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# EEG Spindle and K-Complex Densities During N2 Sleep Increase with Age into Adulthood and are Uncorrelated to Baseline Autonomic Tone

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## Abstract

The non-rapid eye movement (NREM) sleep is considered a true resting state for the brain, yet the physiologic drivers of neural activity in this phase remain undetermined. Neural activity may be endogenously driven to compensate for minimal sensory stimulation, or by physiologic processes associated with development, or the autonomic tone. To address this issue, we examined brain activity and autonomic tone during stage 2, NREM sleep over a cohort of 93 subjects, from toddlers to young adults. Brain activity was quantified as total counts of EEG spindles and spontaneous K-complexes over 30 minutes of uninterrupted sleep. Autonomic tone during sleep was measured as baseline heart rate and amplitude of respiratory sinus arrhythmia.

Blood pressure was also measured at rest. On average, spindle and K-complex counts increased by 8 and 1 units, respectively, with each additional year of development. Furthermore, spindle and K-complex densities were strongly correlated even after adjusting for age, which suggests a common underlying mechanism. Across subjects, spindles were on average 15 times more abundant than spontaneous K-complexes. Gender did not significantly influence any of these trends. Regarding autonomic tone, spindles and K-complex densities were anticorrelated with baseline heart rate and positively correlated with blood pressure, but these correlations are explained by natural covariations with age. Finally, neither respiratory rate nor sinus arrhythmia correlated with spindle or K-complex density, after adjusting for age. These results collectively demonstrate that spindle and spontaneous K-complex densities increase progressively with age during development into adulthood and are unrelated to baseline autonomic tone.

***Keywords:*** *Non-REM Sleep; Heart Rate Variability; Children; Development*

## New and Noteworthy

It is currently known that sleep spindles and K-complexes decline as adults grow old, but it is not known how they change during development into adulthood. It is also known that K-complexes but not spindles are associated with transient changes in heart rate and blood pressure. Our results expand this picture by demonstrating that over periods of uninterrupted sleep, spindle and K-complex counts increase during childhood and adolescence and are uncorrelated to baseline autonomic tone.

## Introduction

The brain is spontaneously active even in the absence of sensory stimulation. This is especially evident during stage 2, NREM (N2) sleep, during which ongoing brain activity is neither driven by external cues nor by vivid dreams. To investigate physiologic drivers of spontaneous neural activity in these epochs, we examined the role of brain development and autonomic tone on the level of brain excitability, quantified as the number of EEG spindles and spontaneous K-complexes over 30 minutes of uninterrupted sleep.

Sleep spindles and K-complex are specific EEG waveforms that distinguish the NREM sleep. Sleep spindles can occur in any NREM stage, with their greatest occurrence during N2 sleep; K-complexes are specific for N2 sleep [1-6]. Opinions regarding the functional role of sleep spindles differ. Schabus., *et al.* [7] report that sleep spindles may play a role in memory consolidation, but others report an influence on cortical development [8] and on the regulation of arousal from slumber [4]. Sleep spindles are known to be generated by the thalamic reticular nucleus, where they propagate through a thalamo-cortical network [6,9-14]. Similarly, K-complexes have a role in memory consolidation but an unclear mechanism of origin. Cash *et al.* [15] showed that K-complexes occur in widespread cortical areas and may be synchronized in the thalamus. Despite the commonalities in sleep spindles and K-Complexes, sleep spindles may antagonize the production of K-complexes [16]. However, another study has disputed that result [3].

To date, very few studies have examined the effect of the aging process on the density of sleep spindles and K-complexes. Of note, Crowley *et al.* [17] conducted a study comparing the number of spindles and K-complexes between elderly individuals and young adults. They found that there is a progressive decrease in the number of spindles and K-complexes as the subjects grew older [17]. Additional studies found similar results when comparing middle-aged subjects to younger participants [18-21]. But surprisingly, to the best of our knowledge, there have been no studies that focused on the occurrence of spindles and K-complexes during development from infant to young adulthood; a relevant question that we address here.

