to test for possible effect modification, where it was hypothesized that the effect of stress on chronic fatigue in women with PASC would be higher due to gendered stress differences.[58,59]

Sleep duration as a possible mediator was analyzed with a path analysis method.[64] The statistical significance and bias-adjusted 95% CIs of the direct effect of perceived stress on chronic PASC fatigue as well as the indirect effect of these variables through the mediation of sleep were estimated using bootstrapping (n=1,000) through the *mediation* software package (R v.3.6.1).[65] The total effect, or the combined direct and indirect effects, was also calculated, with the expectation that the association between stress and fatigue will be at least partially mediated through sleep. In order to adjust for possible confounding, age and pre-existing conditions were included as covariates in the mediation analysis. The proportion mediated was estimated as a measure of the degree to which the relationship between perceived stress and chronic PASC fatigue can be attributed to sleep duration.

We used multiple imputation with chained equations in a sensitivity analysis in order to address missing perceived stress data at baseline and to test the robustness of our original primary analysis model.[66,67] After confirming that the missing data could be assumed to be missing at random and that the prediction model was of good fit, two hundred imputations of the composite PSS-10 total score were pooled to re-estimate the unadjusted and adjusted ORs and 95% CI for the relationship between perceived

stress and chronic PASC fatigue (**Appendix A**). We assessed logistic regression assumptions for linearity in the log-odds in continuous variables with LOESS model smoothing, lack of multicollinearity with variance inflation factors, and influential observations with Cook's distance. Goodness of fit for all models was tested using the C-statistic, and we determined statistical significance using a two-sided Type I error rate of < 0.05. All statistical analyses performed in this study used R v3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

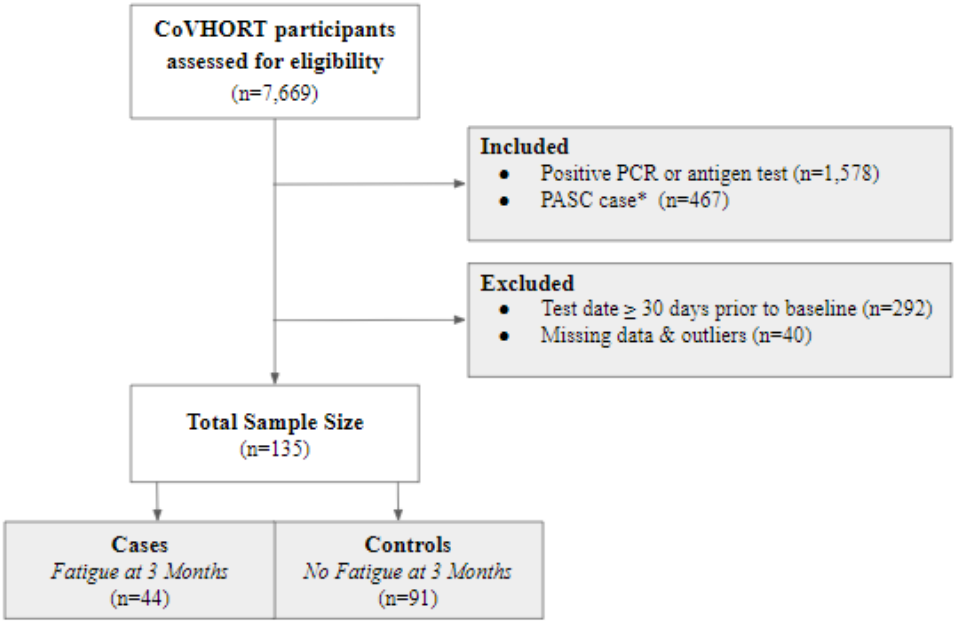

Figure 5. Flowchart of participant exclusion criteria and outcome samples.
*Persisting symptoms for ≥30 days

One hundred and thirty-five CoVHORT participants met the inclusion criteria for the main analyses, with a heavily female and non-Hispanic sample (**Figure 2**). Cases who had PASC fatigue for three months or more (n=44) had an average age of 47.2 ± 17.3 years, while controls with no chronic PASC fatigue for three months or longer (n=91) were on average 44.8 ± 15.2 years old (**Table 1**). Cases also had marginally longer sleep duration (10.0 ± 2.3) than controls (9.4 ± 1.8). Those who experienced moderate illness severity during their acute COVID-19 infection made up 61.4% of cases, while 20.5% had high illness severity. The proportion of controls who experienced moderate illness severity was 49.5%, but only 14.3% had high illness severity. Similarly, 68.2% of cases had at least one pre-existing condition compared to 47.3% of controls.

