BTS guidelines for the insertion of a chest drain

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1 BACKGROUND
In current hospital practice chest drains are used in many different clinical settings and doctors in most specialities need to be capable of their safe insertion. The emergency insertion of a large bore chest drain for tension pneumothorax following trauma has been well described by the Advanced Trauma and Life Support (ATLS) recommendations in their instructor's manual and there have been many general descriptions of the step by step method of chest tube insertion.

It has been shown that physicians trained in the method can safely perform tube thoracostomy with 3% early complications and 8% late. In these guidelines we discuss the safe insertion of chest tubes in the controlled circumstances usually encountered by physicians. A summary of the process of chest drain insertion is shown in fig 1.

2 TRAINING
• All personnel involved with insertion of chest drains should be adequately trained and supervised. [C]

Before insertion of a chest drain, all operators should have been adequately trained and have completed this training appropriately. In all other circumstances, insertion should be supervised by an appropriate trainer. This is part of the SHO core curriculum training process issued by the Royal College of Physicians and trainees should be expected to describe the indications and complications. Trainees should ensure each procedure is documented in their log book and signed by the trainer. With adequate instruction, the risk of complications and patient pain and anxiety can be reduced.

These guidelines will aid the training of junior doctors in the procedure and should be readily available for consultation by all doctors likely to be required to carry out a chest tube insertion.

3 INDICATIONS
Chest tubes may be useful in many settings, some of which are listed in box 1.

4 PRE-DRAINAGE RISK ASSESSMENT
• Risk of haemorrhage: where possible, any coagulopathy or platelet defect should be corrected prior to chest drain insertion but routine measurement of the platelet count and prothrombin time are only recommended in patients with known risk factors. [C]

• The differential diagnosis between a pneumothorax and bullous disease requires careful radiological assessment. Similarly it is important to differentiate between the presence of collapse and a pleural effusion when the chest radiograph shows a unilateral “whiteout”.

• Lung densely adherent to the chest wall throughout the hemithorax is an absolute contraindication to chest drain insertion. [C]

• The drainage of a post pneumonectomy space should only be carried out by or after consultation with a cardiothoracic surgeon. [C]

There is no published evidence that abnormal blood clotting or platelet counts affect bleeding complications of chest drain insertion. However, where possible it is obvious good practice to correct any coagulopathy or platelet defect prior to drain insertion. Routine pre-procedure checks of platelet count and/or prothrombin time are only required in those patients with known risk factors. For elective chest drain insertion, warfarin should be stopped and time allowed for its effects to resolve.

5 EQUIPMENT
All the equipment required to insert a chest tube should be available before commencing the procedure and are listed below and illustrated in fig 2.

• Sterile gloves and gown
• Skin antiseptic solution, e.g. iodine or chlorhexidine in alcohol
• Sterile drapes
• Gauze swabs
• A selection of syringes and needles (21–25 gauge)
• Local anaesthetic, e.g. lignocaine (lidocaine) 1% or 2%
• Scalpel and blade
• Suture (e.g. “1” silk)
• Instrument for blunt dissection (e.g. curved clamp)
6. CONSENT AND PREMEDICATION

- Prior to commencing chest tube insertion the procedure should be explained fully to the patient and consent recorded in accordance with national guidelines. [C]

- Unless there are contraindications to its use, premedication (benzodiazepine or opioid) should be given to reduce patient distress. [B]

Consent should be taken and recorded in keeping with national guidelines. The General Medical Council (GMC) guidelines for consent state that it is the responsibility of the doctor carrying out a procedure, or an appropriately trained individual with sufficient knowledge of a procedure, to explain its nature and the risks associated with it. It is within the rights of a competent individual patient to refuse such treatment. In the case of an emergency, when the patient is unconscious and the treatment is lifesaving, treatment may be carried out but must be explained as soon as the patient is sufficiently recovered to understand. If possible, an information leaflet should be given before the procedure.

Chest drain insertion has been reported to be a painful procedure with 50% of patients experiencing pain levels of 9–10 on a scale of 10 in one study,1 and therefore premedication should be given. Despite the apparent common sense of this approach, there is little established evidence of the effect from these medications. Premedication could be an intravenous anxiolytic—for example, midazolam 1–5 mg titrated to achieve adequate sedation—given immediately before the procedure or an intramuscular opioid given 1 hour before, although neither drug has been shown to be clearly superior. Both these classes of drugs may cause respiratory depression and patients with underlying lung disease such as COPD should be observed as reversal agents—for example, naloxone or flumazenil—are occasionally necessary.

While the use of atropine as part of premedication for fiberoptic bronchoscopy has been assessed, no controlled trial of its use in chest tube insertion has been identified, although it is advocated in some centres. Case reports of vasovagal reactions12 and a death due to vagal stimulation following tube insertion13 may support its use as premedication.
7 PATIENT POSITION
The preferred position for drain insertion is on the bed, slightly rotated, with the arm on the side of the lesion behind the patient’s head to expose the axillary area. An alternative is for the patient to sit upright leaning over an adjacent table with a pillow or in the lateral decubitus position. Insertion should be in the “safe triangle” illustrated in fig 3. This is the triangle bordered by the anterior border of the latissimus dorsi, the lateral border of the pectoralis major muscle, a line superior to the horizontal level of the nipple, and an apex below the axilla.

