Activity-Based Therapy in a Community Setting for Independence, Mobility, and Sitting Balance for People With Spinal Cord Injuries

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ABSTRACT

INTRODUCTION: Activity-based therapy (ABT) aims to activate the neuromuscular system below the level of the spinal cord lesion and promote recovery of motor tasks through spinal reorganisation, motor learning and changes to muscles and sensory system. We investigated the effects of a multimodal ABT program on mobility, independence and sitting balance in individuals with spinal cord injury (SCI).

METHODS: Retrospective clinical data from 91 adults who independently enrolled in four community-based ABT centres in Australia were analysed. The multimodal ABT program was delivered for 3 to 12 months, one to four times per week. Assessments were undertaken every 3 months and included the Modified Rivermead Mobility Index (MRMI), Spinal Cord Independence Measure (SCIM) and seated reach distance (SRD). A linear mixed model analysis was used to determine time-based and other predictors of change.

RESULTS: There was a significant improvement after 12 months for all outcome measures, with a mean change score of 4 points in the SCIM (95% confidence interval [CI]: 2.7-5.3, d = 0.19), 2 points in the MRMI (95% CI: 1-2.3, d = 0.19) and 0.2 in the SRD (95% CI: 0.1-2.2, d = 0.52). Greater improvements occurred in the first 3 months of intervention. There were no interaction effects between time and the neurological level of injury, American Spinal Injury Association Impairment Scale classification, or duration post-injury for most outcomes.

CONCLUSIONS: A community-based ABT exercise program for people with SCI can lead to small improvements in mobility, independence and balance in sitting, with greater improvements occurring early during intervention.

KEYWORDS: Spinal cord injury, activity-based therapy; exercise; recovery, independence, balance, mobility

Introduction

Injuries to the spinal cord result in profound disability and severe physiological changes. Paralysis and deconditioning are not only responsible for reduced mobility and independence, but play a significant role in secondary health complications, such as cardiovascular and respiratory dysfunction, metabolic syndrome, bone demineralization, immunosuppression, and musculoskeletal deterioration. Those factors contribute to limited quality of life and community integration.1,2

Traditionally, physical rehabilitation after spinal cord injury (SCI) aims to optimise motor function and mobility based on the level and severity of the injury. Both factors predict the individual’s ultimate functional capability and needs for rehabilitation.3 This approach relies substantially on providing compensatory strategies focused on the use of the preserved muscles above the level of injury, use of leverage and momentum to move weak or paralysed body parts, and provision of assistive devices to increase independence in activities of daily living (ADL).4,5 While this approach leads to improved functional outcomes, it does not promote recovery of motor control or sensory awareness in the paralysed body parts.5 However, there is growing evidence that suggests that the use of regular and intense activity-based therapy (ABT) directed to activate the paralysed extremities can promote neurological improvements.5,7–13

Activity-based therapy has been defined by Backus et al14 as any intervention that specifically uses tools and interventions to improve muscle activation or sensory function below the level of injury in the spinal cord, and does not rely on compensatory mechanisms for improving function after SCI. Such approach include interventions that combine intensive active movement with one or more of the following: facilitation techniques (use of tactile or vibratory stimulation); electrical stimulation for muscles or nerves (surface or indwelling); body weight supported locomotor training (manual or robotic); upper extremity robotics; massed practice training.

ABT has shown to promote positive effects on motor function of people with complete and incomplete injuries.11,13 Numerous trials have investigated the effects of ABT programs on walking variables and balance in standing.7–10,12,13 ABT interventions often involve weight-bearing through lower
limbs, whole-body coordination exercises, and task-specific training performed mainly out of the wheelchair, in positions where the trunk must work against gravity. Therefore, ABT might contribute to increased strength and control of trunk musculature. Improved trunk control may translate to better general mobility and independence. Therefore, it is important to investigate the effects of ABT in areas of mobility other than walking-related outcomes.

Since there is no cure for SCI, the ultimate aim of rehabilitation is to facilitate the return to a productive and satisfying life. Physical interventions that have the potential to reduce the effects of the impairments are essential to increase mobility. There is evidence that greater mobility contributes for greater independence during ADLs, increased participation in the community and life satisfaction. Hence, it is crucial to explore the potential of ABT as a tool to improve mobility and independence for people with SCI.