Numerous studies have previously investigated the relationship between EEG waveforms and autonomic function. Autonomic correlates of spontaneous K-complexes in young male adults (mean age 22) were identified more than four decades ago using electrodermal recordings and include increased heart rate and frequency of vasoconstrictions [22]. Since then, various interactions have been observed between K-complexes and autonomic processes [23]. In a recent study, healthy adolescents (age 16 - 22 years) were found to have differences in evoked K-complexes based on their gender in response to the cardiac rhythm, suggesting that K-complexes can modulate the cardiovascular system during sleep [24]. This study compared evoked and spontaneous K-complexes to the cardiac regulatory response. Other studies had previously shown that K-complexes, but not spindles, are associated with increased muscle sympathetic-neve activity recorded in humans during sleep from the peroneal nerve [25-27]. In contrast, Monstad and Guilleminault [28] reported that decreases in arterial blood pressure preceded both evoked and spontaneous K-complexes. They associated K-complexes with blood pressure oscillations, or Mayer waves, and noted K-complexes occurred as blood pressure was waning during the Mayer waves. Thus, increases in blood pressure may be a consequence of K-complexes occurring preferentially as blood pressure decreases. In summary, these studies provide insight into transient autonomic effects associated with K-complexes and raise a question on whether baseline autonomic tone influences, or is influenced, by the density of K-complexes. Thus, we also investigated the inherent nature of spontaneous K-complexes and sleep spindles to test if they are significantly related to baseline autonomic tone.

## Methods

### Subject cohort

Data collection from the EEG/ECG database of The Pediatric Epilepsy Unit in the Pediatric Neurology Department at University Hospitals in Cleveland, Ohio, was approved by the Institutional Review Board of University Hospitals and Case Western Reserve University.

Subjects were selected based on availability of sleep EEG and ECG data, from all subjects who had an overnight exam conducted between January 1st, 2008 and November 1st, 2014 at the unit.

Records were reviewed retrospectively. A total of 93 subjects were selected, with ages ranging from 10 months to 21 years. The cohort included 17 subjects that were neurologically normal and 76 subjects with controlled epilepsy. Merging these two groups was justified because the spindle and spontaneous K-complex counts have the same distributions for both groups (See results). Regarding gender, 38 subjects were females (41%) and 55 were males (59%). Gender did not influence the spindle or K-complex densities (See table 1 in results). The resting systolic and diastolic blood pressures, along with ages, were obtained from the monitoring reports. The age of each subject was determined as the difference between the date of data acquisition and the date of birth, expressed in years. Epochs of ECG and EEG data from overnight EEG sleep studies at University Hospitals were obtained for each subject. These clips contained 30 uninterrupted minutes of stage 2 sleep.

## EEG reading

Sleep spindles are defined as a spindle-like waveforms with a frequency of 10-16 Hz, lasting longer than 0.5 s, and with minimum amplitude of 15 μV in the double-banana, bipolar montage (Figure 1A and 1B). K-complexes are defined as a structure that has a short, surface-positive transient followed by a slower, larger, surface-negative complex with peaks at 350 and 550 ms (Figure 1C) and then a final positivity peaking near 900 ms, followed sometimes by 10 to 14-Hz spindles [5,23,29]. Sleep spindles and K-complexes were identified visually and counted manually by a clinician that has several years of experience in EEG interpretation. We chose to do this in order to prevent a systematic underestimation of spindle and K-complex counts by automatized computer algorithms that has been reported in previous studies [30,31].