Table 1. Baseline characteristics of Arizona CoVHORT participants stratified by chronic PASC fatigue (n=135)

Characteristic	Chronic PASC Fatigue (n=44)	No Chronic PASC Fatigue (n=91)	p-value
	n (%)	n (%)	χ^2 test
Gender			0.12
Male	6 (13.6)	23 (25.3)	
Female	38 (86.4)	68 (74.7)	
Ethnicity			0.24
Hispanic	11 (25.0)	15 (16.5)	
Non-Hispanic	33 (75.0)	76 (83.5)	
Pre-existing conditions			0.02
None	14 (31.8)	48 (52.7)	
At least one pre-existing condition[a]	30 (68.2)	43 (47.3)	
Illness Severity[b]			0.09
Low[c]	8 (18.2)	33 (36.3)	
Moderate[d]	27 (61.4)	45 (49.5)	
High[e]	9 (20.5)	13 (14.3)	
	Mean ± SD	Mean ± SD	t-test
Age (years)	47.2 ± 17.3	44.8 ± 15.2	0.32
Post-COVID Sleep Duration (hours)	10.0 ± 2.3	9.4 ± 1.8	0.09
Perceived Stress Score	17.5 ± 6.3	15.4 ± 4.7	0.05

Abbreviations: PASC, post-acute sequelae of SARS-CoV-2; n, number of participants; %, proportion of participants; SD, standard deviation
**Chronic fatigue is considered fatigue persisting past 3 months follow up*
Perceived stress has a possible range of scores from 0-40
[a] Pre-existing conditions with known heightened immune responses to COVID-19 are: cardiovascular disease including myocardial infarction, heart disease, congestive heart failure, stroke, and hypertension, diabetes including type I and type II diabetes, pre-diabetes, and gestational diabetes, obesity defined as having a Body Mass Index (BMI) ≥ 30.0 kg/m2, respiratory disease including asthma, chronic obstructive pulmonary disease (COPD), coccidiomycosis (Valley Fever), and emphysema/chronic bronchitis, and inflammatory bowel disease including ulcerative colitis, Crohn's disease, and general colitis
[b] Based on self-reported COVID-19 severity scale from 1-10
[c] Low;1-3 COVID-19 severity score
[d] Moderate = 4-7 COVID-19 severity score
[e] Severe = 8-10 COVID-19 severity score

In chronic PASC fatigue cases (n=44), the average perceived stress score was 17.5 ± 6.3, while the perceived stress score for controls (n=91) was 15.4 ± 4.7 (**Table 2**). There was an 8% increase in odds for chronic PASC fatigue per 1 unit increase in perceived stress (OR: 1.08; 95% CI: 1.01, 1.16), while for a perceived stress increase of one SD (5.33), odds of chronic PASC fatigue were 49% higher (95% CI: 1.04, 2.16). After adjusting for possible confounders, there was a 7% increase in odds of chronic PASC fatigue per 1 unit increase in perceived stress (adjOR: 1.07; 95% CI: 0.99, 1.15), and for increase in perceived stress per one SD (5.33), odds of chronic PASC fatigue were 41% higher (95% CI: 0.96, 2.07). After adjustment, however, the association between perceived stress and chronic PASC fatigue was no longer statistically significant (*p*-value = 0.08).

