

BMJ Open Protocol of the PLeural Effusion And Symptom Evaluation (PLEASE) study on the pathophysiology of breathlessness in patients with symptomatic pleural effusions

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ABSTRACT

Introduction: Pleural effusion is a common clinical problem that can complicate many medical conditions. Breathlessness is the most common symptom of pleural effusion of any cause and the most common reason for pleural drainage. However, improvement in breathlessness following drainage of an effusion is variable; some patients experience either no benefit or a worsening of their breathlessness. The physiological mechanisms underlying breathlessness in patients with a pleural effusion are unclear and likely to be multifactorial with patient-related and effusion-related factors contributing. A comprehensive study of the physiological and symptom responses to drainage of pleural effusions may provide a clearer understanding of these mechanisms, and may identify predictors of benefit from drainage. The ability to identify those patients whose breathlessness will (or will not) improve after pleural fluid drainage can help avoid unnecessary pleural drainage procedures, their associated morbidities and costs. The PLeural Effusion And Symptom Evaluation (PLEASE) study is a prospective study to comprehensively evaluate factors contributing to pleural effusion-related breathlessness.

Methods and analysis: The PLEASE study is a single-centre prospective study of 150 patients with symptomatic pleural effusions that require therapeutic drainage. The study aims to identify key factors that underlie breathlessness in patients with pleural effusions and develop predictors of improvement in breathlessness following effusion drainage. Participants will undergo evaluation pre-effusion and post-effusion drainage to assess their level of breathlessness at rest and during exercise, respiratory and other physiological responses as well as respiratory muscle mechanics. Pre-drainage and post-drainage parameters will be collected and compared to identify the key factors and mechanisms that correlate with improvement in breathlessness.

Ethics and dissemination: Approved by the Sir Charles Gairdner Group Human Research Ethics Committee (HREC number 2014-079). Registered with

Strengths and limitations of this study

- First prospective study to comprehensively assess breathlessness in patients with pleural effusions.
- Validated assessment tools used can be performed in the clinical setting.
- Developing predictors of improvement in breathlessness following pleural effusion drainage may influence clinical practice.
- Not all of the vast number of potential contributing factors to breathlessness can be included in the study.
- The methods of drainage and the volume of fluid removed cannot be standardised, as the clinical need of individual patients differs.

the Australian New Zealand Clinical Trials Registry (ACTRN12616000820404). Results will be published in peer-reviewed journals and presented at scientific meetings.

Trial registration number: ACTRN12616000820404; Pre-results.

INTRODUCTION

Each year in Australia, pleural effusions are found in around 60 000 patients. There are more than 60 causes of pleural effusions, with malignancy, infection and heart failure being the most common. Breathlessness, often disabling, is the most common symptom in patients with pleural effusions.¹ Pleural fluid drainage is often performed to relieve breathlessness from recurrent (eg, malignant) pleural effusions, but its effectiveness is variable and unpredictable. In a prospective study of patients with malignant

pleural effusion (MPE) undergoing therapeutic pleural fluid drainage, the change in the level of breathlessness after drainage, assessed using a 100 mm Visual Analogue Scale (VAS) score, varied widely; ~15% of patients reported no significant improvement, or worsening, of their breathlessness after drainage.²

Breathlessness arising from pleural effusions is likely to be multifactorial. The pathophysiological mechanism of breathlessness from pleural effusion is unclear. The severity of breathlessness often correlates poorly with the size of the effusion. The modest improvement in forced expiratory volume in 1 s (FEV₁) (~200 mL for every litre of pleural fluid removed) and change in pulmonary mechanics following thoracentesis^{3 4} do not explain the degree of improvement in breathlessness reported by many patients. Most prior studies have focused on specific aetiological factors in isolation,^{3 5–8} and their relevance to the multifactorial nature of effusion-related breathlessness is debated.

The ability to identify patients who are likely to benefit from pleural fluid drainage would represent a significant advance in clinical care. It may enable patients who are unlikely to respond to avoid hospital presentations, pleural interventions, and their associated morbidity/mortality risks and healthcare costs. The PLeural Effusion And Symptom Evaluation (PLEASE) study will evaluate the factors contributing to effusion-related breathlessness in a comprehensive manner.

METHODS AND ANALYSIS

The PLEASE study is a prospective study of 150 consecutive patients with symptomatic pleural effusions treated with pleural effusion drainage. The study aims to:

1. identify key factors that govern the symptom of breathlessness in a patient with pleural effusion;
2. develop predictors of improvement in breathlessness following pleural drainage.