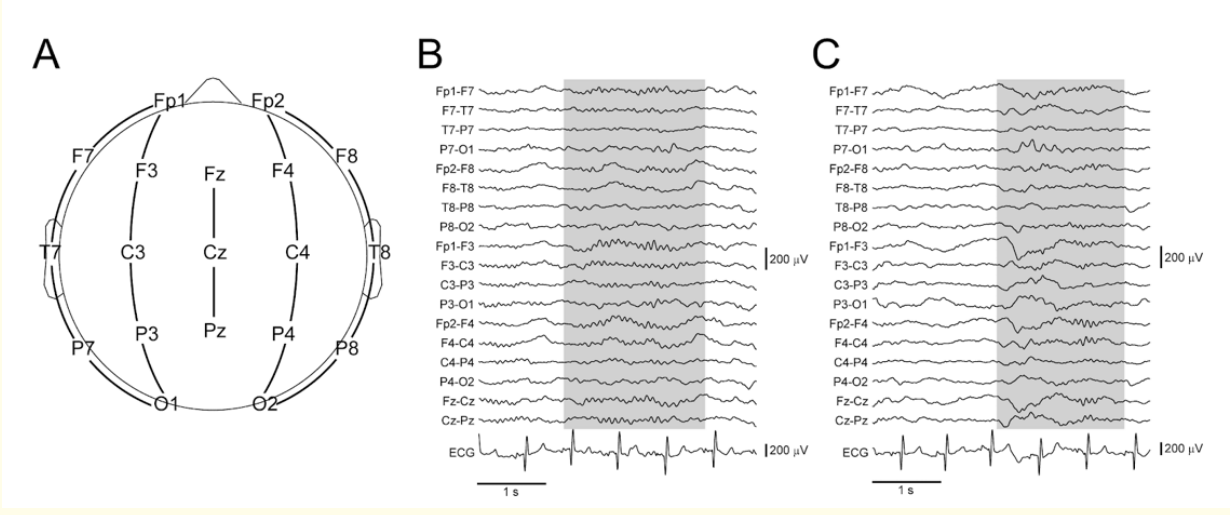


***Figure 1:*** *EEG system and visual detection of sleep waveforms. A) EEG 10-20 system in double-banana montage. B) Representative example of an EEG spindle. C) Representative example of a spontaneous K-complex, including the initial vertex and its trailing spindle.*

## Analysis of heart rate variability

Details for the analysis of heart rate variability can be found in a recent publication of our lab [32]. In short, heart rate time series were calculated as the reciprocal of each inter-beat interval and plotted against the time point of the initial beat in each interval. Cubic-spline interpolation was then employed to generate the instantaneous heart rate with a sampling rate of 200 Hz over the duration of the 30-min-

ute recording. For each subject, the baseline heart rate (in Hz) was calculated as the time average of the heart rate time series. To measure the parameters of respiratory sinus arrhythmia, a zero-phase, digital, high-pass filter with a cutoff frequency of 0.1 Hz was first applied to the heart rate time series in order to eliminate low-frequency variability uniformly across subjects. We then computed the power spectral density of the filtered heart rate time series for each subject. The height of the peak in the power spectral density and its location were recorded for each subject, which quantify the power (in decibels, dB) and frequency (in Hz) of the respiratory sinus arrhythmia, respectively. The frequency of the respiratory sinus arrhythmia corresponds to the respiratory rate.

### Adjustment of sleep structure counts for age

Most of the physiological parameters considered in this study have a natural, linear covariation with age. To correct for this effect, we computed a linear model for each of these waveforms as a function of age, using MATLAB's fitlm function. The residuals of the model provide the spindle or K-complex count adjusted for age. Specifically, the residual for the $n$-th subject is calculated as $y_n - ax_n - b$, where $y_n$ is the spindle or K-complex count, $x_n$ is the age for the $n$-th subject, and $a$ and $b$ are the parameters of the linear regression: $y = ax + b$. Gender was not included in the linear models because it did not have a significant effect on the spindle or K-complex counts.

## Results

EEG traces were recorded with the standard 10 - 20 electrode system, and displayed using a double-banana, bipolar montage (Figure 1A). EEG traces were visually inspected to identify spindles and spontaneous K-complexes, as highlighted in figure 1B and 1C, respectively.

Electrocardiogram (ECG) was simultaneously recorded to monitor heart rate and heart rate variability. Our initial cohort consisted of 17 neurologically normal subjects only. However, in order to increase the size of the cohort for subsequent statistical analyses, we wondered if subjects with controlled epilepsy could be added to the cohort without introducing a bias. Figure 2A and 2B demonstrate that this addition is justified because subjects with controlled epilepsy have the same distribution of spindle and K-complex densities as the neurologically normal subjects (spindles: P = 0.51, Kolmogorov-Smirnov test; K-complexes: P = 0.45, Kolmogorov-Smirnov test). Thus, in total, 93 subjects were present in the cohort for analysis.