Table 2. Results of main and sensitivity analyses using multiple logistic regression for perceived stress in Arizona CoVHORT participants with and without chronic PASC fatigue

	Chronic PASC Fatigue N (%)	Average Perceived Stress Score (Mean ± SD)	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)
Main Analysis (n=135)				
Stress	44 (32.6)	17.5 ± 6.3	1.08 (1.01, 1.16)	1.07 (0.99, 1.15)
Sensitivity Analysis[b] (n=172)				
Stress	51 (29.6)	17.8 ± 6.1	1.08 (1.01, 1.15)	1.08 (1.01, 1.15)

Abbreviations: PASC, post-acute sequelae of SARS-CoV-2; n, number of participants; %, proportion of participants; OR, odds ratio; CI, confidence interval

Logistic regression compared continuous perceived stress levels (possible range 0-40) in chronic PASC fatigue cases to controls (reference group); there were no significant covariates in the adjusted model, and the model also showed slight improvement in discrimination after adjustment (C-stat: 0.59 vs. 0.65)

[a]Adjusted for, age and at least one pre-existing condition (cardiovascular disease, diabetes, obesity, respiratory disease, inflammatory bowel disease, and depression and anxiety).

[b]The sensitivity analysis used multiple imputation with chained equations to impute missing PSS-10 total scores and re-estimate the odds ratio for the relationship between perceived stress and PASC fatigue after 200 imputations.

In the sensitivity analysis using multiple imputation, 172 CoVHORT participants met the inclusion criteria and had missing data. After conducting multiple imputation with chained equations to impute the missing stress data, participants with chronic PASC fatigue (n=51) had an average perceived stress score of 17.8 ± 6.1, while the perceived stress score for those without (n=121) was 15.6 ± 4.8 (**Table 2**). The unadjusted logistic regression using the imputed data estimated an OR of 1.08 (95% CI: 1.01, 1.15), indicating the odds of chronic PASC fatigue increased 8% per 1 unit increase in perceived stress. Similarly, after adjusting for possible confounders, there were 8% higher odds of chronic PASC fatigue per 1 unit increase in perceived stress (adjOR: 1.08; 95% CI: 1.01, 1.15). Both models point to a statistically significant relationship between chronic PASC fatigue and perceived stress during month of illness onset.

The adjusted logistic regression for perceived stress and PASC fatigue was fit with separate interaction terms to test for effect modification by gender and illness severity. Women with chronic PASC fatigue (n=38) had an average perceived stress score of 17.58 ± 6.16, while perceived stress in men with PASC chronic fatigue (n=6) was on average 17.00 ± 7.54 (**Table 3**). When compared to men, women had

a 4% decrease in odds for PASC fatigue per 1 unit increase in perceived stress (adjOR: 0.96; 95% CI: 0.78, 1.18)

Average perceived stress in PASC chronic fatigue cases with low illness severity (n=8) was 16.38 ± 5.53, while cases with moderate illness severity (n=29) and high illness severity (n=9) had a perceived stress level of 16.34 ± 6.36 and 20.67 ± 6.14 respectively (**Table 3**). When compared to those with lower illness severity, those with moderate illness severity had a 4% decrease in odds of chronic PASC fatigue per 1 unit increase in perceived stress (adjOR: 0.95; 95% CI: 0.78, 1.18). In contrast, there was a 5% increase in odds of PASC fatigue per 1 unit increase of perceived stress in those with high illness severity (adjOR: 1.05; 95% CI: 0.82, 1.36).

Table 3. Results of effect modification by gender and illness severity to evaluate perceived stress in Arizona CoVHORT participants with and without chronic PASC fatigue (n=135)

	Chronic PASC Fatigue n (%)	**Average Perceived Stress Score Mean ± SD**	**Adjusted[a] OR (95% CI)**	**Test for Interaction[b]**
Gender				0.30
Males	6 (13.6)	17.00 ± 7.54	1.00 (ref)	
Females	38 (86.4)	17.58 ± 6.13	0.96 (0.78, 1.18)	
Illness severity[c]				0.32
Low severity[d]	8 (18.2)	16.38 ± 5.53	1.00 (ref)	
Moderate severity[e]	27 (61.4)	16.78 ± 6.38	0.96 (0.78, 1.18)	
High severity[f]	9 (20.5)	20.67 ± 6.14	1.05 (0.82, 1.36)	

