Transplant Immunology 52 (2019) 40–44

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Changes in serum KL-6 levels are associated with the development of chronic lung allograft dysfunction in lung transplant recipients

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ARTICLE INFO

Keywords:
Lung transplant
Allograft dysfunction
KL-6

ABSTRACT

Chronic lung allograft dysfunction (CLAD) remains a leading cause of death after lung transplantation. KL-6 is a reliable biomarker for various interstitial lung diseases and levels are increased in lung transplant recipients with versus without bronchiolitis obliterans syndrome. This study investigated whether changes in serum KL-6 levels over time were associated with CLAD.

Twenty-one lung transplant recipients had serum KL-6 measured (NANOPIA®) at baseline and after 7 years. Changes in serum KL-6 levels from baseline were determined. Receiver operating characteristic curves and Kaplan-Meier analysis were used to test the predictive value of changes in serum KL-6 over time. The average increase in KL-6 in patients with CLAD was 15% versus a 28% decrease in non-CLAD patients ($p = .042$). An 11% decrease in serum KL-6 level was determined as the best cut-off value to be associated with the development of CLAD (86% sensitivity, 78% specificity). Kaplan-Meier analysis confirmed the association between this cut-off and the development of CLAD (log rank $p = .013$).

In this small cohort, changes in serum KL-6 over time were associated with the development of CLAD after lung transplantation.

1. Introduction

Lung transplantation (LuTx) is the final therapeutic option for patients with end-stage lung disease. Although survival after LuTx has improved in recent years, chronic rejection remains a major contributor to poor survival [1]. According to the International Society for Heart and Lung Transplantation (ISHLT), bronchiolitis obliterans syndrome (BOS) describes lung transplant recipients with a persistent loss of allograft function starting three or more months after transplantation. The clinical diagnosis of BOS is defined as a persistent decline in forced expiratory volume in 1 s (FEV_1) to ≤80% of the post-transplant baseline FEV_1 that is present for a minimum of 3 weeks, in the absence of confounding factors [2,3].

Chronic lung allograft dysfunction (CLAD) is a description that was introduced in 2010 to cover both, the obstructive and restrictive form of chronic allograft dysfunction [6]. It has been proposed that CLAD should be defined as the irreversible decline of FEV_1 and/or forced vital capacity (FVC) to < 80% of baseline [3,4].

Regarding the pathogenesis of chronic rejection, BOS is associated with inflammation, fibroproliferation and obstruction of small airways [2,4,7,8]. BOS affects > 50% of lung transplant recipients who survive to 5 years after transplantation and remains the leading cause of death of lung transplant recipients who survive the first year after lung transplantation. Therefore, there is an urgent need for biomarkers that facilitate early diagnosis, allowing early interventions to prevent the irreversible loss of lung function.

KL-6, a high-molecular-weight-human MUC1 mucin, is known to be a sensitive biomarker for various interstitial lung diseases, such as idiopathic pulmonary disease (IPF) [9], drug-induced pneumonitis [10], pulmonary alveolar proteinosis [11], and sarcoidosis [12]. KL-6 is

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https://doi.org/10.1016/j.trim.2018.10.006
Received 25 June 2018; Received in revised form 24 October 2018; Accepted 30 October 2018
Available online 01 November 2018
0966-3274/

mainly expressed in the lungs by alveolar type II cells, and to a lesser extent in bronchial epithelial cells [13].

Serum levels of KL-6 are increased in lung transplant recipients with BOS compared to patients without BOS or healthy controls [14]. Furthermore, data from a previous study showed that serum KL-6 levels appeared to correlate with the decline from baseline in FEV_1, and the diagnostic accuracy of serum KL-6 level has been shown to be superior to bronchoalveolar lavage (BAL) neutrophilia for the detection of BOS [15]. The purpose of this study was to investigate whether changes in serum KL-6 levels over time are associated with the development of CLAD in lung transplant recipients.

2. Materials and methods

2.1. Subjects

Patients who underwent a double lung transplant and follow-up at the Ruhrlandklinik, West German Lung Center, University Hospital Essen, Germany were eligible. The initial cohort was recruited and underwent baseline measurements between February and November 2007 (mean time post-transplant 627 ± 161 days).Exclusion criteria were postoperative malignancy, second lung transplantation, BOS grade 1 or greater at the time of enrollment and patient refusal. The study was approved by the local Institutional Review Board (IRB) and written informed consent was obtained from all participants.