This study analysed the clinical data collected by therapists who deliver an ABT exercise program in the community across Australia, with the aim to investigate its effects on general mobility, independence, and sitting balance in people with SCI. A secondary aim was to identify who might be likely to benefit from ABT according to the characteristics of the injury, such as neurological level, completeness, and duration. A further aim was to seek the optimal duration of treatment, if changes in the outcomes of interest were detected. A retrospective study design was employed to provide preliminary data on the possible effects of ABT for people with SCI and could provide basis for a larger study with a comparison group to be conducted.

Methods

Study design

This study employed a retrospective, multicentre, non-controlled design and consists of an interrogation of the clinical data collected by the therapists in an ABT program. Participants who independently enrolled in the NeuroMoves program in four community-based clinics in Australia were consecutively recruited. The NeuroMoves program was established in 2008 in response to requests from people with SCI to participate in an ABT program after their discharge from hospital. Data collected by therapists from each centre between January 2012 and March 2016 were screened for analysis. The centres were located in New South Wales (The University of Sydney, Cumberland Campus; Lidcombe, Sydney), Victoria (Whitten Oval, Footscray; Melbourne), Queensland (Sporting Wheelies Disabled Association; Baulking Hills, Brisbane) and Western Australia (Edith Cowan University; Joondalup, Perth). This study was approved by The University of Sydney Human Research Ethics Committee (HREC 2013/824).

Participants

Assessment data from 91 participants were extracted from each centre's electronic database. The data contained in the electronic database were compared with the data recorded by the therapists on paper files to ensure the accuracy of the data.

The participants were adults (aged >16 years old), who had sustained a non-progressive SCI at any level or completeness according to the American Spinal Injury Association Impairment Scale (AIS A, B, C, and D).

Participants attended the ABT program for at least 3 months for a maximum of 12 months, one to four times weekly. They underwent assessments of physical impairments, activity level, and participation at every 3 months as part of their standard ABT program. Data related to mobility, independence, and sitting balance were extracted from each clinic's electronic database.

ABT intervention

Each participant had their exercise program individually tailored according to their goals and functional abilities by an exercise physiologist or physiotherapist trained in ABT. The intervention involved three key elements: task-specific training, weight-bearing tasks, and whole-body muscle strengthening. This approach involved training in different positions such as sitting on the edge of the bed; 4-point kneeling, kneeling, standing with partial or full body weight; body-weight supported treadmill training; active-assisted exercises; resistance training; neuromuscular electrical stimulation and, balance and coordination tasks. All of the exercises were performed out of the wheelchair, incorporating whole-body movements (refer to Appendix A for detailed exercise interventions).

The incorporation and sequencing of the various exercise modalities differed across participants according to the individual's level of physical function and neurological impairment. Participants were encouraged to perform all modalities of exercise to their maximum capacity, with 1-5 min for recovery, if required, between exercises. Participants attended the program 1 to 4 times per week for 2-h sessions.

Outcome measures

Data from baseline, 3-, 6-, 9-, and 12-month assessments were extracted from the electronic databases, according to each client’s time in the program.

General mobility. The Modified Rivermead Mobility Index (MRMI) was used to measure mobility. It evaluates bed mobility, postural transfers, and walking ability, including eight activities from turning over in bed to climbing stairs. The MRMI has not yet been validated in a SCI population; however, it has demonstrated excellent inter-rater reliability (ICC = 0.93) in a mixed neurological population. Furthermore, it has shown high responsiveness and adequate predictive validity with a sensitivity of 71% and specificity of 70% in determining independent walking ability in a stroke population. The authors opted to use the MRMI; once no specific measures of general mobility were found among the measures validated to the SCI population.
Independence. The Spinal Cord Independence Measure version II (SCIM) was used to assess the ability to accomplish activities of daily living. This scale assesses three areas: (1) self-care (feeding, grooming, bathing, and dressing); (2) respiration and sphincter management; and (3) mobility (bed, transfers, and indoor/outdoor). The SCIM III has demonstrated high internal consistency (Cronbach's $\alpha = 0.77-0.91$) and inter-rater reliability (ICC = 0.96). Moreover, it has shown responsiveness similar to the Functional Independence Measure (FIM). Ceiling effects were observed in three items: Feeding, Respiration, and Bed mobility, whereas floor effects were observed in 11 items: feeding, bathing (upper and lower body), dressing (upper and lower body), use of toilet, bed mobility, transfers from wheelchair (to bed, toilet, car, and ground), and stair management.