Abbreviations: PASC, post-acute sequelae of SARS-CoV-2; n, number of participants; %, proportion of participants; SD, standard deviation; OR, odds ratio; CI, confidence interval
Logistic regression compared continuous perceived stress levels (possible range 0-40) in chronic PASC fatigue cases to controls (reference group)
[a]Adjusted for age and least one pre-existing condition (cardiovascular disease, diabetes, obesity, respiratory disease, inflammatory bowel disease, and depression and anxiety).
[b]Likelihood ratio chi-square test
[c]Based on self-reported COVID-19 severity scale from 1-10
[d]Low;1-3 COVID-19 severity score
[e]Moderate = 4-7 COVID-19 severity score
[f]Severe = 8-10 COVID-19 severity score

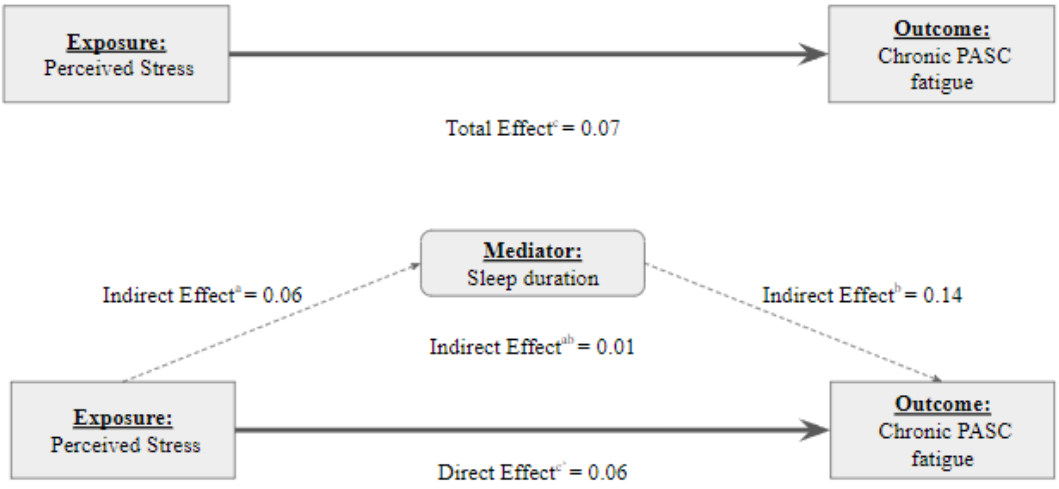

Figure 6. Effect of perceived stress on chronic PASC fatigue via sleep duration as illustrated by directed acyclic graph.

The mediation analysis pathways for the direct and indirect effects of sleep on stress and chronic fatigue can be found in Figure 3. After adjusting for age and pre-existing conditions, the direct effect of perceived stress on chronic PASC fatigue was 0.06 (95% CI: -0.04, 0.13), and the indirect effect of these variables through the mediation of sleep duration was 0.01 (95% CI: -0.01, 0.10) (**Table 4**). The combined direct and indirect effects gave a total effect of 0.07 (95% CI: -0.02, 0.14) with 11.8% of the effect of the exposure on the outcome attributed to sleep, indicating no statistically significant mediation.

Table 4. Results of mediation analysis on the effect of sleep duration on the relationship between perceived stress and chronic PASC fatigue in adult Arizona CoVHORT participants (n=135)

	β (95% CI[b])
Total effect of perceived stress on chronic PASC fatigue	0.07 (-0.02, 0.14)
Direct effect (independent of sleep duration)	0.06 (-0.04, 0.13)
Indirect effect (attributed to sleep duration)	0.01 (-0.01, 0.10)
Proportion mediated by sleep duration[c]	11.8%

Abbreviations: PASC, post-acute sequelae of COVID-19; 95% CI, 95% confidence interval; OR, odds ratio
Perceived stress has a possible range of scores from 0-40
[a] *Adjusted for age and least one pre-existing condition (cardiovascular disease, diabetes, obesity, respiratory disease, inflammatory bowel disease, and depression and anxiety).*
[b] *Bias-adjusted confidence intervals calculated via bootstrapping technique (n=1,000).*
[c] *Proportion mediated calculated as indirect effect/(total effect+indirect effect).*