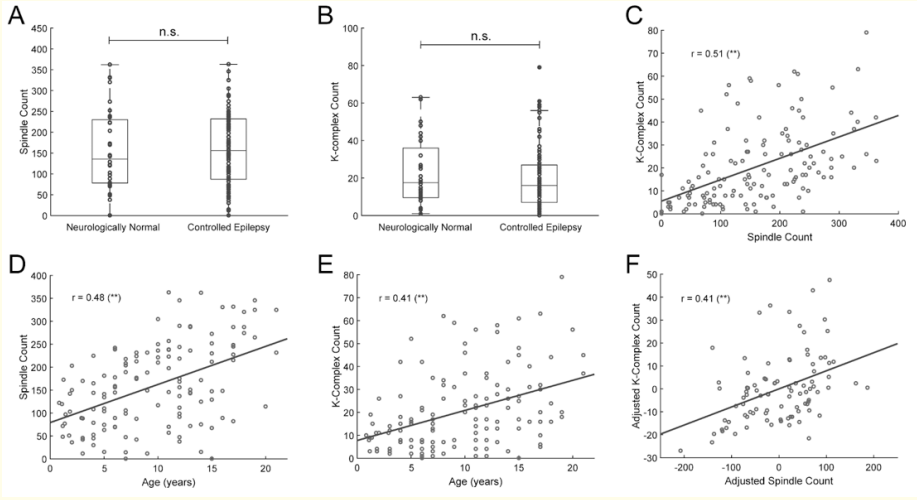


***Figure 2:*** *Dependence of spindle and K-complex densities with development. A&B) Spindle and K-complex densities have the same distributions in neurologically normal subjects and subjects with controlled epilepsy, and therefore, both groups can be merged in this study. C) Spindle and K-complex densities are strongly correlated across subjects. D&E) Both densities increase steadily with age. F) After adjusting for age, the correlation between both densities remains highly significant. Asterisks denote P-values: 0.01< *P < 0.05; **P < 0.01. "n.s." stands for not significant, P > 0.05.*

Across subjects, the spindle density is significantly correlated with the spontaneous K-complex density (Pearson's correlation: r = 0.51, P = 2e-7; Figure 2C). Both densities increase steadily with age (spindles: r = 0.48, P = 1e-6; K-complexes: r = 0.37, P = 2e-4; Figure 2D and 2E, respectively). These trends are well described by linear regression models provided in table 1. On average, the spindle count increases by 8 units per year (Figure 2D and table 1), whereas the K-complex count increases by 1 unit per year (Figure 2E and table 1). Table 1 does not include gender as an independent variable because gender did not have a significant effect on those trends. Importantly, after adjusting for age, the spindle and K-complex densities were still significantly correlated with each other (r = 0.41, P = 5e-5; Figure 2F), which suggests a common underlying mechanism, such as overlapping thalamo-cortical circuits.

| | | Estimate | Standard error | t-statistic | P-value |
|---|---|---|---|---|---|
| Spindle Count | Intercept | 89.46 | 16.71 | 5.35 | 6.4e-7 (**) |
| | Age | 7.89 | 1.52 | 5.20 | 1.2e-6 (**) |
| K-Complex Count | Intercept | 10.00 | 3.23 | 3.10 | 0.0025 (**) |
| | Age | 1.14 | 0.29 | 3.88 | 0.0002 (**) |

***Table 1:*** *Linear Models of Spindle and K-complex Counts with Age. Asterisks denote P-values: **P < 0.01.*

We next investigated the relationship between the spindle and K-complex density with parameters of the autonomic tone. Focusing on spindles first (Figure 3), we found that the spindle density is negatively correlated with the baseline heart rate during the 30-minute periods in which the EEG and ECG were recorded (Figure 3A; r = -0.30, P = 0.004). However, this relationship may be due to the natural covariation of these two parameters with age: heart rate decreases with age, whereas the spindle count increases, as noted above. Indeed, after adjusting the spindle count for age, the negative correlation with the heart rate disappeared (Figure 3B; r = 197 0.00, P = 0.99). We also compared the spindle count with blood pressure measures at rest and observed a positive correlation with systolic (Figure 3C; r = 0.26, P = 0.014) but not diastolic blood pressures (Figure 3E; r = 0.14, P = 0.18). However, after adjusting for age, the correlation with the systolic blood pressure disappeared (Figure 3D; r = 0.03, P = 0.75) and with the diastolic blood pressure became even less significant (Figure 3F; r = -0.07, P = 0.53). This indicates that the spindle density is unrelated to the subject's sympathetic tone. The respiratory frequency was not correlated with the spindle count either before (Figure 3G; r = -0.15, P = 0.16) or after adjusting for age (Figure 3H; r 205 = -0.02, P = 0.87). Finally, we found no correlation with the amplitude of respiratory sinus arrhythmia (Figure 3I; r = -0.12, P = 0.31) as a measure of parasympathetic, vagal tone.