8 CONFIRMING SITE OF DRAIN INSERTION
• A chest tube should not be inserted without further image guidance if free air or fluid cannot be aspirated with a needle at the time of anaesthesia. [C]
• Imaging should be used to select the appropriate site for chest tube placement. [B]
• A chest radiograph must be available at the time of drain insertion except in the case of tension pneumothorax. [C]

Immediately before the procedure the identity of the patient should be checked and the site and side for insertion of the chest tube confirmed by reviewing the clinical signs and the chest radiograph. Fluoroscopy, ultrasonography, and CT scanning can all be used as adjunctive guides to the site of tube placement. Before insertion, air or fluid should be aspirated; if none is forthcoming, more complex imaging than a chest radiograph is required.

The use of ultrasonography guided insertion is particularly useful for empyema and effusions as the diaphragm can be localised and the presence of loculations and pleural thickening defined. Using real time scanning at the time of the procedure can help to ensure that the placement is safe despite the movement of the diaphragm during respiration. The complication rate following image guided thoracocentesis is low with pneumothoraces occurring in approximately 3% of cases. Success rates of image guided chest tube insertion are reported to be 71–86%. If an imaging technique is used to indicate the site for drain insertion but the procedure is not carried out at the time of imaging, the position of the patient at the time must be clearly documented to aid accurate insertion when the patient returns to the ward. It is recommended that ultrasound is used if the effusion is very small or initial blind aspiration fails.

9 DRAIN INSERTION SITE
The most common position for chest tube insertion is in the mid axillary line, through the “safe triangle” illustrated in fig 3 and described above. This position minimises risk to underlying structures such as the internal mammary artery and avoids damage to muscle and breast tissue resulting in unsightly scarring. A more posterior position may be chosen if suggested by the presence of a locule. While this is safe, it is not the preferred site as it is more uncomfortable for the patient to lie on after insertion and there is a risk of the drain kinking.

For apical pneumothoraces the second intercostal space in the mid clavicular line is sometimes chosen but is not recommended routinely as it may be uncomfortable for the patient and may leave an unsightly scar. Loculated apical pneumothoraces are not uncommonly seen following thoracotomy and may be drained using a posteriorly sited (suprascapular) apical tube. This technique should be performed by an operator experienced in this technique—for example, a thoracic surgeon. If the drain is to be inserted into a loculated pleural collection, the position of insertion will be dictated by the site of the locule as determined by imaging.

10 DRAIN SIZE
• Small bore drains are recommended as they are more comfortable than larger bore tubes [B] but there is no evidence that either is therapeutically superior.
• Large bore drains are recommended for drainage of acute haemothorax to monitor further blood loss. [C]

The use of large bore drains has previously been recommended as it was felt that there was an increase in the frequency of drain blockage, particularly by thick malignant or infected fluid. The majority of physicians now use smaller catheters (10–14 French (F)) and studies have shown that these are often as effective as larger bore tubes and are more comfortable and better tolerated by the patient. There remains intense debate about the optimum size of drainage catheter and no large randomised trials directly comparing small and large bore tubes have been performed.

In pneumothoraces 9 F catheters have been used with success rates of up to 87%, although in a few patients the air leak seems to exceed the capacity of this small catheter. In the event of failure to drain a pneumothorax due to excessive air leakage, it is recommended that a larger bore tube be inserted. There is no evidence to suggest that surgical emphysema rates vary between the size of drains. Ultrasonographically guided insertion of pigtail catheters for treatment of malignant pleural effusions has been particularly well studied with good effect. The use of small bore pigtail catheters has allowed outpatient treatment of malignant pleural effusions which have not responded to chemotherapy. Empyemas are often successfully drained with ultrasonically placed small bore tubes with the aid of thrombolytic agents.

In the case of acute haemothorax, however, large bore tubes (28–30 F minimum) continue to be recommended for their dual role of drainage of the thoracic cavity and assessment of continuing blood loss.

11 ASEPTIC TECHNIQUE
• Aseptic technique should be employed during catheter insertion. [C]
• Prophylactic antibiotics should be given in trauma cases. [A]

As a chest drain may potentially be in place for a number of days, aseptic technique is essential to avoid wound site infection or secondary empyema. Although this is uncommon, estimations of the empyema rate following drain insertions for trauma are approximately 2.4%. While the full sterile technique afforded by a surgical theatre is usually unnecessary, sterile gloves, gown, equipment and the use of sterile towels after effective skin cleansing using iodine or chlorhexidine are recommended. A large area of skin cleansing should
be undertaken. In a study of chest tubes inserted in trauma suits using full aseptic technique, there were no infective complications in 80 cases. Studies of the use of antibiotic prophylaxis for chest tube insertion have been performed but have failed to reach significance because of small numbers of infectious complications. However, a meta-analysis of these studies has been performed which suggested that, in the presence of chest trauma (penetrating or blunt), the use of prophylactic antibiotics reduces the absolute risk of empyema by 5.5–7.1% and of all infectious complications by 12.1–13.4%. The use of prophylactic antibiotics in trauma cases is therefore recommended. The antibiotics used in these studies were cephalosporins or clindamycin.

