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Collagen Metabolism Biomarkers and Health Related Quality of Life in Pulmonary Arterial Hypertension

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Abstract

Objectives—The goal of this study was to investigate the association between collagen metabolism biomarkers and health related quality of life (HRQoL) in PAH patients.

Methods—We prospectively enrolled 68 stable idiopathic, anorexigen-associated, and hereditary PAH subjects and 37 healthy controls. Serum samples were analyzed for N-terminal propeptide of type III procollagen (PIIINP), c-terminal telopeptide of collagen type I (CITP), matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1). The Minnesota Living with Heart Failure (MLWHF), Euro QoL-5D (EQ-5D), Cambridge Pulmonary Hypertension Outcome Review (CAMPOR) and Short Form (SF-36) general health survey were administered at the time of blood draw. General linear models, as well as logistic regression models were used to assess associations between variables.

Results—CITP, PIIINP, MMP9, and TIMP1 levels, and all HRQoL domains were significantly different between controls and PAH patients ($p < 0.001$ for each). Interestingly, PIIINP levels were significantly associated with MLWHF physical (coef=1.63, and $p=0.02$), SF-36 physical (coef=−2.93, $p=0.004$), and EQ-5D aggregate (coef=0.34, $p=0.001$) scores. Several of the CAMPOR scores strongly linearly associated with PIIINP. The odds of obtaining a walk distance 330 meters decrease by 38% per unit increase in PIIINP (OR=0.62; 95% CI=0.43, 0.90) and a PIIINP cutoff of 5.53 $\mu\text{g/L}$ provided 81% sensitivity and 82% specificity.

Conclusions—PIIINP is a good predictor of disease severity, and is strongly related to HRQoL scores in PAH patients. These relationships suggest PIIINP as a promising tool for PAH clinicians to determine or confirm the level of disease severity.

Introduction

Pulmonary Arterial Hypertension (PAH) is a progressive disease of the pulmonary vasculature that interferes with the normal functioning of the heart and lungs. Advances in PAH treatment in the past decade have improved patient outcome [1], but the number of

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tools available to physicians for the assessment of PAH patient health remain limited. Patient reported health related quality of life (HRQoL) surveys provide a good method for gauging patient health and functional status. Few studies have been done to determine the usefulness and accuracy of various measures of HRQoL [1,2–9] in PAH patients, though none have compared these to collagen biomarkers.

The Medical Outcome Study 36-item Short Form Health Survey (SF-36) has previously been administered to patients with cardiopulmonary disease. However, since it was designed to assess the health outcome of patients with a wide variety of health issues, it may not be an effective tool for the evaluation of PAH patients. The Minnesota Living With Heart Failure questionnaire (MLHFQ) is among the most useful measurements for the impact of heart disease on HRQoL [2,3] and has been shown to be useful in assessing QoL in PAH [4,10]. The Euro-QOL 5D (EQ-5 D) survey is another useful tool, developed for the purpose of general health assessment, and has been used recently in studies on heart failure, with good results [11]. The newly developed Cambridge Pulmonary Hypertension Outcome Review (CAMPHOR) is a reliable PH specific quality of life assessment tool [12–14].

The most prominent pathophysiological feature of PAH progression is an increase in vascular remodeling and fibrosis, primarily in the pulmonary vascular bed. Patients with severe disease have been shown to experience excess smooth muscle proliferation, and collagen production and metabolism in the affected pulmonary vasculature [15–17]. N-terminal propeptide of procollagen III (PIIINP) and Carboxy-terminal telopeptide of collagen I (CITP) can be found circulating in the bloodstream, and has been used as peripherally measurable markers of collagen metabolism [18–19]. Matrix metalloproteinase-9 (MMP-9) is a gelatinase whose primary function is the breakdown of basement membranes. However, the protein has been shown to play an active role in the inflammatory response, as well as in the regulation of angiogenesis [20–23]. Meanwhile, tissue inhibitor of metalloproteinase I (TIMP-1) is an inhibitor of several matrix metalloproteinases, including MMP-9. Recent publications provide evidence for excess collagen metabolism in patients with hypertension and heart failure by the discovery of elevated levels of circulating MMP-9 and TIMP-1, and that the proportion of tissue comprised of collagen can be assessed by serum analysis for markers of collagen breakdown and metabolism [24].

