

# Exploring correlates of improved depression symptoms and quality of life following tai chi exercise for patients with heart failure

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## Abstract

**Aims** Tai chi exercise has been shown in a prior randomized controlled trial to improve depression symptoms and quality of life (QoL) in patients with heart failure (HF), but correlates of these improvements are not well known. The purpose of this secondary analysis was to explore whether tai chi is associated with improvements in biopsychosocial and behavioural measures and whether such improvements are correlated with improved depression and QoL.

**Methods and results** Participants were  $n = 100$  adults with chronic systolic HF (mean age = 67.4, SD = 12.0; 64% male; 96% White; New York Heart Association class = 1–3) randomized to a 12 week tai chi exercise intervention or health education control. Constructs of interest included social support, exercise self-efficacy, activity engagement, sense of coherence, and inflammatory biomarkers. Tai chi was associated with increased everyday activity engagement compared with the health education group ( $P < 0.05$ ), but there were no group differences in social support or sense of coherence. Among tai chi participants, improved self-efficacy was correlated with QoL ( $r = 0.31$ ,  $P = 0.05$ ), and there was a trend toward improved depression symptoms and social support ( $r = -0.22$ ,  $P = 0.13$ ). Among all participants, controlling for intervention group, improved sense of coherence, and inflammation (C-reactive protein) were associated with improved depression symptoms, and improved self-efficacy, sense of coherence, and frequency of activity engagement were associated with improved QoL.

**Conclusions** Tai chi exercise promotes inter-related psychosocial improvements for patients with HF. A range of biopsychosocial and behavioural variables are relevant to mood management in patients with HF.

**Keywords** Tai chi; Heart failure; Depression; Social support; Self-efficacy; Activity

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## Introduction

Up to 40% of patients with heart failure (HF) meet the criteria for major depressive disorder,<sup>1</sup> rates substantially higher than those of the general population (7%).<sup>2</sup> Greater depression symptoms<sup>3</sup> and poorer quality of life (QoL)<sup>4</sup> among patients with HF are associated with increased medical and cardiac morbidity, hospitalizations, and mortality. The co-morbidity between HF and emotional outcomes is well established, but the processes underlying this complex mind–body interaction are poorly understood.

Tai chi is a movement-based meditation practice rooted in ancient Chinese martial arts that integrates slow, gentle movements with mindful awareness and visualization to promote physical and psychological well-being.<sup>5</sup> Randomized controlled trials (RCTs) have shown greater improvements in QoL and depression symptoms for patients with HF following tai chi as compared with a control group intervention (e.g. usual care and aerobic exercise<sup>6,7</sup>). We previously conducted an RCT of a 12 week tai chi programme in patients with chronic medically stable systolic HF that yielded greater improvements in depression

symptoms and QoL as compared with an attention-matched education control.<sup>8</sup>

As a group-based multi-modal mind–body exercise, tai chi might target psychosocial, behavioural, and biological processes. Theoretical models and evidence in other populations suggest that tai chi can improve exercise self-efficacy (i.e. confidence in one's ability to exercise), sense of coherence (i.e. one's orientation to life, including manageability and meaningfulness), social support, activity engagement (including both physical and non-physical lifestyle and social activities), and inflammation, each of which is associated with improvements in depression symptoms and QoL. Participating in slow, gentle physical movements can build self-efficacy for the ability to exercise,<sup>9</sup> which is associated with better mood and QoL.<sup>10</sup> Tai chi practitioners can experience a state of flow during the practice, which increases positive emotions and promotes a greater sense of coherence.<sup>11</sup> Among older adults with depression, improvements in social support following tai chi partly accounted for improvements in depression symptoms.<sup>12</sup> Several studies have focused on exercise endurance and functional capacity,<sup>13</sup> but there may be separate benefits for engagement in everyday lifestyle activities, which may not confer aerobic activity but are still key factors for mood management.<sup>14</sup> We previously reported greater improvements in exercise self-efficacy and moderate-intensity physical exercise following tai chi,<sup>8</sup> but there is no research exploring effects on activity engagement. Lastly, inflammation is a key biological process underlying depression,<sup>15</sup> and tai chi can reduce inflammation.<sup>16</sup>

There is very limited research exploring correlates of improved mood and QoL following tai chi for patients with HF. We are aware of two trials, which showed that improvements in sleep or fatigue were correlated with improved QoL<sup>17</sup> and depression symptoms.<sup>18</sup> Further exploring the variables correlated with improved depression and QoL will help guide future mechanistic studies, inform treatment rationale, and identify targets of tai chi treatments for patients with HF. This work could also advance the theoretical foundation and scientific evidence base for movement-based meditation practices, as well as help tease apart the nature of the intriguing co-morbidity of depression and HF.