DISCUSSION

Chronic PASC fatigue appears to have a positive relationship with perceived stress levels during the month of acute COVID-19 illness onset from the results of this study's primary analysis. While this association is only marginally statistically significant after adjusting for possible confounding by age and pre-existing conditions, the sensitivity analysis using multiple imputation with chained equations also demonstrates that the odds of chronic PASC fatigue significantly increase with increased perceived stress, indicating that the assumptions made in the primary analysis are robust. Although research on PASC is still fairly limited due to the lack of a consistent case definition and the new nature of this condition, previous studies have consistently found similar results where chronic fatigue symptoms are strongly correlated with post-infection stress levels.[68] Additionally, previous studies on viral infections such as SARS, poliomyelitis, and rotavirus indicate that high stress levels during illness onset are predictive of post-viral fatigue and CFS-like symptoms.[26,27,50,51,69,70] As far as we know, this is one of the first studies on post-acute sequalae of COVID-19 to attempt to examine the role of perceived stress levels during acute infection and the development of chronic fatigue.

The decrease in odds for chronic PASC fatigue in female cases is unexpected as previous studies of stress have indicated that female stress is a significant predictor of burnout fatigue, while the same effect is not observed with male stress.[71] However, while it is true that women report stress more often and have greater associated symptoms than men, they also cope with stress in more effective and healthy ways which could explain this different result.[72] Similarly, while there was no significant modification of the effect of stress on chronic PASC fatigue by illness severity, the increased odds of chronic fatigue for those with higher illness severity is consistent with the established literature that severe illnesses modify illness perceptions and perceived stress level.[11,26,73,74] The lack of significant effect modification may be influenced by the restricted size of the sample used in this study, so additional research on the impact of gender and illness severity on stress and chronic PASC fatigue is needed.

Finally, while there appeared to be a partial mediation of the effect of perceived stress on chronic fatigue in PASC cases by sleep duration, the mediating effect and the proportion mediated were not statistically significant. This result does not agree with the previous research that found that sleep duration can mediate the effect of stress on both acute and chronic fatigue through its restorative impact on immune system function.[44–46] However, this lack of mediation may be explained by the increased duration of sleep reported by the general public during the pandemic compared to prior sleep duration.[75,76] In addition to this, many studies looking at mediation by sleep use sleep quality rather than sleep duration.[77,78] Because of the established literature on sleep as a mediator and the evidence that some degree of the relationship between perceived stress and chronic PASC fatigue can be attributed to sleep duration, continued research on this mediation pathway may provide more conclusive results.

Strengths of this study include its contribution to the limited research on PASC and specifically chronic PASC fatigue. The consistency of the results between the main analysis and sensitivity analysis after multiple imputation indicates that the assumptions made in this analysis are robust. The nested case control design of this study is also a strength, as it allowed PASC cases from the same population-based cohort study to be selected as controls. In addition to this, the temporal relationship between stress during illness onset period and the later development of PASC chronic fatigue is more clearly established than it would be in a regular case control study.[79,80] This also limits recall bias as the outcome had not yet occurred when the baseline data was collected. Finally, while the PSS-10 assesses perceived stress using self-report responses from participants, many studies have validated it as a consistent and accurate measure of stress for both English and Spanish speaking individuals.[56]

However, the use of self-reported measures for all other covariates is a limitation of this study. Similarly, the wording of the sleep duration question may have resulted in measurement error when participants selected their wake time and bedtime after COVID-19, which could influence the results of this study's mediation analysis. Selection bias may also be present, as we were unable to fully restrict the sample to those without pre-existing conditions due to small sample size. The reduced sample also limited

the number of covariates included in the adjusted model, which means that not all confounders may have been appropriately controlled. The sample used was primarily female and non-Hispanic, so the results of this study may not be completely generalizable, and sparse cells for Hispanic and male cases may have influenced the effect modification analysis. Lastly, while three months of persisting fatigue has been used to categorize chronic fatigue, the general recommendation is at least six months.[18] This study was unable to use this criterion due to limited follow-up data after six months, so additional research using long-term fatigue in PASC cases with the six-month definition is needed.