Recently, we showed that circulating levels of PIIINP, CITP, MMP9 and TIMP1 were elevated in our cohort of PAH patients and that the elevated PIIINP level indicated worse disease [25]. The aim of this study was to determine the relationship between HRQoL and collagen metabolites in PAH, as well as confirm the role that collagen biomarkers may play in determining disease severity in PAH in terms of poor HRQoL. Determining relationships between collagen biomarkers and HRQoL scores in PAH patients could help in elucidating disease severity.

Methods

Subject selection and enrollment

This is a prospective, non-interventional single center study conducted at the Baylor College of Medicine Pulmonary Hypertension Program. This study was undertaken after obtaining Baylor IRB approval and informed consent. PAH was defined by a historical right heart catheterization documenting mean pulmonary artery pressure of ≥ 25 mmHg and pulmonary artery wedge pressure of ≥ 15 mmHg. The inclusion criteria included 18 years of age or older, able to provide written informed consent and stable PAH therapy for at least one month prior to study enrollment. Sixty-eight patients with hereditary, anorexigen associated or idiopathic PAH were prospectively enrolled for the study. Thirty-nine healthy controls were enrolled in the study that had similar distributions of age, gender, and BSA as PAH patients, 2 were subsequently excluded due to systemic hypertension. For a more detailed list of the inclusion/exclusion criteria, see reference 25.

Health Related Quality of life questionnaires

HRQoL questionnaire administration and blood for collagen biomarker measurement was drawn at the same clinic visit. The MLWHF questionnaire (Regents of University of Minnesota, Minneapolis, MN), SF-36 general health survey version 1 (QualityMetric, Lincoln, RI), EQ-5D-3L (EQ-5D) (EuroQOL, Rotterdam, The Netherlands), and the CAMPHOR questionnaire (Galen Research Ltd, Manchester, UK) were used for assessment of HRQoL in our PAH patients and healthy controls. The CAMPHOR questionnaire was implemented about halfway through the study, so about 60% of PAH patients and 92% of controls were administered this survey at the time of blood draw.

The MLWHF survey is divided into three separate scores: the total score is the sum of all responses, with a maximum score of 100. It is divided into Physical and Emotional domains, each of which has maximum scores of 50. The SF-36 is divided in a similar fashion, into Physical and Mental health domains. The raw SF-36 scores for each question were converted to a 0–100 scale, and the average across questions for one of the domains was taken to be the value for that domain. Missing values for the SF-36 responses ($<5\%$ for each question) were taken to be the average score for that domain, so as not to disrupt the average score of the domain. The EQ-5D questionnaire had two parts: the aggregate score, ranging from 5–15, and the visual analogue scale (VAS), a patient selected number between 0–100 describing the patient's own perception of their overall health. The CAMPHOR questionnaire is divided into several categories in order to capture each of the individual and unique symptoms of PH. The domains were: Energy, Breathlessness, Mood, Activity, and Quality of Life. The Energy, Breathlessness, and Mood categories are then combined to create the Symptoms category. The scores are presented as raw scores. Missing values in the CAMPHOR questionnaire ($<5\%$ of answers) were handled by calculating a score for each of the domains that was weighted based on the number of missing values. For all questionnaires, higher scores indicated worse outcomes except for SF-36 and the VAS score of the EQ-5D questionnaire where lower scores indicated worse outcomes.

Biochemical measurements of Indices of collagen metabolism

A one-time blood draw was used to collect peripheral blood samples. The specimens were transferred immediately into a holding tube, allowed to clot and stored at -80°C until simultaneous analysis of collagen biomarkers was undertaken. Amino-terminal propeptide of procollagen type III (PIIINP) and carboxy-terminal telopeptide of collagen type I (CITP) levels were measured using double antibody radioimmunoassays (RIA) according to manufacturer's specifications (Orion Diagnostica, Finland, purchased through Immunodiagnostic System, Fountain Hills, Arizona). The sensitivity (lower detection limit) of both the CITP and the PIIINP assays were approximately 0.3 ng/mL . For both CITP and PIIINP assays, the coefficients of variation (CV) derived from duplicate measurements were $<10\%$. MMP-9 and TIMP-1 were measured by 2-site sandwich ELISAs (R & D Systems, Inc., Minneapolis, MN) per manufacturer's protocol and the CV derived from duplicate measurements were $<5\%$ for these assays.