Utilizing data from our previously described clinical trial,<sup>8</sup> the current study aims to (i) quantitatively explore between-group differences in social support, activity engagement, and sense of coherence; (ii) explore whether changes in social support, exercise self-efficacy, sense of coherence, activity engagement, and inflammatory biomarkers (C-reactive protein and TNF- $\alpha$ ) are correlated with improvements in depression symptoms and QoL among tai chi participants; and (iii) if these variables are correlated with mood outcomes among all patients with HF, controlling for intervention group. The overall goal of this study is to better understand tai chi's potential pathways of impact on depression and QoL in HF in order to inform theoretical models and identify

potential targets for future explanatory work. The secondary goal is to explore variables related to general mood management in patients with HF.

## Methods

Details of the source study are published elsewhere.<sup>8</sup> In brief, patients with chronic systolic HF ( $n = 100$ ) were randomized to tai chi or a time-matched health education control. Both groups met twice weekly for 12 weeks. Outcome measures were collected at baseline and 6 and 12 weeks. Full details of the intervention protocols, CONSORT diagram, and intervention components are previously described.<sup>8</sup> The investigation conforms with the principles outlined in the *Declaration of Helsinki*. Study procedures were approved by the Beth Israel Deaconess Medical Center ethics committee, and all study subjects gave written informed consent to participate in the study.

## Measures

### *Depression symptoms*

The depression subscale of the EDITS Profile of Mood States (EPOMS)-Brief<sup>19</sup> was used to assess depression symptoms. The EPOMS is 30-item abbreviation of the original POMS that shows strong psychometric properties and includes a subscale for depression symptoms.<sup>19</sup> The depression subscale includes five items, and scores range from 0 to 20, where higher scores indicate greater depression symptoms. Research supports the reliability and validity of the depression subscale.<sup>20</sup>

### *Quality of life*

We used the well-validated Minnesota Living with Heart Failure Questionnaire (MLHFQ<sup>21</sup>) to measure cardiac disease-specific QoL. The MLHFQ is a 21-item measure of physical, psychological, and socio-economic aspects of QoL related to HF (e.g. fatigue, difficulty climbing stairs, and spending money on treatment). Scores range from 0 to 105, and lower scores indicate better QoL.

### *Social support*

The Multidimensional Scale of Perceived Social Support<sup>22</sup> was used to assess social support. This is a reliable and well-validated 12-item measure that asks participants to indicate how much they agree with various statements about accessing and utilizing a social support network. Example items include 'there is a special person who is around when I am in need' and 'I get the emotional help and support I need from my family'. Scores range from 12 to 84, and higher scores indicate greater perceived social support.

### Cardiac exercise self-efficacy

The Cardiac Exercise Self-Efficacy Instrument is a 16-item measure used to assess an individual's self-efficacy for exercise-related activities.<sup>23</sup> Scores range from 16 to 80, where higher scores indicate greater self-efficacy. The measure shows good psychometric properties including internal consistency reliability and construct validity.<sup>23</sup>

### Activity variables

The Community Healthy Activities Model Program for Seniors (CHAMPS) was used to measure number and frequency of engagement in various activities over the past month.<sup>24</sup> The CHAMPS is a well-validated measure that asks participants to indicate participation in 41 different activities over the past month (e.g. visit with friends or family, dance, do household chores, do volunteer work, read, and garden). This instrument is typically used to report frequency and caloric expenditure per week for physical activity, and in particular moderate-intensity or greater-intensity physical activity. In this analysis, we examined the total number of activities (physical and non-physical; range of 0–41 activities) and the frequency of each activity (open-ended question reporting number of hours/week) as measures of activity engagement, allowing the inclusion of non-exercise activities such as going to the senior centre, attending a concert or movie, and playing a musical instrument.