2.2. Clinical definitions

CLAD was defined as an irreversible decline in FEV_1 and/or FVC to < 80% of baseline in the absence of confounding factors [4]. BOS was diagnosed according to ISHLT criteria (significant decrease in FEV_1 to ≤80% of the post-transplant baseline and forced expiratory flow at 25–75% of FVC [$FEF_{25–75\%}$] > 75% of post-transplant baseline from the average of the two best measurements made at least 3 weeks apart, without use of an inhaled bronchodilator) [2]. RAS was diagnosed by a persistent decline in FEV_1 (by > 20% compared with the best post-operative value) and an associated restrictive pulmonary function defect (defined as a > 20% decline in total lung capacity [TLC] versus baseline) [4].

2.3. Sample collection

Serum samples for the measurement of KL-6 were obtained by venipuncture at baseline (2007) and during the latest follow-up (2014); samples were stored at −80 °C until analysis. The average time between the two measurements was 2409 ± 183 days.

2.4. Biomarker measurements

KL-6 was measured using the NANOPIA® KL-6 assay (Sekisui diagnostics) according to the manufacturer's protocol. All samples were measured in duplicate, and mean values were used for the subsequent analysis. Serum lactate dehydrogenase (LDH) was measured during routine blood examinations and used as a comparison parameter.

2.5. Pulmonary function tests

Lung function tests were performed by a trained technician using Zan500 Body (nSpire Health, Oberthulba, Germany) according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [16].

2.6. Statistical methods

Normality of distribution for assessed variables was evaluated with the Kolmogorov-Smirnov test. Data are expressed as mean ± standard deviation (SD). Between-group differences in numerical variables were evaluated using unpaired *t*-test or Mann-Whitney *U* test for normally-distributed and skewed data, respectively. Comparisons between groups were performed with one-way analysis of variance (ANOVA) for normally distributed data and with the Kruskal-Wallis test for skewed data, with appropriate post-hoc tests to adjust for multiple comparisons (Bonferroni). The association of the changes in KL-6 over time with the development of CLAD was tested using a Receiver Operating Characteristic (ROC) curve, calculating the area under the curve (AUC) as an effective measure of accuracy, and the predictive accuracy of these changes was evaluated using Kaplan-Meier analysis. Uni- and multivariate analyses for possible predictors of CLAD were performed using a Cox-proportional hazard model. Statistical analyses was performed using SPSS (IBM SPSS Version 21). A p-value of < 0.05 was defined as statistically significant.

3. Results

3.1. Demographics and patients characteristics

Of 38 patients who were CLAD-free at time of the baseline measurement, 22 were still alive for follow-up after 7 years. All enrolled patients were Caucasians and non-smokers. Of the 22 surviving patients, 21 had serum KL-6 levels measured at baseline and follow-up and were included in the analysis (Table 1). During the follow-up period, 9/21 patients (43%) met the diagnostic criteria for CLAD in the form of BOS. No patients in this cohort developed RAS. At the time of follow-up assessment, 19 patients were clinically stable, one had a cytomegalovirus reactivation and one had a pulmonary infection.

3.2. Serum KL-6 levels

Serum levels of KL-6 at baseline and follow-up are detailed in Table 2. Mean serum KL-6 levels at follow-up did not differ significantly between patients with and without CLAD (331.1 ± 87.01 U/mL vs. 358.3 ± 165.1 U/mL, respectively; p = .712) (Fig. 1). Mean serum LDH levels at follow-up were also not statistically different between CLAD and non-CLAD patients (82.1 ± 114.6 U/L in CLAD vs. 184.5 ± 243.9 U/L, respectively; p = .153).

Changes in KL-6 over time were calculated as percentage values. Average serum KL-6 levels decreased over time in lung transplant recipients without CLAD but increased over time in those with CLAD (average change: −28% for non-CLAD vs. 15% for CLAD; p = .042) (Fig. 2). There was no significant difference between CLAD and non-CLAD patients in the average change in LDH levels (−16% vs. +21%, respectively; p = .113).