Balance in sitting. The seated reach distance (SRD) test was used to evaluate sitting balance. The test consists of measuring the ability to reach in different directions as far as possible without falling. The participant is seated in front of a large table with its closest edge in line with the greater trochanters and at the height of the iliac crests. The table is covered with a large paper sheet with five lines: (1) lateral right (3 o'clock); (2) lateral left (9 o'clock); (3) 45° right (1:30 o'clock); (4) 45° left (10:30 o'clock); and (5) forwards (12 o'clock). With a marker pen placed in the thumb web space in both hands, participants are asked to reach in each direction as far as possible and mark the sheet at the most distal reach. The greatest reach distance for each direction is recorded in centimetres and then divided by the arm length to constitute the score. A final score was obtained by calculating the mean score of all directions. The SRD test has been validated in a spinal cord population and has been shown excellent test-retest reliability with an ICC ranging from 0.80 to 0.89 for each reach direction. It has also shown significant correlation with International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) motor scores and was able to discriminate between people with acute and chronic SCI with a sensitivity of 80% and specificity of 90%.

Statistical analysis

A linear mixed model (LMM) fixed-effects analysis of variance (ANOVA) was used in this study to investigate the change across time for all outcome measures. The LMM ANOVA calculated the estimated marginal means from the arithmetical means at each time point for all three outcomes of interest. The approach was chosen due to the significant amount of missing data points at follow-up.

Bivariate regression analyses were conducted to examine possible predictors of treatment outcomes, such as AIS, level, and duration post-injury. Lesions were categorised into motor complete and motor incomplete for the comparisons of the effect of completeness. A comparison was also conducted between individuals with tetraplegia (above T1 level) and paraplegia (T1 level and below). For duration post-injury, the participants were allocated into 4 groups for analysis: less than 1 year, 1 to 5 years, 6 to 10 years, and over 10 years post-injury. All posteriori pairwise contrasts were conducted using estimated marginal means derived from the LMM ANOVA.

In the linear mixed models ANOVA, an unstructured covariance matrix was selected since it presented the lowest $-2$ LL (log likelihood). All data analyses were performed using SPSS 22. Statistical significance was set at $p < 0.05$ for all analyses.

The effect sizes for each pairwise outcome measured at 3 and 12 months vs baseline were calculated using Cohen’s $d$ to verify the early and late effects of the program. Cohen’s $f^2$ was calculated to verify the magnitude of the interaction effects. To test for the interaction between total number of sessions and the three outcomes of interest, the total number of sessions was divided in percentiles, resulting in four groups corresponding to 25th, 50th, 75th, and 100th percentiles. Pairwise contrasts were conducted for each outcome measure and group. All calculations were performed using IBM SPSS Statistics 22 software.

The minimally important difference (MID) was calculated according to the recommendations of Musselman et al., using a distribution-based approach, based on the effect size for all variables. This was accomplished by multiplying the effect size representing a MID, Cohen’s $d = 0.2$ by the pooled standard deviation. Those values reflect the amount of change necessary to detect differences beyond expected measurement error and provide an estimate of clinically significant improvement.

Results

Cases extracted from databases

Figure 1 details the flow of participants included in this study, with their reason for exclusions. Not all 91 included participants had data available for all outcome measures at all five assessment time points. There was a substantial drop in the number of cases for each outcome measure as time progressed. The outcome measure with the greatest loss of cases over time was level of independence, followed by mobility. The assessment time point with the lowest number of cases was at 9 months. Table 1 demonstrates the number of cases per outcome measures at each time point. It is worth noting that the missing points were due to the lack of assessment data and do not always represent drop outs.

Participants’ characteristics

Demographic data, number of sessions attended, and the weekly frequency of sessions are displayed in Table 2.