### Sense of coherence

Sense of coherence was measured using the validated 13-item Orientation to Life Questionnaire,<sup>25</sup> which assesses an individuals' global orientation to life in terms of three related domains: confidence that the environment is understandable and predictable (i.e. comprehensibility); ability to cope with challenges using available resources (i.e. manageability); and the perspective that challenges are worth engaging in (i.e. meaningful). Scores range from 13 to 91, and higher scores indicate a greater sense of coherence.

### Inflammatory biomarkers

Serum samples were collected from an intravenous catheter of patients resting in supine position for 20 min, and sample were stored on ice. These samples were analysed for C-reactive protein and TNF- $\alpha$ , both established biomarkers of stress-related inflammation. Full methods are described elsewhere.<sup>8</sup>

### Data analysis

Frequencies and descriptive statistics were examined to assess normality for baseline and endpoint variables and change scores for each variable [calculated as Time 2 (12 weeks) – Time 1 (baseline)]. Change scores for depression, QoL, and social support were all found to be non-normal owing to outliers (defined as >3 standard

deviations above the mean); non-parametric tests were therefore used for all analyses. Wilcoxon rank sum tests were used to compare changes in social support, number of activities, activity frequency, and sense of coherence between the tai chi and control groups. We used bivariate Spearman correlations of changes in depression symptoms and QoL with changes in social support, exercise self-efficacy, sense of coherence, activity engagement, C-reactive protein and TNF- $\alpha$  to explore individual-level associations among participants in the tai chi group.

To obtain a broader perspective on the relationship between emotional factors and HF in general, we used multivariable regression to explore the relationship between each psychosocial, behavioural, and biological variable and changes in depression and QoL, controlling for intervention group. Change in depression symptoms and change in QoL were separate dependent variables. We ran separate regression models for each independent variable (i.e. change in social support, self-efficacy, sense of coherence, activity engagement, C-reactive protein, and TNF- $\alpha$ ). Covariates were group (tai chi vs. control), gender, age, and baseline value of the outcome. All covariates were entered simultaneously along with the predictor of interest. Trends were considered ( $P < 0.15$ ) given the relatively small sample size and exploratory, hypothesis-generating nature of the study. All analyses were conducted using SPSS (Version 25).

## Results

Participants were 100 patients with chronic systolic HF (New York Heart Association Class = 1–3, ejection fraction < 40%). The average age was 67.4 years (SD = 12.0), and most were male (64%) and White (96%). Please see *Table 1* for demographic and clinical characteristics of the sample. Further details are provided in the source paper.<sup>9</sup>

*Table 2* provides baseline and endpoint (12 week) summary scores for each outcome by group. There was a significant difference in change in number of activities between groups with the tai chi group increasing and the education group decreasing the number of activities engaged in per week ( $P = 0.04$ ). There was also a significant difference in change in frequency of activity engagement between groups such that both groups increased, but this increase was greater in the tai chi group ( $P = 0.01$ ). There were no significant group differences in change in social support ( $P = 0.78$ ) or sense of coherence ( $P = 0.18$ ).

See *Table 3* for bivariate correlations among tai chi participants ( $n = 50$ ). Improvements in exercise self-efficacy were correlated with improvements in QoL ( $r = -0.31$ ,  $P = 0.05$ ). There was a trend toward an association between decreases in depression symptoms and increases in social support ( $r = -0.22$ ,  $P = 0.13$ ). There were no significant correlations

**Table 1** Baseline demographic and clinical characteristics of the sample

	Tai chi	Control
Age, <i>M</i> (SD)	68.1 (11.9)	66.6 (12.1)
Male sex, <i>n</i> (%)	28 (56)	36 (72)
Race, <i>n</i> (%)		
White	43 (86)	43 (86)
Black	5 (10)	5 (10)
Asian/Pacific Islander	1 (2)	2 (4)
American Indian	1 (2)	0
Screening LVEF, <i>M</i> (SD)	28.3 (8.0)	29.8 (7.3)
NYHA heart failure class, <i>n</i> (%)		
I	10 (20)	10 (20)
II	31 (62)	32 (64)
III	9 (18)	8 (16)
Cardiovascular co-morbidities, <i>n</i> (%)		
Myocardial infarction	24 (48)	34 (68)
Arrhythmia	33 (66)	32 (64)
Hypertension	35 (70)	35 (70)
Mood outcomes at baseline, median (Q1, Q3)		
Depression symptoms (POMS)	2 (0, 5)	3 (1, 6)
Quality of life (MLHFQ)	28 (12, 47)	21 (11, 52)

LVEF, left ventricular ejection fraction; MLHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association; POMS, Profile of Mood States—Depression subscale.