Statistical Analysis

Patient characteristics and outcomes of the study population are presented as mean $\pm$ standard deviation, medians with 25th and 75th percentiles or frequencies with percentages, as appropriate. Summary statistics were stratified by patients and controls. Characteristics and outcome measures were compared between patients and controls using Chi-squared test, Fisher's exact test, t-test, or Wilcoxon rank sum test, as indicated. The correlations between the pairwise HRQoL measures were evaluated using Spearman's rank correlation. Cronbach's alpha was calculated for each of the questionnaire domains and for total scores, in order to determine the internal consistency (or reliability) of the HRQoL. The disease severity was defined in terms of poor 6 Minute Walk Distance 6MWD (< 330 meters), worse right atrial pressure (RAP) with a cutoff of 15 mmHg and worse WHO FC (I, II versus III and IV).

The association between HRQoL questionnaires and biomarkers was investigated using general linear models. This analysis was performed without and with adjustment for disease group. Regression coefficients (β) describe the linear association between predictor variables and a continuously measured outcome variable. Multivariable regression models simultaneously adjust for the effect of covariates included in the model. The p-values associated with regression coefficients test the two-sided null hypothesis that the coefficient is equal to zero. If the coefficient is statistically significantly different from zero, then there is a significant association between the predictor and dependent variable. We used linear regression tests as it provides an estimate of the magnitude of this association and also allows for the opportunity to adjust for the effects of confounding variables. We developed odds ratio estimates from a logistic regression fit by using the 95th or 5th percentile (whichever was in the direction of a worse outcome) HRQOL score from control distribution as a binary outcome.

Receiver Operating Curves (ROCs) were constructed by plotting the true positive rate (sensitivity) versus the false positive rate for predicting poor 6MWD. Of note, the use of 330 meters as a threshold for poor 6MWD is a conservative measure of disease severity. Furthermore, a recent study suggests that no specific 6MWD threshold has greater

prognostic value than any other [26], while others have shown 330 meters to be a good threshold [27]. All statistical analyses were performed in Stata (version 12.1, College Station, TX).

Results

Baseline characteristics of the PAH patients and healthy controls are summarized in Table 1 and for more detailed information see reference 25. Interestingly, we found that in our cohort of healthy controls and PAH patients, there were significant differences in the values of the meaningful HRQoL questionnaire scales and subscales ($p = 0.001$ for all; Table 2).

Quality of life measurements

We used Cronbach's alpha to determine reliability (internal consistency) and found a strong internal consistency of these questionnaires. We found that the physical and mental domains of the MLWF questionnaire were highly correlated with one another and with the MLWHF total score ($rs = 0.89$ for each pair), while they were negatively correlated with SF-36 measures and EQ-5D VAS ($-0.70 < rs < -0.88$ and $-0.66 < rs < -0.70$ respectively). MLWHF Physical, Emotional, and Total scores were also positively correlated with EQ-5D Aggregate ($0.73 < rs < 0.80$) and CAMPHOR measures (Energy: $0.60 < rs < 0.69$, Breathlessness: $0.69 < rs < 0.77$, Symptoms: $0.70 < rs < 0.76$, Activity: $0.75 < rs < 0.83$, QOL: $0.70 < rs < 0.75$). MLWF measures were not strongly correlated with CAMPHOR mood unlike other CAMPHOR measures. SF-36 was negatively correlated with all other quality of life measures in the study, except for EQ-5D VAS. There appeared to be higher correlations between SF-36 Physical and CAMPHOR measures.

Biomarkers and quality of life

Linear regression models constructed using biomarker values as the predictor for HRQoL scores adjusted for disease state showed that PIIINP related with many of these questionnaires (Table 3). Importantly, several of the CAMPHOR scores were strongly linearly associated with PIIINP. Also of note, MLWHF physical and EQ-5D aggregates positively associated with PIIINP while SF-36 physical is negatively associated with PIIINP. BNP also was negatively associated with the SF-36 Physical domain, but with no other scores.