Level of independence

There was a significant improvement over 12 months in the participants’ level of independence with an overall small to medium effect size ($P = .000, f^2 = 0.13$). The change score in the SCIM after 12 months, based on the estimated marginal
mean, was 4 points (95% CI: 2.7-5.3, d = 0.19), corresponding to an 8% increase. The distribution-based MID calculated in this sample for SCIM was 4.2 points. Major improvements were seen between baseline and 3 months, with a change score of 1.5 points (95% CI: 0.6-2.4, d = 0.07). No significant changes were observed between 9 and 12 months (Figure 2).

Comparison of outcomes between participants with para-plegia and tetraplegia revealed significant main effects for time and impairment group, without a group vs time interaction ($P= .658$). There were significant main effects for time and motor completeness with a completeness vs time interaction ($P= .018$). The motor complete group showed a gain of 3 points (95% CI: 2.46-3.54) in the SCIM, whereas the motor incomplete group had a gain of 7 points (95% CI: 5.82-8.18) after 12 months of intervention compared with baseline.

In terms of duration post-injury, there was a significant main effect for time over 12 months, but no significant effect or any interaction between time and duration post-injury. Individuals between 1 and 5 years after SCI showed the greatest improvements in their independence (estimated mean SCIM = 5 points, 95% CI: 4.17-5.83), whereas individuals between 6 and 10 years post-injury had little to no improvement in SCIM (estimated SCIM = 0.6 points, 95% CI: −1.83 to 3.03). For supplemental data, refer to Appendix B.
There was a significant improvement over 12 months in overall mobility ($P = .000$, $f^2 = 0.12$). The change score in the MRMI, based on the estimated marginal means, was 2 points (95% CI: 1-2.3, $d = 0.19$), corresponding to a 10% change. The distribution-based MID calculated in this sample for MRMI was 2.02 points. Greater improvements were observed between baseline and 3 months with a change score of 0.7 (95% CI: 0.3-1.2, $d = 0.52$), corresponding to 14% change. All changes in score over time were significant when compared with the baseline score; however, no significant changes were observed between 3 and 6 months, or 6 and 12 months, or 9 to 12 months (Table 1 in Appendix B).

Analysis comparing individuals with paraplegia vs tetraplegia revealed a significant main effect for time and impairment group, with no group vs time interaction ($P = .907$). A significant main effect for time and extent of motor completeness was found with no interaction between these main effects ($P = .12$) (Table 2 in Appendix B).

There was a significant improvement over 12 months in sitting balance ($P = .000$, $f^2 = 0.26$). The change score in the SRD after 12 months, based on the estimated marginal means, was 0.2 (95% CI: 0.1-0.22, $d = 0.52$), corresponding to 14% change. The distribution-based MID calculated in this sample for SRD was 0.5 points; 95% CI: –1.13 to 2.13 (Tables 3a and 3b in Appendix B).

Analysis contrasting individuals with paraplegia vs tetraplegia demonstrated significant main effects for time and impairment group, but no group vs time interaction ($P = .174$). Comparing motor complete (AIS grade A and B) vs motor incomplete (AIS grade C and D) lesions revealed significant main effects for time and motor completeness, with no interaction effect ($P = .528$).

The analysis of duration post-injury on sitting balance showed a significant main effect for time, with no effect for duration of injury, but with a significant interaction effect for