Note. Further details of sample characteristics are reported in Yeh *et al.*<sup>8</sup>

**Table 2** Median [Q1, Q3] for each outcome by group at baseline and endpoint

	Baseline	Endpoint
<i>Social support</i>		
Tai chi	70.0 [56.0, 78.5]	71.0 [64.5, 76.0]
Control	63.0 [53.75, 72]	63.5 [53.75, 73.25]
<i>Sense of coherence</i>		
Tai chi	69.5 [60.8, 77]	75.0 [64.8, 80.0]
Control	66.0 [57.0, 76.3]	67.0 [54.0, 76.0]
<i>Number of activities*</i>		
Tai chi	9.0 [6.0, 11.3]	10.0 [6.75, 11.25]
Control	9.0 [7.0, 12.0]	8.0 [6.0, 11.0]
<i>Activity frequency*</i>		
Tai chi	13.5 [8.0, 23.0]	29.5 [21.25, 48.50]
Control	15.5 [7.50, 21.0]	27.5 [20.5, 35.0]

Note. Summary scores for exercise self-efficacy have been previously published and showed greater improvement in the tai chi group.<sup>8</sup>

\* $P < 0.05$  for the difference between change scores in Wilcoxon rank sum tests.

between changes in activity engagement and outcomes, or between biomarkers and outcomes among tai chi participants.

In multivariable regression models controlling for intervention type, for change in depression symptoms (Table 4), the significant independent variables were change in sense of coherence [ $b = -0.31$ ,  $P = 0.001$ ,  $R^2 = 0.41$  (full model accounting for 41% of variance)] and change in C-reactive protein ( $b = 0.24$ ,  $P = 0.02$ ,  $R^2 = 0.41$ ). Change in frequency of activity engagement showed a non-significant trend ( $b = -0.17$ ,  $P = 0.07$ ,  $R^2 = 0.35$ ). Change in social support, exercise self-

efficacy, and TNF- $\alpha$  was not significant. For change in QoL (Table 5), significant independent variables were change in exercise self-efficacy ( $b = -0.44$ ,  $P < 0.001$ ,  $R^2 = 0.46$ ); change in sense of coherence ( $b = -0.23$ ,  $P = 0.01$ ,  $R^2 = 0.36$ ); and change in frequency of activity engagement ( $b = -0.19$ ,  $P = 0.05$ ,  $R^2 = 0.32$ ). Change in social support, number of activities engaged in, C-reactive protein, and TNF- $\alpha$  were not significant.

## Discussion

The purpose of this study was to explore changes in biopsychosocial and behavioural processes following tai chi for patients with HF and to explore potential correlates of improvements in depression symptoms and QoL to inform theoretical models and identify testable targets for future mechanistic work. Results indicated that tai chi showed benefit for improving overall activity engagement (including non-exercise behaviours and social activities), and improvements in depression symptoms and QoL may be correlated with improvements in other psychosocial, behavioural, and biological processes.

Tai chi participants reported that they engaged in a greater number of everyday activities and engaged in these activities more frequently, as compared with participants in the health education group. Building on prior work focused on moderate-intensity physical activity, these results demonstrate greater behavioural activation in daily life and increased engagement in leisure and social activities, which can have direct positive impacts on social, cognitive, and emotional functioning. Indeed, a gold-standard treatment for depression is behavioural activation, which involves helping people identify, plan, and engage in everyday activities that promote a sense of pleasure or mastery, such as visiting with friends and family or completing household chores.<sup>25</sup> As a behavioural intervention, tai chi may promote engagement in other activities by allowing patients to learn that they are able to initiate and successfully execute desired behaviours, despite the variety of uncomfortable physical or emotional symptoms that many patients experience. Increased activity during tai chi may also be self-reinforcing, as people notice improved emotional or social functioning, motivating them to become more engaged in activities outside of the tai chi class as well.