Using the score distribution from the control questionnaire scores, cutoffs were constructed from the 95th or 5th percentile, as appropriate. Multiple logistic regressions were used to estimate odds ratios for having a poor outcome measure. After adjusting for disease severity, a unit increase in PIIINP appeared to increase the odds of having a HRQoL score above the 95th percentile of the control population by a significant amount for several of the HRQoL domains (Table 4). MMP9 increased the odds of having a CAMPHOR Breathlessness score of above the 95th percentile of the control score distribution after adjustment ($OR=1.01$, $p=0.045$). BNP also increased the odds of both CAMPHOR Breathlessness and QOL scores being above the 95th percentile of the control patient score distribution after adjustment ($OR = 0.99$ and 1.15 , $p=0.009$ and $p=0.015$ respectively).

Biomarkers and clinical outcome measurement tools

An ROC curve analysis was undertaken for 6MWD with a threshold of 330 meters as this walk distance has been shown to be associated with worse outcome. For each unit increase in PIIINP, the odds of a patient obtaining a distance of ≥ 330 decreases by 38% (OR=0.62; 95% CI=0.43, 0.90; $p=0.011$). The area under the curve was 0.8142. A PIIINP cutoff of 5.53 $\mu\text{g/L}$ provided a sensitivity of 81.32% and specificity of 81.82% (Figure 1). For RAP, each unit increase in PIIINP increases the odds by 40% (95% CI=1.06, 1.85; $p=0.018$). The area under the ROC for RAP was 0.6647. For the WHO functional class, the odds of a patient being class III or IV was 2.11 times greater per unit increase in PIIINP (95% CI=1.53, 2.91; $p<0.001$). The area under the ROC for this analysis was 0.7557.

Discussion

Research on PAH has gained momentum over the past several years, and data supports a role of collagen metabolism in the pathophysiology of PAH. Meanwhile, there are studies that have investigated HRQoL in PAH, but only enough to discern that the PH symptoms resemble a mixture of heart, lung, and systemic issues [24]. Our study investigated the relationship between clinically relevant, peripherally measurable markers of collagen metabolism and patient reported HRQoL in a cohort of idiopathic, anorexigen associated and hereditary PAH.

PIIINP and other collagen biomarkers

PIIINP is a marker of type III collagen turnover in the heart and lungs, and can be used as a measure of collagen metabolism [19]. Our recent data demonstrated PIIINP levels to be a prognostic indicator in PAH [25]. PIIINP is well known for its excellent storability and stability [28], and low between subject variability [29], both of which make it a reliable candidate for a disease indicator biomarker. Interestingly, PIIINP produced the most striking results, compared to the other collagen biomarkers. Our data showed that, in particular, PIIINP was strongly associated with the physically oriented domains of the CAMPHOR questionnaire. Similarly, higher PIIINP values increased the odds of having an abnormal (high) CAMPHOR score, estimated via logistic regression. As the CAMPHOR questionnaire is the most specific to PAH, this result provides promising evidence of the utility of PIIINP as a marker of disease severity and prognostic tool in PAH. Other studies done on the same patient population have provided evidence of relationships between PIIINP and the hemodynamic and clinical parameters which are typically used to assess disease state in PAH [25]. The relationship between PIIINP and the EQ-5D aggregate score is interesting in light of the recent findings of Roman A., et al. From their study, they concluded that the questionnaire adequately followed trends in PAH severity, with patients obtaining consistently worse scores as severity increases [30]. This association, therefore, supports the evidence for a relationship between PIIINP and disease severity in PAH. The trends seen with PIIINP in this study are similar to results of previous studies aiming to improve diagnosis and assessment of disease severity and progression in PAH [31–33]. The additional information provided by the ROC analysis provides even stronger evidence for the validity of PIIINP as a clinical tool for the PAH physician. PIIINP showed good

predictive capabilities with respect to the levels of RAP, and worsening WHO functional class and 6MWD, all three of which are widely accepted parameters of PAH severity.