### Table 2. Participants characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants (N=91)</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>35.3 ± 15.9 (17-77)</td>
</tr>
<tr>
<td>Sex</td>
<td>M=65/F=26</td>
</tr>
<tr>
<td>Duration post-injury (mo)</td>
<td>43.1 ± 51.4 (3-244)</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>32</td>
</tr>
<tr>
<td>1 to 5 years</td>
<td>39</td>
</tr>
<tr>
<td>6 to 10 years</td>
<td>10</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>10</td>
</tr>
<tr>
<td>Tetraplegia/paraplegia</td>
<td>49/42</td>
</tr>
<tr>
<td>AIS classification</td>
<td>A = 31/B = 36/C = 15/D = 9</td>
</tr>
<tr>
<td>Number of sessions attended</td>
<td>56.4 ± 37.2 (15-227)</td>
</tr>
<tr>
<td>25th percentile (&gt;=26 sessions)</td>
<td>23</td>
</tr>
<tr>
<td>50th percentile (27-51 sessions)</td>
<td>24</td>
</tr>
<tr>
<td>75th percentile (52-72 sessions)</td>
<td>22</td>
</tr>
<tr>
<td>100th percentile (&gt;=72 sessions)</td>
<td>22</td>
</tr>
<tr>
<td>Frequency of sessions per week</td>
<td>2 ± 0.7 (1-4)</td>
</tr>
<tr>
<td>1×</td>
<td>19</td>
</tr>
<tr>
<td>2×</td>
<td>52</td>
</tr>
<tr>
<td>3×</td>
<td>18</td>
</tr>
<tr>
<td>4×</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; M, male; F, female. Values are mean ± SD (range). Raw number of participants is provided for duration post-injury divided into four categories, tetraplegia/paraplegia, gender, AIS classification, number of sessions attended divided in percentiles and frequency of sessions per week.
duration of injury vs time (P = .025). Individuals injured for less than 1 year showed an estimated mean improvement in balance only of 0.06 (95% CI −0.06 to 0.16), whereas all other groups had an estimated mean improvement of 0.2 points over 12 months (1-5 years 95% CI: 0.09 to 0.31; 6-10 years 95% CI: −0.14 to 0.54; over 10 years 95% CI: −0.13 to 0.53).

Frequency and total number of sessions

There was no statistical effect of frequency of sessions on the three outcomes measured. However, a statistical significant interaction effect between total number of sessions and independence was identified, indicating that individuals that attended more than 72 sessions demonstrated greater improvements than individuals who attended less than 26 sessions (P = .01).

Minimally important difference

The distribution-based MID calculated in this sample was 0.05 for SRD, 2.02 for MRMI, and 4.2 points for SCIM.

Discussion

This study is the first to investigate the effects of a multimodal ABT program on general mobility, functional independence, and sitting balance in people with SCI, by assessing clinical data from a community program. Improvements of medium and small magnitude were seen in the three outcome measures analysed. Most changes occurred early during the first 3 months after commencing ABT, although significant changes were detected for up to 9 months of participation. The overall change scores over time met or surpassed the MID calculated for this sample for all three outcome measures, suggesting that changes were clinically significant.

Several studies have demonstrated positive effects of ABT in muscle strength, mobility, balance, and hand function in SCI populations, mainly analysing a single modality of ABT, such as locomotor training and electrical stimulation. The first trial to investigate a combination of ABT interventions was Harness et al., who compared a multimodal intense ABT program to self-regulated exercises over a 6-month period. The authors reported significant positive effects of ABT on motor function of people with complete and incomplete SCI, supporting the positive changes in mobility found in the present study. However, no effect sizes were reported by Harness et al. Furthermore, a randomised controlled trial conducted by Jones. Furthermore, a randomised controlled trial (RCT) conducted by Jones emplyed a multimodal ABT program to 38 people with chronic incomplete SCI. the findings revealed that ABT had the potential to promote neurological recovery, verified by changes in the lower extremity motor score that lead to enhanced walking ability after chronic motor incomplete SCI.

In contrast, a more recent randomised controlled trial compared a 12-week multimodal ABT intervention, including locomotor training, functional electrical stimulation (FES)-assisted cycling, trunk, upper and lower extremity exercise with an upper-body exercise program. The results showed no neurological recovery after ABT and no significant differences between groups for functional or behavioural variables. Similarly, a case series conducted by Padula et al investigated the long-term effects of an 18-month multimodal ABT program and showed no effects of ABT on activities of daily living or participation outcomes.

In the present study, the underlying mechanisms explaining the participants’ improvements in balance, mobility, and independence are unclear. One possibility is that the repetitive and intensive nature of the ABT program may have led
to motor learning and improved motor control. Participants could have learned new compensatory strategies to move their body, using their non-paralysed muscles in a more efficient way.45,46 Moreover, ABT interventions are highly repetitive and involve low load tasks, which may have improved the strength and endurance of innervated or partially innervated muscles, increasing participant’s physical capacity and contributing to the gains in mobility, independence, and balance. Another hypothesis is that ABT might have resulted in neuroplastic changes in the spinal cord or brain. Such changes have been reported to occur following ABT interventions to the upper limb and body weight-supported treadmill training.34,47–49

The ABT program had similar effects on the rates of improvement in mobility, independence, and balance in sitting over time. Our data conflict with the findings of a previous RCT, where individuals with paraplegia showed greater responsiveness to ABT treatment.42 There is substantial evidence that ABT is effective for individuals with motor incomplete injuries in areas of mobility, balance, and independence.32,44,50–53 However, the present study showed that individuals with motor complete (AIS grade A and B) and motor incomplete (AIS grade C and D) lesions had similar rates of improvement in mobility and balance in sitting. The exception was independence, where greater changes were seen in individuals with motor incomplete injuries.