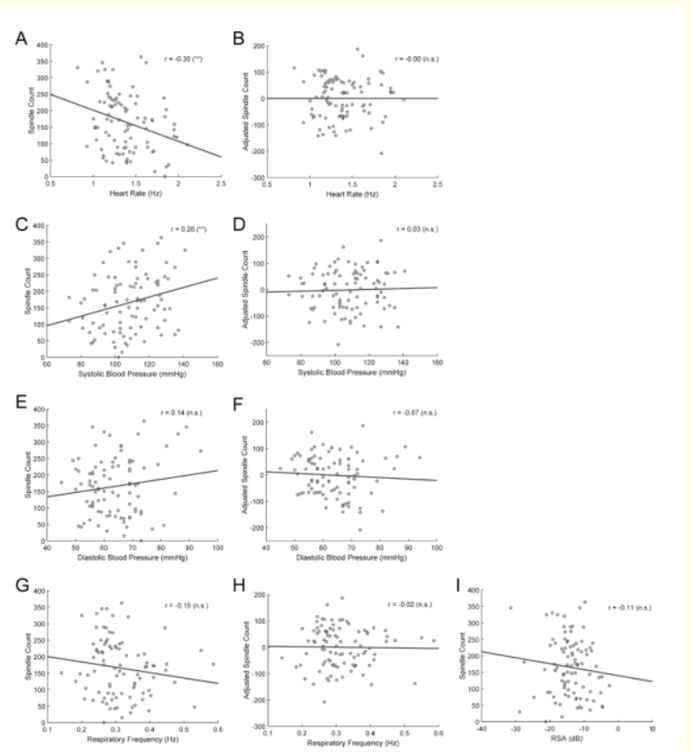


***Figure 3:*** *Spindle density and autonomic tone. A) Spindle density correlates negatively with heart rate, and positively with systolic C) but not diastolic E) blood pressures. B, D&F) However, all these trends are insignificant after adjusting for age. G&H) Spindle density does not correlate with respiratory frequency, nor with I) respiratory sinus arrhythmia. Asterisks denote P-values: 0.01 < *P < 0.05; **P < 0.01. "n.s." stands for not significant, P > 0.05.*

We performed similar analyses for the spontaneous K-complex densities (Figure 4). As for the spindles, the K-complex count correlated negatively with heart rate (Figure 4A; r = -0.29, P = 0.004). Heart rate correlated positively with the diastolic (Figure 4E; r = 0.21, P = 0.049), but not the systolic (Figure 4C; r = 0.13, P = 0.21) blood pressure. However, after adjusting the K-complex counts for age, the correlations with heart rate (Figure 4B; r = -0.07, P = 0.53), systolic blood pressure (Figure 4D; r = -0.05, P = 0.63) and diastolic blood pressure (Figure 4F; r = 0.05, P = 0.61) became largely insignificant. Similarly, K-complex counts correlated negatively with the respiratory frequency (Figure 4G; r = -0.28, P = 0.01) but the correlation weakened below significance after adjusting for age (Figure 3G; r = -0.20, P = 0.06). Finally, as it was the case with spindles, we found no correlation across subjects between the K-complex densities and the amplitude of respiratory sinus arrhythmia (Figure 3I; r = -0.02, P = 0.85) as a measure of parasympathetic tone.