Circulating biomarkers are an attractive tool as non-invasive means of prognostication in PAH. Several studies have tested biomarkers such as endothelin-1, isoprostanes, IL6, VEGF and vWF in small studies in an effort to prognosticate PAH [34–37]. Endothelin 1 level is elevated in PAH whereas increased urinary isoprostanes, biomarker of lipid peroxidation, are associated with increased mortality in PAH patients [34–36]. Platelet levels of VEGF also shown to be elevated in PH and similarly vWF levels correlated with mortality in PAH [37]. However, no single biomarker has stood the test of time except BNP in prognostic studies. The specificity of PIIINP in PAH remains to be determined in large studies. Nevertheless, its good stability, its low within-subject variation, its circulating concentration easily measurable, and above all its quantification on lab analyzers, now possible with analytical robustness and reproducibility, make PIIINP a prime candidate to evaluate in future PAH prognostic studies.

Limitations

An interesting point not covered in this study is the role that PAH specific medications play in determining or altering HRQoL scores and how those effects compare to the effects that the medications have on the physically manifested symptoms of PAH. One study by Rival G., et al. showed that certain PAH treatments have dramatic effects on patient perceived HRQoL [38]. A consideration for interpreting our result involves the sample size used for the logistic regression estimation of the odds ratio. While logistic regression is a valid method for estimating odds ratio, the confidence interval for such estimation is wide for a small sample size. Another point is the apparent (though not statistically significant) difference in mean age between the control and patient groups (Table 1). It seems plausible that the difference in PIIINP concentrations between the two groups could be age related. However, PIIINP levels tend to decrease until the age of 50, and then increase very slightly thereafter into old age [33]. Since our patient population was, on average, older one would assume that, given no other differences between the two groups, the PIIINP levels of the control group would be slightly higher. However, the exact opposite was seen (Table 1), which is concurrent with the findings that PIIINP is elevated in a diseased state [15–19,25]. In contrast to the PIIINP related results, associations between the other collagen biomarkers and the HRQoL scores were either uncommon or weak. Thus these biomarkers may not be as good as PIIINP as indicators of disease state, as perceived by the patient.

Conclusion

As shown, PIIINP is a good predictor of disease severity, and is strongly related to HRQoL scores in PAH patients. These relationships suggest PIIINP as a promising tool for PAH clinicians to determine or confirm the level of disease severity. However, a larger study is needed to firmly establish these findings. It would be interesting to explore whether these collagen biomarkers specifically PIIINP could have any role in forecasting the risk of PAH, not only to predict the disease severity. Further studies are needed to fully establish PIIINP as a prognostic and severity marker in PAH.

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Figure 1.

ROC curves of PIIINP predicting worse walk distance. ROC analysis was conducted using 330 meters as six-minute walk distance threshold using PIIINP as the predictor variable. The area under the curve was 0.8142 and the PIIINP cutoff that gave the highest sensitivity and specificity was 5.53 $\mu\text{g/L}$.

Table 1

Data presented as either mean $\pm$ SD or as median (25th, 75th percentiles), or as N (%), depending on data type.

	Anx, Fam, idiopathic PAH	Controls	p-value
	N=68	N=37	
CITP (ng/ml)	3.93 $\pm$ 1.78	2.21 $\pm$ 1.21	<0.001
PIIINP (μ g/L)	5.30 $\pm$ 1.87	3.081 $\pm$ 0.93	<0.001
MMP9 (ng/ml)	434.3 (292.1, 662.7)	236.5 (170.7, 369.8)	<0.001
TIMP1 (ng/ml)	203.96 $\pm$ 60.39	128.03 $\pm$ 33.57	<0.001
BNP (pg/ml)	53 (23, 134)	16 (10, 22)	<0.001
Gender, Female	62 (91%)	33 (89%)	0.739
Age (yrs)	45.46 $\pm$ 15.35	49.54 $\pm$ 14.18	0.1841
Race, Caucasian	37 (54%)	23 (62%)	0.207
BSA (m2)	1.84 $\pm$ 0.24	1.79 $\pm$ 0.22	0.3448
WHO functional class III–IV	32 (47%)	0	<0.001
Edema	24 (35%)	1 (3%)	<0.001
6 Minute Walk Distance (meters)	414.34 $\pm$ 104.33	468.77 $\pm$ 63.23	0.0057
Borg dyspnea Score	2 (1, 3)	0 (0, 0.5)	<0.001
Lowest oxygen saturation (%)	87 (84, 92)	95 (89, 96)	<0.001
HR Max (beats/min)	126.30 $\pm$ 20.50	107.31 $\pm$ 13.56	<0.001

P values <0.05 are considered statistically significant

Table 2

HRQoL questionnaire scales in PAH patients and Controls.