Table 1
Patient characteristics at baseline.

Variables[a]	Patients (n = 21)
Sex, n (%)	
Male	14 (67)
Female	7 (33)
Age at transplant, years	45 ± 9
Age at CLAD diagnosis, years	52 ± 9
Time from transplant to KL-6 measurement, days	627 ± 161
Time from KL-6 measurement to CLAD onset, days	2252 ± 563
Initial diagnosis, n (%)	
Cystic fibrosis	4 (19)
COPD	9 (43)
Pulmonary fibrosis	5 (24)
Pulmonary artery hypertension	1 (5)
Bronchiolitis obliterans syndrome	2 (9)

CLAD: chronic lung allograft dysfunction; COPD: chronic obstructive pulmonary disease.

[a] Data are shown as mean ± standard deviation, or number of patients (%).

Table 2
Changes of KL-6, lactate dehydrogenase levels in serum, and forced expiratory volume in 1 s over time in the 21 included patients.

Variables[a]	Baseline	Follow-up	p-value
KL-6, U/mL	364 ± 25	350 ± 29	0.817
LDH, U/L	194 ± 24	234 ± 45	0.257
FEV_1, % predicted	77 ± 6	71 ± 8	0.301

FEV_1: forced expiratory volume in 1 s; LDH: lactate dehydrogenase.
[a] Data are shown as mean ± standard deviation.

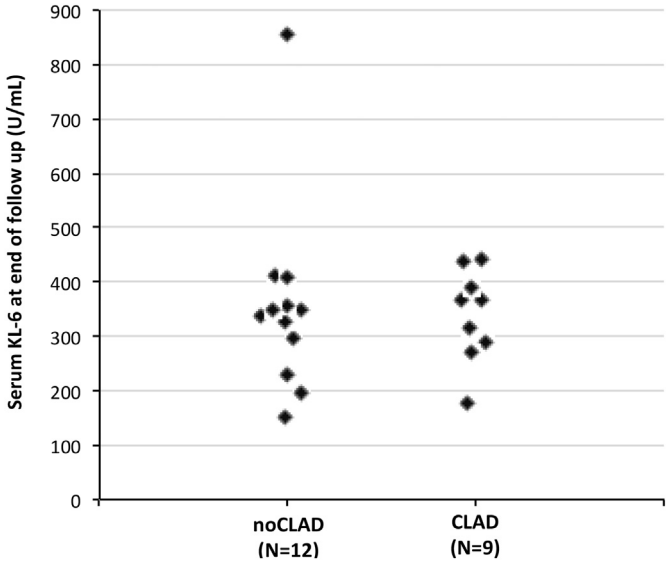

Fig. 1. Scatter plot showing individual serum KL-6 levels at follow-up in patients with or without chronic lung allograft dysfunction (CLAD).

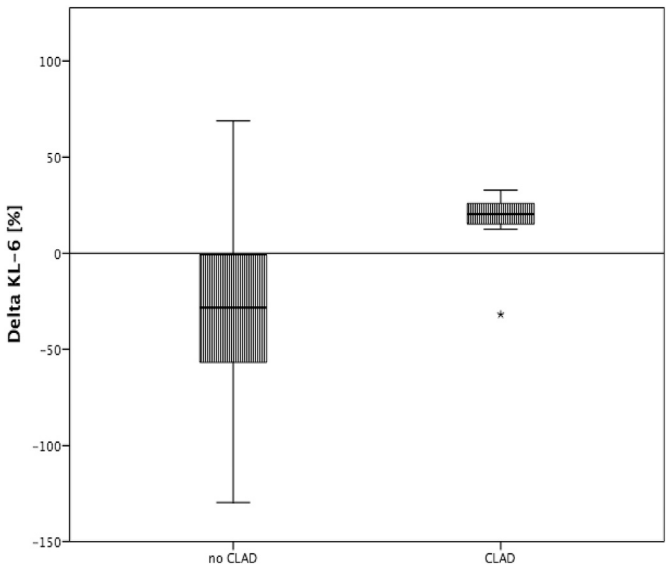

Fig. 2. Change in serum KL-6 levels over time in patients with or without chronic lung allograft dysfunction (CLAD).