The primary study hypothesis is that improvement in breathlessness and functional exercise capacity from pleural fluid drainage can be assessed using a combination of factors related to the patient and the effusion (and its impact on lung and diaphragm mechanics).

Trial details are as per the PLEASE study trial protocol V.3 (date 25 July 2015). A series of pre-drainage and postdrainage measures will be performed on the participants to evaluate:

1. the severity of their breathlessness at rest and during exercise,
2. their respiratory functions,
3. their diaphragmatic characteristics on pleural ultrasonography.

The PLEASE study will assess changes in diaphragm function following fluid drainage using advanced imaging (eg, thoracic ultrasound). The potential importance of mechanical function of the diaphragm and rib cage in the physiological impairment of individuals with benign asbestos-related pleural disease has been

highlighted previously.⁹ Specifically, drainage-related changes in diaphragm shape and mechanics may play a key role in the symptoms of breathlessness in patients with pleural effusion.^{8 10}

Participant screening and selection

The study aims to recruit 150 participants over a period of 24 months at Sir Charles Gairdner Hospital, Perth, Western Australia. Following on a recent internal audit, this enrolment target can provide a representative cross-section of pleural effusions of varying sizes, chronicity and aetiology (eg, malignant, heart failure, hepatic hydrothorax).

Patients presenting with a symptomatic pleural effusion requiring therapeutic pleural drainage will be identified as potential study participants by the treating clinicians and screened according to specific criteria (see below). Consecutive eligible patients will be offered participation in the study and a screening log will be maintained. The study will be performed according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and national and local regulatory and legal requirements. The study doctor will obtain informed consent from the patient prior to their participation in the study.

Inclusion criteria: Suitable participants are those with symptomatic pleural effusion who require therapeutic pleural drainage (by needle aspiration, indwelling pleural catheter (IPC), intercostal catheter (ICC) including those undergoing pleuroscopy) as part of standard clinical management of the effusion and are able to provide written informed consent.

Exclusion criteria include patients who:

1. are <18 years;
2. require urgent pleural drainage performed before pre-drainage assessment tests can be completed;
3. are unable to perform lung function and/or exercise tests, or are unable to comply with the protocol;
4. are pregnant or lactating.

Study measurements

1. *Baseline assessment:* All participants will be interviewed and examined prior to pleural drainage (see [figure 1](#)).
 - i. *Patient data:* Demographics, comorbidities (eg, chronic obstructive pulmonary disease (COPD), heart disease), underlying cancer (type, stage, treatment, etc) and relevant clinical data including cardiopulmonary status (respiratory rate, heart rate, blood pressure and oxygen saturation by pulse oximetry) will be recorded.
 - ii. *Pleural effusion characteristics:* This will include (if known) the aetiology of the effusion and the details of any prior pleural procedures.
 - iii. *Measurement of breathlessness:* The severity of breathlessness will be assessed using three instruments: (a) VAS,^{11 12} (b) Dyspnea-12 (D-12) Questionnaire^{13–15} and (c) Modified Borg 0–10