Tai chi did not, however, have a significant effect on sense of coherence or social support when compared with group education. It is possible that the 12 week dose was not sufficient to change broad lifestyle perspectives or that tai chi does not target cognitive change directly enough to have an effect for this older adult population. The lack of group differences in social support is consistent with the results of our qualitative study among these participants, which found themes of

**Table 3** Inter-correlations between changes in psychosocial, behavioural, and biological variables among tai chi participants

	1	2	3	4	5	6	7	8	9
1. $\Delta$ Depression	—	0.04	-0.22*	0.19	-0.16	-0.12	-0.08	0.14	-0.07
2. $\Delta$ QoL		—	-0.06	-0.31**	0.02	0.03	-0.14	-0.04	0.23
3. $\Delta$ Social support			—	0.21	0.41***	0.01	0.04	0.28*	-0.03
4. $\Delta$ Exercise self-efficacy				—	0.19	-0.02	-0.03	-0.15	-0.05
5. $\Delta$ Sense of coherence					—	0.12	-0.09	-0.05	0.20
6. $\Delta$ Number of activities						—	0.48***	-0.06	0.03
7. $\Delta$ Activity frequency							—	0.26*	0.09
8. $\Delta$ CRP								—	0.01
9. $\Delta$ TNF- $\alpha$									—

CRP, C-reactive protein.

Note. These are Spearman correlations. All change scores are in reference to the baseline and endpoint of the 12 week intervention (calculated as Time 2 – Time 1).

\* $P < 0.10$ .

\*\* $P < 0.05$ .

\*\*\* $P < 0.001$ .

**Table 4** Multivariable regression models for change in depression symptoms

	$R^2$	$b$	$t$	$P$
<i>Model 1</i>	0.41			<0.001
Gender		0.05	0.53	0.60
Age		0.01	0.16	0.88
Tai chi		-0.22	-2.45	0.0
Baseline depression		-0.56	-6.38	<0.001
$\Delta$ Sense of coherence		-0.31	-3.61	0.001
<i>Model 2</i>	0.41			<0.001
Gender		0.07	0.74	0.46
Age		0.07	0.71	0.48
Tai chi		-0.32	-3.26	<0.001
Baseline depression		-0.62	-6.19	<0.001
$\Delta$ CRP		0.24	2.49	0.02
<i>Model 3</i>	0.35			<0.001
Gender		0.05	0.55	0.58
Age		-0.02	-0.26	0.80
Tai chi		-0.24	-2.67	0.01
Baseline depression		-0.56	-6.36	<0.001
$\Delta$ Number of activities		-0.22	-2.50	<0.01
<i>Model 4</i>	0.35			<0.001
Gender		0.04	0.46	0.64
Age		-0.03	-0.31	0.75
Tai chi		-0.25	-2.69	0.01
Baseline depression		-0.59	-6.35	<0.001
$\Delta$ Activity frequency		-0.17	-1.84	0.07

CRP, C-reactive protein.

Note. All change scores are in reference to the baseline and endpoint of the 12 week intervention (calculated as Time 2 – Time 1). Social support, exercise self-efficacy, and TNF- $\alpha$  were not significant predictors. Only results for significant predictors or trends are presented owing to space constraints, but the full results can be obtained upon request.

improved social support in both groups.<sup>26</sup> Thus, the group setting may be particularly salient in promoting social support, consistent with a large body of literature.<sup>27</sup> Alternatively, these results may relate to the measure used in the current study, which assessed the type of support that individuals receive outside of the tai chi group and included dimensions that may not be likely to change over a 12 week period (e.g. receiving support from family members and having access to a special person to share joys and sorrows). Measures that assess the cohesiveness of the group itself may be more informative

**Table 5** Multivariable regression models for change in quality of life

	$R^2$	$b$	$t$	$P$
<i>Model 1</i>	0.46			<0.001
Gender		0.14	1.61	0.11
Age		0.04	0.45	0.66
Tai chi		-0.10	-1.13	0.26
Baseline QoL		-0.52	-5.98	<0.001
$\Delta$ Exercise self-efficacy		-0.44	-4.79	<0.001
<i>Model 2</i>	0.36			<0.001
Gender		0.08	0.85	0.40
Age		-0.06	-0.63	0.53
Tai chi		-0.17	-1.83	0.07
Baseline QoL		-0.54	-5.69	<0.001
$\Delta$ Sense of coherence		-0.23	-2.54	0.01
<i>Model 3</i>	0.32			<0.001
Gender		0.06	0.60	0.55
Age		-0.10	-1.01	0.31
Tai chi		-0.15	-1.56	0.12
Baseline QoL		-0.56	-5.65	<0.001
$\Delta$ Activity frequency		-0.19	-2.01	0.05

CRP, C-reactive protein.