As COVID-19 continues to be an issue throughout the world, post-acute sequelae of COVID-19 will also persist in the population, so it is crucial to understand why these symptoms occur and what external factors may worsen or improve their severity and duration. The results of this study indicate that stress as a contributing factor to persistent post-infection fatigue should be explored further with a larger sample size and additional follow-up data past six months. The effect of sleep on stress and chronic PASC fatigue should also be examined as sleep duration partially mediated the relationship between outcome and exposure. While total prevention of PASC is dependent on the prevention of COVID-19 infection, it may be possible to use previous research on chronic fatigue and stress management to improve quality of life of those suffering from post-COVID fatigue.[81–84] In conclusion, this study adds to the growing body of literature on PASC, and the results can be used to inform future research on how stress and sleep may influence chronic fatigue in recovered COVID-19 cases.

APPENDIX A: MODEL ASSUMPTIONS

Multiple Imputation with Chained Equations Assumptions

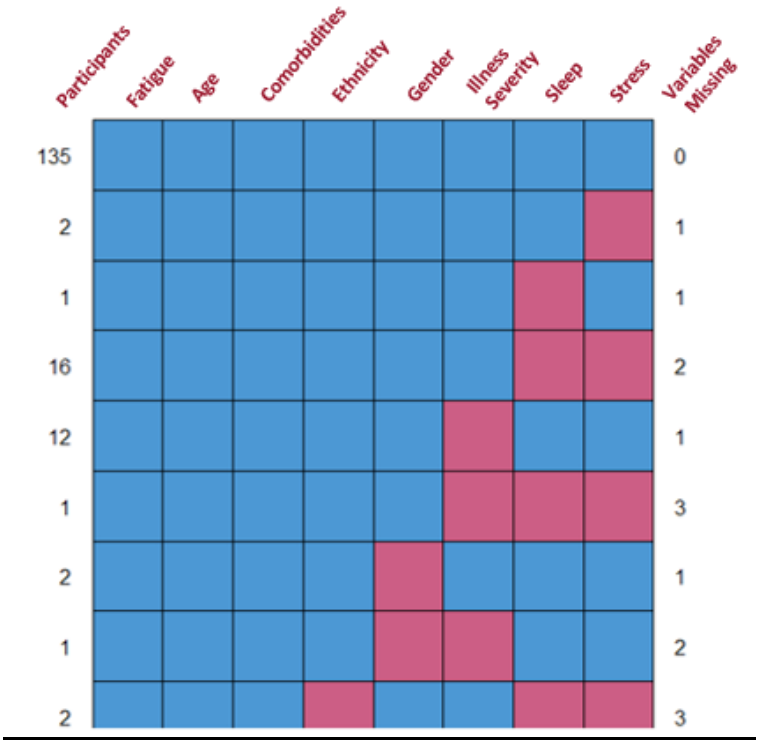

Figure 4. Missingness patterns within multivariate sample data (n=172), where blue is observed, and red is missing