3.3. ROC analysis

This analysis showed that an 11% decrease in KL-6 levels over time (AUC 0.810; p = .014), which had a sensitivity of 86% and a specificity of 78%, was the best cut-off value to be associated with the development of CLAD (Fig. 3). No significant cut-off values for the association with CLAD were found for FEV_1 at baseline or changes over time (p = .535 and p = .186 respectively), or for serum LDH at baseline or changes over time (p = .860 and p = .191).

3.4. Uni- and multivariate analyses for prediction of CLAD

In univariate analysis, a > 24% reduction in FEV_1 and a < 11% change in serum KL-6 level were significantly associated with the development of CLAD (hazard ratio [HR] 6.331; p = .020) (Table 3), and serum levels of LDH and KL-6 at baseline were associated with a decreased risk of CLAD (HR 0.996 and 0.996, respectively). In multivariate analysis, only a < 11% change in serum KL-6 could be still associated with the development of CLAD after adjustment for age, gender, change in FEV_1, and baseline serum levels of LDH and KL-6 (HR 17.61; p = .049). There was a trend towards statistical significance for age as parameter associated with CLAD, with risk increasing in parallel with increasing age (HR 1.163; p = .064) (Table 3).

3.5. Kaplan-Meier analysis

Patients with a < 11% decrease in serum KL-6 levels over time were at greater risk of developing CLAD compared to those with a ≥ 11% decrease in serum KL-6 from baseline (log rank p = .013) (Fig. 4).

4. Discussion

The present study showed that serum KL-6 levels increased or remained stable over time in lung transplant recipients who developed CLAD, whereas KL-6 levels decreased over time in those who did not develop CLAD. A decrease of 11% in KL-6 levels over time was determined to be the best cut-off value to be associated with the development of CLAD; this was confirmed in a subsequent analysis.

To our knowledge this is the first study evaluating long-term changes in KL-6 serum levels in lung transplant recipients and correlating these with the development of CLAD after transplantation. A previous study showed that KL-6 serum levels were higher in lung transplant recipients with BOS compared to those without BOS and healthy individuals, and that KL-6 levels correlated with the decline in FEV_1 after transplantation [14]. We confirmed these results in our previous work and also demonstrated the superiority of KL-6 over the percentage of neutrophilia in BAL fluid as a predictor of BOS [15]. Both studies have illustrated that KL-6 levels are not elevated during episodes of acute rejection or during infection, implying that KL-6 might be a useful predictive biomarker for the diagnosis of BOS after lung transplantation [15,17]. A recent study showed higher KL-6 levels in pediatric patients with versus without BOS after allogenic stem cell transplantation, suggesting that measurement of KL-6 in serum may also identify patients at higher risk for the development of BOS in this specific population [18].

The present study showed that an increase in KL-6 levels over time is associated with the development of CLAD with high sensitivity and specificity (86% and 78% respectively). These findings are in agreement with a previous study indicating a possible role of KL-6 in predicting the development of CLAD. Haberman et al. reported that a 200 U/mL increase in KL-6 serum levels from baseline was seen in lung transplant recipients with versus without BOS [17]. However, in that study KL-6 changes were assessed over a follow-up period of about 2 years, mainly in a pediatric population, and did not reach statistical significance. An interesting observation was that peak KL-6 levels occurred either soon before, or coincided with, the diagnosis of BOS. We cannot verify this finding in our study because KL-6 was not measured at CLAD onset but over long-term follow-up.

KL-6 is produced in type II pneumocytes and bronchial epithelial cells of the lungs. Low concentrations of this protein can be measured in the serum of healthy subjects, presumably due to its leakage through the air-blood barrier into the circulation. KL-6 is believed to cause