Note. All change scores in reference to the baseline and endpoint of the 12-week intervention (calculated as Time 2 – Time 1). Social support, number of activities, CRP, and TNF- $\alpha$  were not significant. Only results for significant predictors or trends are presented owing to space constraints, but the full results can be obtained upon request.

for understanding how tai chi promotes social connectedness among participants, and how these social connections relate to health outcomes. There is some literature to suggest that tai chi involves participants' coordination of their own body movements with the body movements of others, and inter-personal motor coordination can increase social connectedness.<sup>28</sup> Researchers might therefore consider using measures of group cohesion to capture unique aspects that traditional social support measures do not.

While we did not detect group differences in social support, there was a trend toward an association between individual-level improvements in social support and improvements in depression symptoms for tai chi participants. That is, tai chi participants who reported increased social support

also tended to report corresponding decreases in depression symptoms, suggesting that social support may be an important factor for mood management in adults with HF. Improvements in exercise self-efficacy were also correlated with improvements in QoL for tai chi participants, which supports prior literature on the importance of self-efficacy and association between self-efficacy and QoL in patients with HF.<sup>29</sup> These results may suggest that changes in psychosocial processes are most relevant to tai chi-related mood improvements, and thus, future studies on mechanisms of tai chi for mood outcomes should focus on measuring other psychosocial variables such as social support and self-efficacy.

While psychosocial variables might be most relevant to tai chi-related mood improvements, a broader range of psychosocial, behavioural, and biological variables may all be important for general mood management in patients with HF, irrespective of intervention type. The finding regarding decreased C-reactive protein and decreased depression symptoms is consistent with the literature linking depression and inflammation among patients with cardiovascular disease.<sup>30</sup> In clinical practice, it may be most feasible to target psychosocial and behavioural outcomes, but knowledge of potential biological processes underlying improved depression symptoms may be encouraging for patients and providers.

Limitations to the current study include a relatively small sample size, use of a non-depressed sample, limited depression measures, predominantly White participants, and a focus on secondary analyses unplanned in the initial study. The results should be considered in light of the fact that these were post-hoc analyses. While the use of a non-depressed sample may have limited our ability to detect some associations (e.g. between activity engagement and depression symptoms), it also provided a more generalizable sample of patients with HF. Future trials using larger and more demographically diverse samples with clinically significant levels of depression may further extend these results. Not explored in the current analyses is the effect of postural aspects of movement and behaviour, which may be impacted by tai chi and have a beneficial impact on mood, highlighting a need for further research into behavioural processes.<sup>31</sup> Similarly, future research should explore the impact of exercise capacity and functional outcomes (e.g. 6 min walk distance and peak VO<sub>2</sub>) on mood, including mediation analyses to assess whether exercise capacity may be a causal mechanism explaining the effects of tai chi on mood outcomes.

Overall, these results suggest that tai chi may promote greater activity engagement for patients with HF and that self-efficacy and social support may be relevant to tai chi-related improvements in depression symptoms and QoL. Providers might consider tai chi, or other multidimensional practices that target mind–body–behaviour interactions (e.g. yoga and meditative walking), as an accessible way to promote behavioural engagement and target psychosocial processes important for emotional-well-being. More broadly, the current results also suggest that a range of psychosocial, behavioural, and biological processes may be relevant to general mood management in patients with HF. Clinically, these findings highlight the importance of addressing psychosocial and behavioural processes, in addition to biological processes targeted through medical management, to promote well-being in patients with HF, and that tai chi may be one appropriate mind–body approach.

## Conflict of interest

Peter Wayne is the founder and sole owner of the Tree of Life Tai Chi Center. His interests were reviewed and managed by the Brigham and Women's Hospital and Partner's HealthCare in accordance with their conflict of interest policies. The other authors declare no competing interests.

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## Ethics Statement

This study is in compliance with the *Declaration of Helsinki*. The Beth Israel Deaconess Medical Center Institutional Review Board approved the research protocol. Informed consent was obtained from all subjects.

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