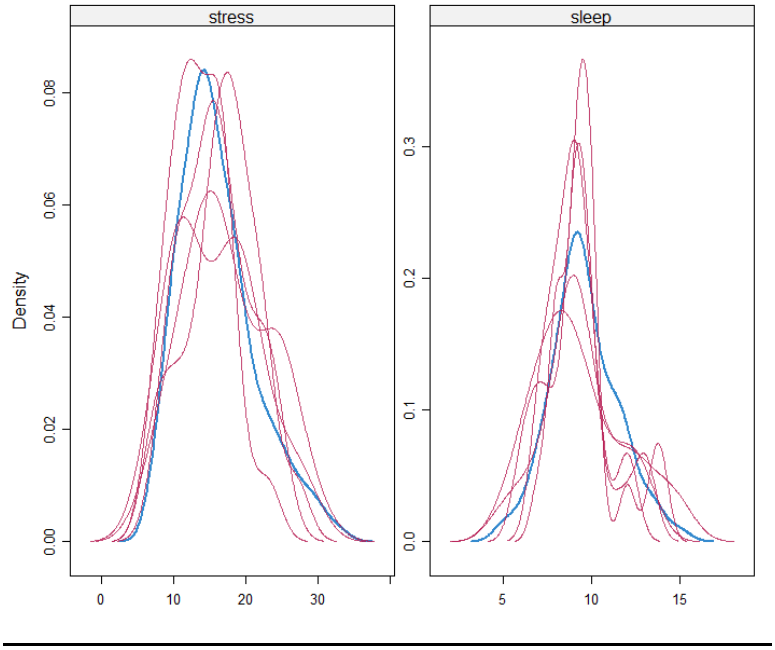

Figure 5. Kernel density estimates for the marginal distributions of the original observed data (blue) and the m= 5 densities per continuous variables calculated from the imputed data (thin red lines).

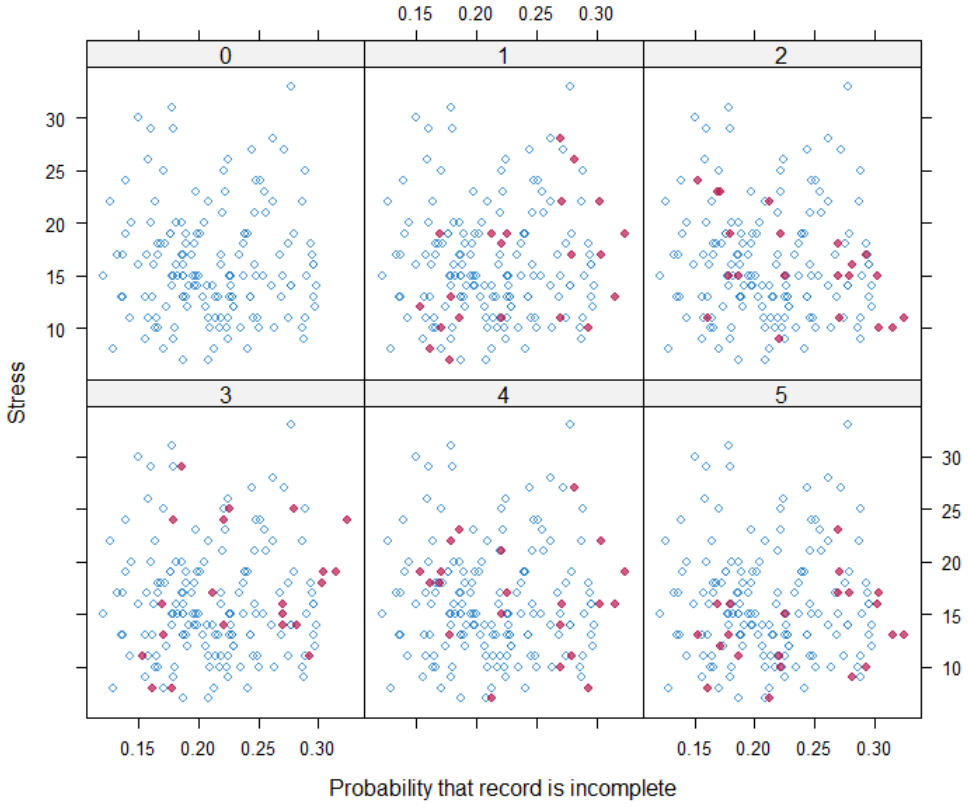

Figure 6. Stress against missingness probability for observed and imputed values to determine if predictive imputation model is of good fit.