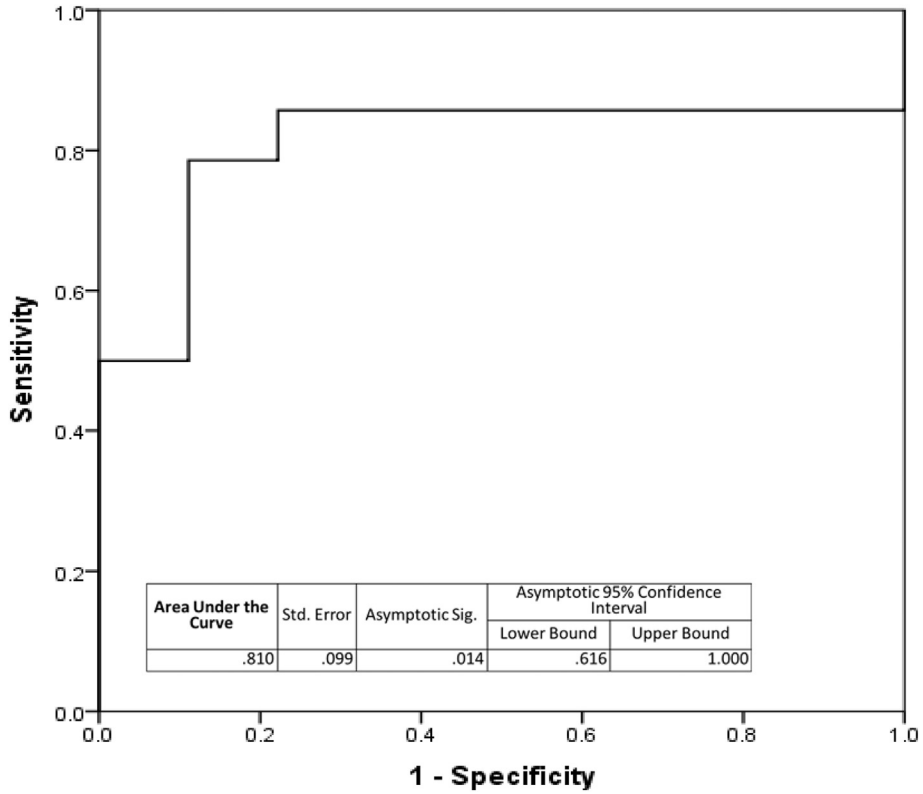

Fig. 3. Receiver operator characteristic (ROC) curve for an 11% decrease in serum KL-6 levels over time to identify patients with CLAD. Se, Sp are reported in the text of the results.

Table 3
Univariate and multivariate analyses for prediction of chronic lung allograft dysfunction.

Variables	Hazard ratio (95% CI)	p value[a]
Univariate analysis		
Age, years (continuous)	1.005 (0.968, 1.042)	0.807
Gender (female = 1)	1.165 (0.539, 2.518)	0.697
FEV_1 at baseline (continuous)	0.992 (0.977, 1.007)	0.271
Change in FEV_1 (continuous)	1.030 (1.002, 1.059)	0.035
Serum KL-6 at baseline (continous)	0.996 (0.993, 1.000)	0.041
≤11% change in serum KL-6 (categorical)	6.331 (1.331, 30.111)	0.020
Serum LDH at baseline (continuous)	0.996 (0.993, 1.000)	0.031
Change in serum LDH (continuous)	0.989 (0.970, 1.008)	0.243
Multivariate analysis[b]		
≤11% change in serum KL-6 (categorical)	17.283 (0.024, 12,626.369)	0.049
Age, years (continuous)	1.163 (0,992, 1.363)	0.064

CI: confidence interval; FEV_1: forced expiratory volume in 1 s; LDH: lactate dehydrogenase.

[a] The results are shown as hazard ratios (HR), representing the relative risk to develop CLAD as a specific characteristic at baseline. P values were obtained by using backward and forward stepwise (Conditional LR) analyses (Cox proportional model).

[b] Covariates included in the multivariate mode and not influencing: gender, Δ (delta) FEV1, serum LDH and serum KL-6 at baseline.

fibrotic changes in the lungs because elevated serum levels of KL-6 are found in various interstitial lung diseases [10,11,19,20]. Therefore, KL-6 is currently used as clinical biomarker for these conditions. Ohshimo et al. reported that KL-6 accelerated proliferation and inhibited apoptosis of human lung fibroblasts in vitro [21]. It is assumed that increased production of KL-6 in the regenerating type II pneumocytes and that increased permeability through the damaged alveolar/blood surface into the circulation leads to the increased levels of KL-6 measured in patients with interstitial lung diseases [17]. This increase in KL-6 is believed to result in fibrotic changes in the lungs via the proliferative and anti-apoptotic effects on lung fibroblasts [15].