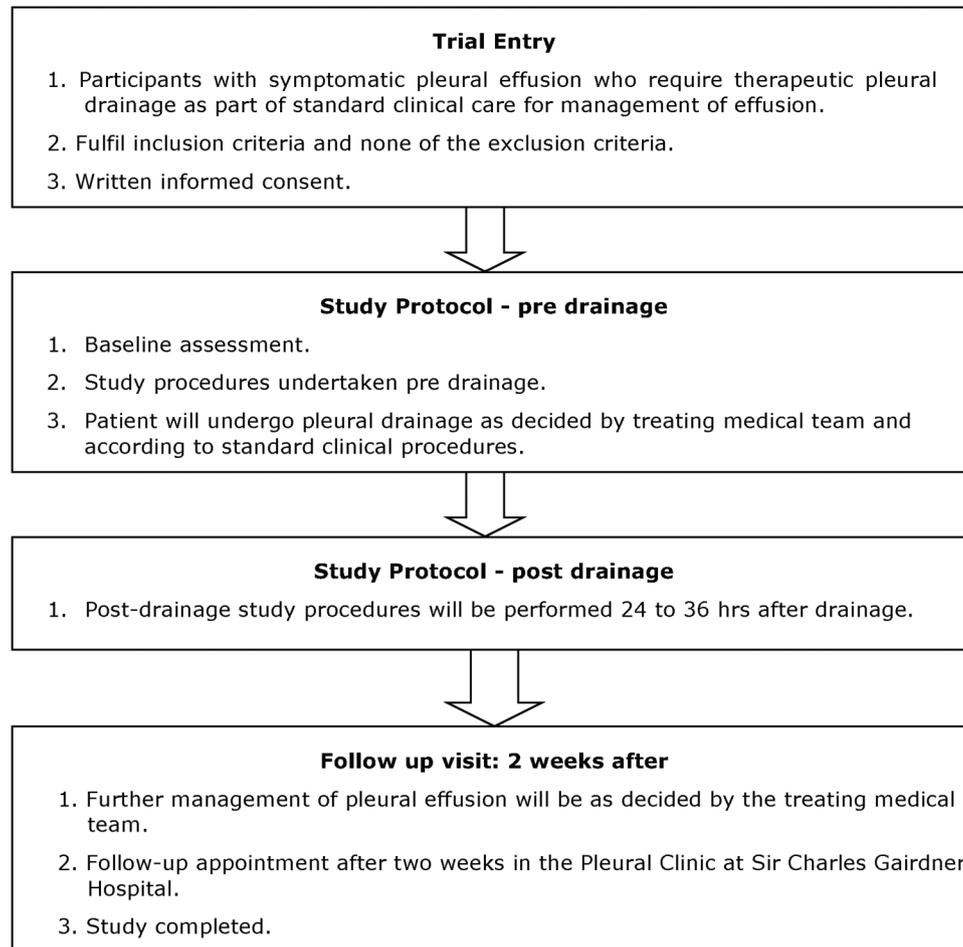


Figure 1 Trial entry, trial treatment and postprocedure care.

scale.¹⁶ The VAS score is a validated measure of breathlessness in MPE² and has been used in clinical trials involving MPE cohorts.^{11 17} The participant places a mark on the 100 mm vertical scale to indicate the severity of their breathlessness. A score of 0 mm indicates the most severe breathlessness. The D-12 Questionnaire was developed in people with chronic lung disease and chronic heart failure¹⁵ and has demonstrated validity and reliability in COPD, asthma, interstitial lung disease and pulmonary arterial hypertension.^{13–15 18} It consists of 12 questions with physical (7 questions) and affective (5 questions) components. The D-12 Questionnaire assesses current severity of dyspnoea and does not depend on activity limitation. Each question is scored on a 4-point scale (0–3 points), with a total maximum score of 36 points indicating the most severe level of dyspnoea. The modified Borg 0–10 scale will be used to assess breathlessness at rest prior to the 6 min walk test and the peak level of breathlessness evoked during the test.¹⁶

- iv. *Measurement of functional exercise capacity:* Functional exercise capacity will be assessed using

the 6 min walk test. The test will be supervised by trained operators and carried out in accordance with international guidelines.¹⁹ The distance walked (ie, 6 min walk test, 6 min walk distance (6MWD)) will be expressed as an absolute distance (m) and as a percentage of the patient's predicted 6MWD derived from a reference equation developed locally in healthy individuals.²⁰

- v. *Measurements of lung physiology:* Spirometry and, wherever possible, detailed lung functions, including lung volumes and gas transfer measurements, will be performed as per the American Thoracic Society recommendations,²¹ provided that there are no contraindications.
- vi. *Measurements of diaphragmatic morphology and function:* Pleural ultrasound will be employed to assess diaphragm (1) shape—normal (domed), flattened or inverted and (2) movement²²—normal, reduced or paradoxical.
- vii. *Radiological assessment of size of effusion:* Effusions will be graded based on their radiographic appearance into five categories (where 0=no effusion and 5 more than >75% of hemithorax) as published by Light *et al.*²³ The presence of other radiographic abnormalities (eg, presence of



- mediastinal shift, trapped lung and fluid loculations) will also be recorded.
2. *Pleural drainage*: The fluid will be removed at a controlled rate to ensure patient safety as per international clinical guidelines.²⁴ Data collected at the time of pleural drainage will include:
 - i. Total volume and duration of pleural fluid drainage.
 - ii. *Measurements of pleural pressure*. In a subgroup of patients, end-inspiratory and end-expiratory pleural pressure measurements will be recorded using a digital manometer (Compass; Mirador Biomedical, Seattle, Washington, USA) at opening pressure, after every 100 mL of drainage (up to 1000 mL), and at closing pressure.
 - iii. *Pleural fluid macroscopic appearance and biochemical analysis* (eg, pleural fluid pH and levels of protein, lactate dehydrogenase and glucose).²⁵
 3. *Repeat testing*: The pre-drainage tests will be repeated between 24 and 36 hours after completion of drainage.