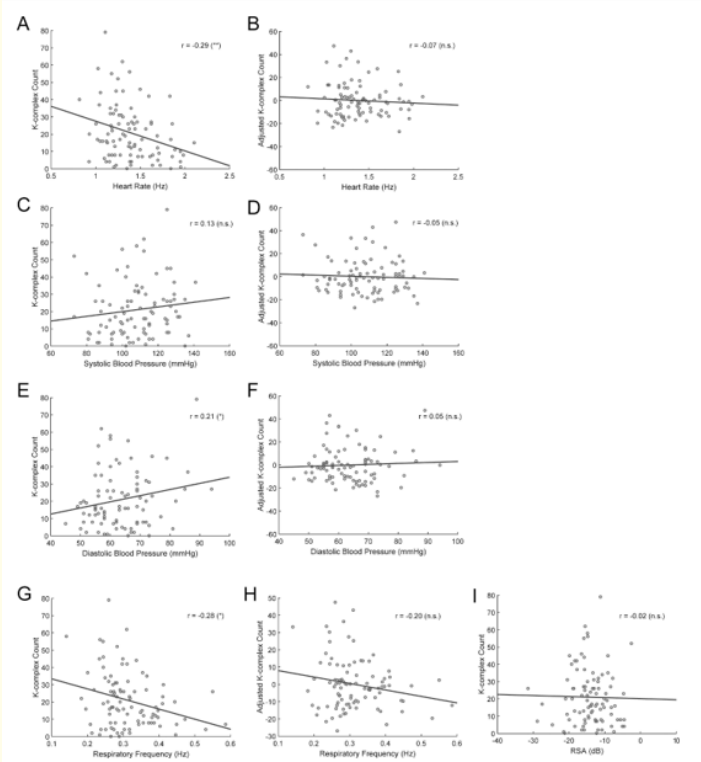


***Figure 4:*** *K-complex density and autonomic tone. A) K-complex density correlates negatively with heart rate, and positively with diastolic E) but not systolic C) blood pressures. B, D&F) However, all of these trends are insignificant after adjusting for age. G&H) K-complex density correlates with respiratory frequency before but not after adjusting for age. I) K-complex density does not correlate with respiratory sinus arrhythmia. Asterisks denote P-values: 0.01 < *P < 0.05; **P < 0.01. "n.s." stands for not significant, P > 0.05.*

## Discussion and Conclusion

Spindles and K-complexes are the defining characteristic of N2 sleep. Their physiologic role is not fully understood, and the exact origination of the K-complex remains unknown. In this study, we show that the counts of sleep spindles and spontaneous K-complexes are positively correlated to age, and to each other independently of age, but not to gender. The sleep structure counts also have a positive

relationship with blood pressure and a negative trend with mean heart rate, although these correlations are fully accounted for by age. Similarly, the spindle and K-complex densities are not correlated with respiratory sinus arrhythmia or the respiratory rate. K-complexes are thought to provide "sleep protection" while consolidating memory [15]. Sleep spindles have an unclear functional role, but have been suggested to promote sleep protective mechanisms, assist in memory consolidation, and regulate arousals [4,7,8]. We have shown that the number of sleep spindles and K-complexes over a fixed period of N2 sleep varies with age. Based on previous literature, it is plausible that this relationship may be related to the amount of sleep an individual gets per night: sleep spindle and K-complex counts have been found to depend on the duration of sleep and the presence of any unstable arousals [33]. As humans age, we tend to require less and less sleep-in order to feel refreshed, and this trend is most prominent through childhood and adolescence [34]. We show an overall significant, positive correlation between the age and the density of both sleep spindles and K-complexes in our study population that spans from infants into young adulthood. Other literature demonstrates that sleep spindle occurrence decreases with age in the adult and elderly populations [17,19-21]. These complementary observations are likely explained by the end of brain development at around age 25, when the frontal lobe completes its maturation. At this point, human development is said to be complete and the brain is fully mature [35]. The relationship between sleep structures and autonomic function has been investigated for several decades [22,23] and it is well established by now that K-complexes but not spindles are associated with transient changes in cardiovascular [24,28], and sympathetic function [25-27]. However, those studies have focused on fast time scales, that is, on transient interactions between brain activity (i.e. K-complex waveforms) and autonomic changes, and have ignored tonic effects on larger time scales, that is, the relationship between baseline autonomic tone and baseline EEG activity. Here, we have investigated these tonic effects by focusing on the spindle and K-complex counts over long periods of uninterrupted N2 sleep and demonstrated that they are uncorrelated to autonomic parameters after adjusting for natural covariations of all of these variables with age. We thus conclude that tonic neural activity during N2 sleep, quantified as the densities of sleep and K-complexes, increases progressively with age during development into adulthood, but is unrelated to the baseline autonomic tone.

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## Author Contributions

RFG conceived the project. AMZ, AGN, SSS, MI and RFG, collected and analyzed data. AMZ, AGN, SSS and RFG wrote the manuscript. RFG and SSS made the figures. All authors reviewed and approved the manuscript.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Ethical Considerations

This research project was conducted in accordance with the ethical standards guidelines and regulations set by the Institutional Review Board at University Hospitals and Case Western Reserve University.

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