Overall, the individuals who were injured up to 5 years experienced more benefits from the ABT intervention. However, individuals injured for over 10 years also presented changes of similar magnitude in independence and sitting balance; the exception was for mobility, where minimal improvements were shown. Hence, suggesting that chronicity may reduce the capacity to improve mobility. These findings are consistent with research by Jones42 who verified that individuals who were 3 years post-injury were more responsive to a multimodal ABT intervention and support the fact that ABT can be beneficial for people with SCI, regardless the chronicity of injury.

Most of the improvements occurred during the initial 3 months of exposure to the intervention for all outcomes, albeit with small effect sizes. However, individuals continued to display significant changes in the level of functional independence and mobility until 6 and 9 months, respectively. Balance in sitting did not change significantly after 3 months of treatment. Similar results were reported by Padula et al.,44 who analysed the effects of a long-term multimodal ABT intervention, finding greater improvements in functional independence in the first 3 months of treatment.

In our sample, the total number of sessions was only related to changes in independence, demonstrating that changes in balance in sitting and mobility can happen even with low volume of sessions. There was no statistical significant correlation between frequency of intervention and the outcomes of interest. The lack of statistical power can be attributed to the uneven sample sizes in each subgroup. Only two individuals participated in the program for four times per week, whereas 62% attended the program two times per week, which is below the recommended dose for ABT interventions of three to five times per week.4 This finding shows that high intensity, high volume therapy is hardly feasible in a community setting.

The main limitation of the current study was the significant amount of missing data in the database, reducing the statistical power of the analysis, and the validity of the findings. The considerable amount of missing data reflects the reality of a community-based exercise program where people participate for different lengths of time according to their goals, medical, social, and financial status. In this sample, some of the reasons why people have left the program were due to medical complications, such as urinary tract infections and pressure injuries. Other common cause of exit was the distance to travel to the program, financial burden, and acquisition of the desired level of functionality.

Another reason of missing data was due to therapists not conducting all assessments at the appropriate time points due to forgetting about it, participant refusing to be reassessed, and participant cancelling the session where the assessment should have been conducted. Since the aim of this study was to investigate the outcomes obtained by people attending a community-based exercise program without manipulation of the intervention, we expected to have missing data. The statistical analysis accounted for missing data by estimating the missing data points.

The lack of a control group was another relevant source of bias that reduced the methodological power of this study and averted the generalisation of the results as it was not possible to establish a comparison of the effectiveness of ABT interventions with other physical interventions. However, the present study may provide the basis for an RCT to be conducted. Furthermore, the data were collected by therapists trained in each of the outcome measures. However, bias in the consistency of the tests may have arisen across the sites.

**Conclusions**

This study provided preliminary evidence about the positive effects of ABT on functional independence, mobility, and balance in sitting for individuals with SCI. Improvements of greater magnitude happened in earlier phases of ABT exposure, with changes in mobility continuing until 9 months. Individuals experienced benefits regardless their level, severity, or duration post-injury. Those aspects must be taken into consideration when prescribing ABT interventions delivered in the community and planning discharge. Furthermore, the present study has provided insight of some of the barriers of implementing ABT programs in the community according to the evidence-based recommendations, such as frequency, volume of therapy, and inconsistent data collection between therapists.
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Author Contributions
Conceived and designed the experiments: All authors. Analyzed the data: CQO and GD. Wrote the first draft of the manuscript: CQO. Contributed to the writing of the manuscript: JM, KR and GD. Agree with manuscript results and conclusions: All authors. Jointly developed the structure and arguments for the paper: CQO, GD, JM. Made critical revisions and approved final version: All authors. All authors reviewed and approved of the final manuscript.

Supplemental Material
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