Control/comparator

All participants will serve as their own control. The post-drainage parameters will be compared with the pre-drainage parameters in the same patient.

Standard care

All participants will receive standard care for their respective conditions as determined by their attending physicians. Participation in the PLEASE study will not interfere with the administration of other treatments or interventions necessary to manage the underlying condition. Pre-procedure workup, for example, blood tests and chest imaging, will be decided by the managing clinician (s), as is the choice of pleural drainage procedure. It is expected that most patients will have fluid evacuated by needle aspiration, chest tube or IPC insertion, performed in accordance with standard practice and international guidelines. Patients with pleural effusions undergoing pleuroscopy and patients with an IPC already placed for regular pleural fluid drainage may also participate.

Drainage procedures will be performed by the attending clinicians or their team members. The method of drainage, volume (and speed) of fluid removal, monitoring procedures during fluid evacuation, decisions to terminate fluid evacuation during procedure (if needed) and postprocedural care (including analgesia) are determined by the patients' clinical team. Participants will be monitored by standard continuous observations during and after the pleural drainage procedure.

The participants will conclude their involvement in the study with a clinic visit 2 weeks after the pleural drainage procedure.

Statistical analysis plan

Descriptive statistics of baseline characteristics of patients and measured responses will be provided. The magnitude and direction of the change between the pre-drainage and post-drainage measurements (eg, responses in

breathlessness and functional capacity (6MWD)) will be estimated using mean differences with 95% CIs provided. Formal statistical modelling will take two approaches in order to (1) predict response in breathlessness and (2) model the mechanistic relationships between responses in breathlessness and the changes in pre-drainage and post-drainage measurements.

Multiple linear regression will be used to model and predict the change in breathlessness after fluid drainage. Logistic regression, with receiver operating characteristic (ROC) curve analyses, will be used to model the dichotomous response of whether a clinically important change in breathlessness has been achieved. These analyses will investigate the impact of baseline measurements of variables included in the clinical data, measurements of lung physiology and diaphragmatic morphology (and function) and baseline demographics. A prognostic index will be generated to identify patients and patient characteristics that would predict a positive outcome from pleural effusion drainage. To model the mechanistic relationships between the change in measurements from pre-procedure to post-procedure, correlations and multiple linear regression analyses will be used to describe associations between the breathlessness variables and the changes in the aforementioned variables, as well as pleural effusion characteristics, diaphragmatic morphology and the total volume and duration of pleural drainage.

While it is expected the missing data will be minimal, standard imputation techniques will be used if necessary, with regression analyses adjusted accordingly.

The sample size for this study was based on standard regression modelling strategies²⁶ and is deemed sufficient given the number of predictors to be considered in the modelling.

All data will be analysed using the R environment for statistical computing (R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2015. <http://www.R-project.org/>).

ETHICS AND DISSEMINATION

Data collection and management

Data will be entered into a secure study database and a system of data validation checks will be implemented and applied to the database. The accuracy of the data will be verified by comparing study data to source documents. All procedures for the handling and analysis of data will be conducted using GCP meeting ICH guidelines and the Australian Human Research Ethics Committee for the handling and analysis of data for clinical trials.

Safety reporting

All adverse events (AEs) occurring during the study period will be documented and reported. A Data and Safety Monitoring Committee, comprising three independent members, will oversee the monitoring of all

AEs. For each AE, the investigator will provide the onset, end, intensity, treatment required, outcome, seriousness and action taken. Serious AEs (SAEs) will be reported immediately to the local ethics committee and the Data and Safety Monitoring Committee using the Serious Adverse Event Report Form including a documented causal relationship assessment. An SAE is defined as any AE that results in death; is life-threatening; results in persistent or significant disability/incapacity; prolongs hospitalisation by ≥ 24 hours; is deemed serious for any other reason such that it is thought to jeopardise the patient and may require medical or surgical intervention to prevent one of the other outcomes listed in the above SAE definitions.

Trial monitoring and oversight

A Trial Steering Committee, comprising the investigators and trial coordinators, will be responsible for supervision of the trial in its entirety. It will be responsible for ensuring completion of the trial to clinical and ethical standards. The Data and Safety Monitoring Committee will oversee the monitoring of AEs and the ethical conduct of the study.

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Competing interests None declared.