	Possible Range	Cronbach's Alpha	Anx, Fam, Idiopathic PAH	Controls	p-value
MLWF Physical, Mdn (25th, 75th)	0–50	0.970	16 (9.5, 29.5)	0 (0, 0)	<0.001
MLWF Emotional, Mdn (25th, 75th)	0–50	0.924	8 (2, 14)	0 (0, 0)	<0.001
MLWF Total, Mdn (25th, 75th)	0–100	0.974	26 (13, 42)	0 (0, 0)	<0.001
SF36 Physical, Mean(SD) N=94	0–100	0.955	45.77 (20.69)	92.47 (7.92)	<0.001
SF36 Mental, Mean(SD) N=94	0–100	0.908	62.07 (21.58)	84.98 (10.05)	<0.001
EQ5D Aggregate, Mdn (25th, 75th) N=97	5–15	0.815	7 (6, 9)	5 (5, 5)	<0.001
EQ5D VAS, Mean(SD) N=99	0–100		63.81 (21.88)	88.72 (12.19)	<0.001
CAM Energy, Mdn (25th, 75th) N=75	0–10	0.917	3 (0, 6)	0 (0, 0)	<0.001
CAM Breathless, Mdn (25th, 75th) N=75	0–8	0.825	3 (2, 4)	0 (0, 0)	<0.001
CAM Mood, Mdn (25th, 75th) N=75	0–7	0.878	1 (0, 2)	0 (0, 0)	<0.001
CAM Symptoms, Mdn (25th, 75th) N=75	0–25	0.938	7 (3.125, 10)	0 (0, 0)	<0.001
CAM Activity, Mdn (25th, 75th) N=75	0–30	0.898	7 (5, 9)	0 (0, 0)	<0.001
CAM QOL, Mdn (25th, 75th) N=75	0–25	0.942	4 (2, 9)	0 (0, 0)	<0.001

Table 3

Linear Regression of Quality of Life measures in PAH patients and controls

β	MLWHF			SF36			EQ5D			Camphor		
	Total	Physical	Mental	Physical	Mental	Aggregate	VAS	Energy	Breathlessness	Mood	Symptoms	Activity
PHNP	1.92 [†]	1.63 [*]	0.29	-2.93 ^{**}	-3.15 [*]	0.34 ^{**}	-s1.70	0.51 ^{**}	0.41 ^{**}	0.29 [†]	1.21 ^{**}	0.62 [*]
ICTP	0.09	0.19	-0.10	-2.48 [*]	-1.13	0.13	-0.22	0.21	0.25 [*]	-0.00	0.45	0.40 [†]
MMP9	0	0	0	0	0	-0.00	0.01	-0.00	-0.00	-0.00	0.00	-0.01
TTMP1	-0.02	-0.01	-0.01	-0.07 [*]	0.01	0	-0.02	-0.00	-0.00	-0.00	-0.01	-0.00
BNP	0.01	0.01 [†]	0	-0.03 ^{**}	-0.02 [†]	0.00 [*]	0	-0.00	-0.00	-0.00	0.00	-0.00

Note: All values are linear regression coefficients, after adjusting for the PAH diagnosis.

[†] p<0.10;

^{*} p<0.05;

^{**} p<0.01

Table 4

PHINP Odds ratios for 95% cutoff from control HRQoL score distribution.

		Odds ratio	95% C.I.	p-value
MLWHF	Total	1.04	0.71 1.51	0.84
	Physical	1.04	0.71 1.51	0.84
	Emotional	1.11	0.86 1.43	0.43
SF-36	Physical	1.23	0.89 1.69	0.21
	Mental	1.52	1.10 2.10	0.01*
EQ-5D	Aggregate	1.52	1.02 2.28	0.04*
	VAS	1.25	0.88 1.76	0.21
Camphor	Energy	1.53	1.03 2.27	0.03*
	Breathlessness	1.40	0.75 2.59	0.30
	Mood	1.71	1.05 2.78	0.03*
	Symptoms	1.36	0.84 2.22	0.213
	Activity	1.83	0.69 4.89	0.227
	QOL	1.46	0.80 2.63	0.214

Note: The logistic regression models used to compute all odds ratio estimates included adjustment for disease severity.