Logistic Regression Assumptions

Linearity in the Log Odds

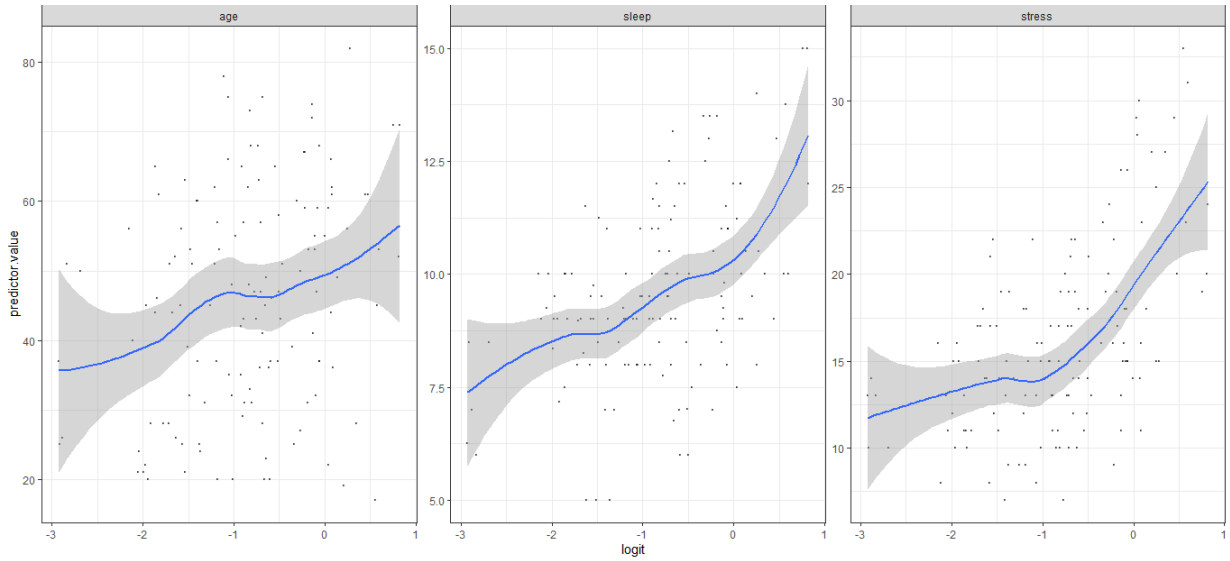

Figure 7: Linearity in the log odds using LOESS model smoothing

Multicollinearity

	GVIF	Df	GVIF^(1/(2*Df))
stress	1.148355	1	1.071613
age	1.147639	1	1.071279
sleep	1.081583	1	1.039992
severe	1.179589	2	1.042156
comorbidity	1.096804	1	1.047284
gender	1.018508	1	1.009212

Influential Observations

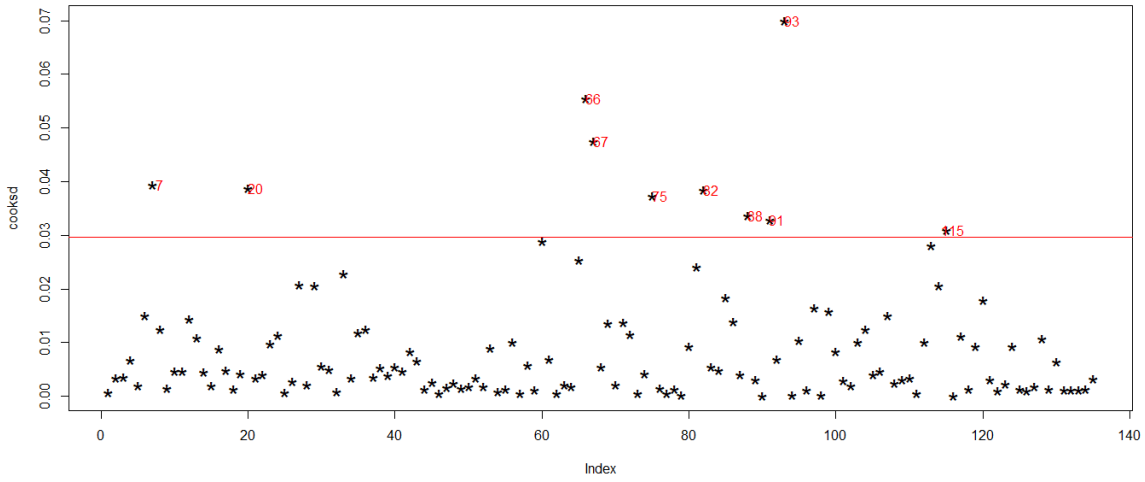

Figure 8. Influential observations using Cook's distance

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