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REFERENCES

- Lee YC, Light RW. Pleural effusion: overview. In: Laurent G, Shapiro S, eds. *Encyclopedia of respiratory diseases*. 4th edn. Oxford, UK: Elsevier, 2006:353–8.
- Mishra EK, Corcoran JP, Hallifax RJ, *et al*. Defining the minimal important difference for the visual analogue scale assessing dyspnea in patients with malignant pleural effusions. *PLoS ONE* 2015;10:e0123798.
- Estenne M, Yernault JC, De Troyer A. Mechanism of relief of dyspnea after thoracentesis in patients with large pleural effusions. *Am J Med* 1983;74:813–19.
- Light RW, Stansbury DW, Brown SE. The relationship between pleural pressures and changes in pulmonary function after therapeutic thoracentesis. *Am Rev Respir Dis* 1986;133:658–61.
- Cartaxo AM, Vargas FS, Salge JM, *et al*. Improvements in the 6-min walk test and spirometry following thoracentesis for symptomatic pleural effusions. *Chest* 2011;139:1424–9.
- Krell WS, Rodarte JR. Effects of acute pleural effusion on respiratory system mechanics in dogs. *J Appl Physiol* 1985;59:1458–63.
- Mulvey RB. The effect of pleural fluid on the diaphragm. *Radiology* 1965;84:1080–6.
- Wang JS, Tseng CH. Changes in pulmonary mechanics and gas exchange after thoracentesis on patients with inversion of a hemidiaphragm secondary to large pleural effusion. *Chest* 1995;107:1610–14.
- Singh B, Eastwood PR, Finucane KE, *et al*. Effect of asbestos-related pleural fibrosis on excursion of the lower chest wall and diaphragm. *Am J Respir Crit Care Med* 1999;160:1507–15.
- Light RW, Lee YC, eds. *Textbook of pleural diseases*. 2nd edn. London, UK: Hodder Arnold, 2008:54–5.
- Davies HE, Mishra EK, Kahan BC, *et al*. Effect of an indwelling pleural catheter vs chest tube and talc pleurodesis for relieving dyspnea in patients with malignant pleural effusion: the TIME2 randomized controlled trial. *JAMA* 2012;307:2383–9.
- Ries AL. Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale. *COPD* 2005;2:105–10.
- Yorke J, Russell AM, Swigris J, *et al*. Assessment of dyspnea in asthma: validation of The Dyspnea-12. *J Asthma* 2011;48:602–8.
- Yorke J, Swigris J, Russell AM, *et al*. Dyspnea-12 is a valid and reliable measure of breathlessness in patients with interstitial lung disease. *Chest* 2011;139:159–64.
- Yorke J, Moosavi SH, Shuldham C, *et al*. Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12. *Thorax* 2010;65:21–6.
- Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377–81.
- Fysh ET, Thomas R, Read CA, *et al*. Protocol of the Australasian Malignant Pleural Effusion (AMPLE) trial: a multicentre randomised



- study comparing indwelling pleural catheter versus talc pleurodesis. *BMJ Open* 2014;4:e006757.
18. Yorke J, Armstrong I. The assessment of breathlessness in pulmonary arterial hypertension: reliability and validity of the Dyspnoea-12. *Eur J Cardiovasc Nurs* 2014;13:506–14.
 19. Holland AE, Spruit MA, Troosters T, *et al.* An official European Respiratory Society/American Thoracic Society Technical Standard: field walking tests in chronic respiratory disease. *Eur Respir J* 2014;44:1428–46.
 20. Jenkins S, Cecins N, Camarri B, *et al.* Regression equations to predict 6-minute walk distance in middle-aged and elderly adults. *Physiother Theory Pract* 2009;25:516–22.
 21. Miller MR, Crapo R, Hankinson J, *et al.* General considerations for lung function testing. *Eur Respir J* 2005;26:153–61.
 22. Ueki J, De Bruin PF, Pride NB. In vivo assessment of diaphragm contraction by ultrasound in normal subjects. *Thorax* 1995;50:1157–61.
 23. Light RW, Rogers JT, Cheng D, *et al.* Large pleural effusions occurring after coronary artery bypass grafting. Cardiovascular Surgery Associates, PC. *Ann Intern Med* 1999;130:891–6.
 24. Roberts ME, Neville E, Berrisford RG, *et al.* Management of a malignant pleural effusion: British Thoracic Society Pleural Disease Guideline 2010. *Thorax* 2010;65(Suppl 2):ii32–40.
 25. Light RW, Macgregor MI, Luchsinger PC, *et al.* Pleural effusions: the diagnostic separation of transudates and exudates. *Ann Intern Med* 1972;77:507–13.
 26. Cohen J, Cohen P. *Applied multiple regression/correlation analysis for the behavioural sciences*. Hillsdale, New Jersey: Taylor & Francis, 1983.

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