The pathogenesis of BOS remains poorly understood, but is assumed to be characterized by fibrosis of the small airways [4,7]. This could be the explanation for the increased levels of KL-6 over time found in these patients. Early diagnosis of CLAD is clinically important because this allows earlier initiation of treatment that could possibly improve patient survival. Therefore, the need for biomarkers to facilitate early detection of chronic rejection is of high clinical value.

A major limitation of this study is that changes in serum KL-6 levels were not measured at the onset of CLAD. As a result, it is not possible to evaluate the clinical role of KL-6 as a predictor of the onset of CLAD. Furthermore, this study did not include patients with RAS in the follow-up measurement because all those with RAS died prior to the follow-up investigation. The evaluation of whether changes in KL-6 over time could predict the development of RAS should therefore be the subject of future studies. Finally, this cohort consisted of a relatively small number of patients. Therefore, further work is required to analyze long-term changes in KL-6 serum levels in larger patient cohorts.

In summary, this study showed that serum KL-6 levels increased or remain stable over time in lung transplant recipients who develop CLAD, but decreased in lung transplant recipients without CLAD. In our small cohort, these changes were associated with the development of CLAD, but validation of these findings in larger populations is required. The availability of additional data will help to determine the potential utility of changes in serum KL-6 levels over time as an easy-to-measure biomarker for the development of CLAD, and clarify its role in the clinical management of patients after lung transplantation.

Funding

This research did not receive any specific grant funding from agencies in the public, commercial, or non-for-profit sectors.

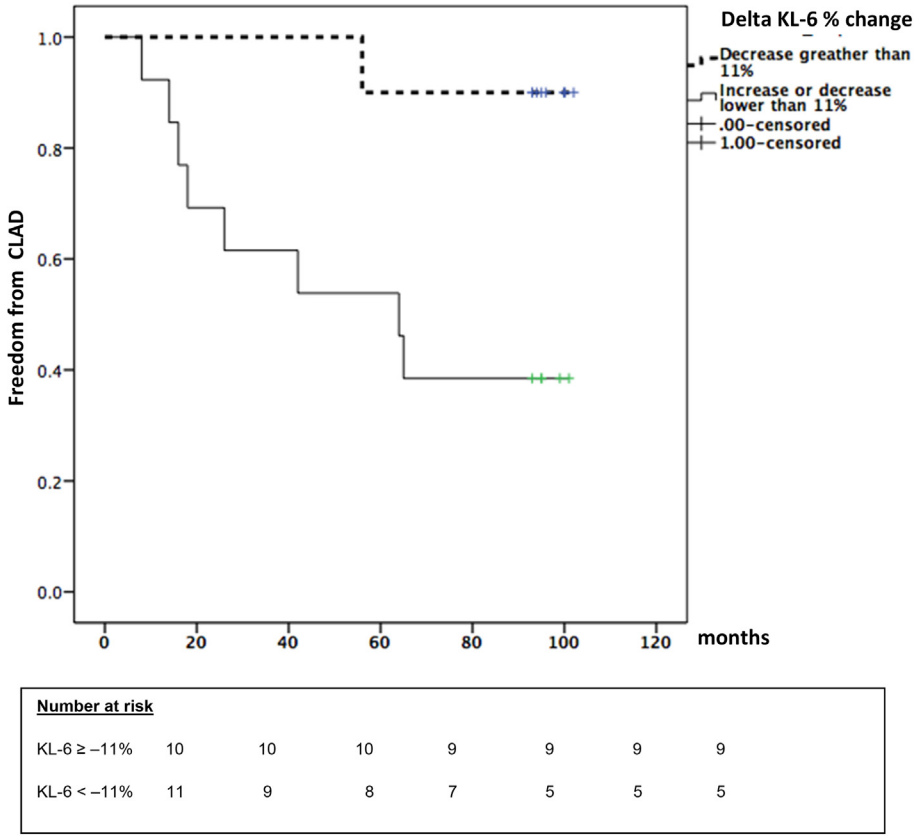

Fig. 4. Risk of developing chronic lung allograft dysfunction (CLAD) is based on change in serum KL-6 level over time (< 11% decrease versus ≥11% decrease).

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

English language editing assistance was provided by Nicola Ryan, independent medical writer.

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