In one study only one infectious complication (in the chest cavity along the wire. These have been successfully used for pneumothorax, effusions, or loculated empyemas.

13.2 Medium bore tube (16–24 F)
Medium sized chest drains may be inserted by a Seldinger technique or by blunt dissection as outlined below. As the incision size should afford a snug fit around the chest tube, it is not possible to insert a finger to explore the pleura when inserting this size of tube. Exploration with a finger is felt to be unnecessary for the elective medical insertion of these medium sized chest tubes.

13.3 Large bore tube (>24 F)
- Blunt dissection into the pleural space must be performed before insertion of a large bore chest drain. [C]

13.3.1 Incision
- The incision for insertion of the chest drain should be similar to the diameter of the tube being inserted. [C]

Once the anaesthetic has taken effect an incision is made. This should be slightly bigger than the operator’s finger and tube. The incision should be made just above and parallel to a rib.

13.3.2 Blunt dissection
Many cases of damage to essential intrathoracic structures have been described following the use of trocars to insert large bore chest tubes. Blunt dissection of the subcutaneous tissue and muscle into the pleural cavity has therefore become universal and is essential. In one retrospective study only four technical complications were seen in 447 cases using blunt dissection. Using a Spencer-Wells clamp or similar, a path is made through the chest wall by opening the clamp to separate the muscle fibres. For a large chest drain, similar in size to the finger, this track should be explored with a finger through into the thoracic cavity to ensure there are no underlying organs that might be damaged at tube insertion.

The creation of a patent track into the pleural cavity ensures that excessive force is not needed during drain insertion.

13.3.3 Position of tube tip
- The position of the tip of the chest tube should ideally be aimed apically for a pneumothorax or basally for fluid. However, any tube position can be effective at draining air or fluid and an effectively functioning drain should not be repositioned solely because of its radiographic position. [C]

In the case of a large bore tube, after gentle insertion through the chest wall the trocar positioned a few centimetres from the tube tip can afford support of the tube and so help its positioning without incurring organ damage. A smaller clamp can also be used to direct the tube to its desired position. If possible, the tip of the tube should be aimed apically to drain air and basally for fluid. However, successful drainage can still be achieved when the drain is not placed in an ideal position, so effectively functioning tubes should not be repositioned simply because of a suboptimal radiographic appearance.

13.3.4 Securing the drain
- Large and medium bore chest drain incisions should be closed by a suture appropriate for a linear incision. [C]

“Purse string” sutures must not be used. [C]

Two sutures are usually inserted—the first to assist later closure of the wound after drain removal and the second, a stay suture, to secure the drain. The wound closure suture should be inserted before blunt dissection. A strong suture such as “1” silk is appropriate. A “mattress” suture or sutures across the incision are usually employed and, whatever closure is used, the stitch must be of a type that is appropriate for a linear incision (fig 4). Complicated “purse string” sutures must not be used as they convert to...
a linear wound into a circular one that is painful for the patient and may leave an unsightly scar. A suture is not usually required for small gauge chest tubes.

The drain should be secured after insertion to prevent it falling out. Various techniques have been described, but a simple technique of anchoring the tube has not been the subject of a controlled trial. The chosen suture should be stout and non-absorbable to prevent breaking (e.g. “1” silk), and it should include adequate skin and subcutaneous tissue to ensure it is secure (fig 4).

Large amounts of tape and padding to dress the site are unnecessary and concerns have been expressed that they may restrict chest wall movement or increase moisture collection. A transparent dressing allows the wound site to be inspected by nursing staff for leakage or infection. An omental tag of tape has been described which allows the tube to lie a little away from the chest wall to prevent tube kinking and tension at the insertion site (fig 5).

14 MANAGEMENT OF DRAINAGE SYSTEM
14.1 Clamping drain

- A bubbling chest tube should never be clamped. [C]
- Drainage of a large pleural effusion should be controlled to prevent the potential complication of re-expansion pulmonary oedema. [C]
- In cases of pneumothorax, clamping of the chest tube should usually be avoided. [B]
- If a chest tube for pneumothorax is clamped, this should be under the supervision of a respiratory physician or thoracic surgeon, the patient should be managed in a specialist ward with experienced nursing staff, and the patient should not leave the ward environment. [C]

- If a patient with a clamped drain becomes breathless or develops subcutaneous emphysema, the drain must be immediately unclamped and medical advice sought. [C]

There is no evidence to suggest that clamping a chest drain prior to its removal increases success or prevents recurrence of a pneumothorax and it may be hazardous. This is therefore generally discouraged. Clamping a chest drain in the presence of a continuing air leak may lead to the potentially fatal complication of tension pneumothorax. A bubbling drain therefore should never be clamped. However, many experienced specialist physicians support the use of the clamping of non-bubbling chest drains inserted for pneumothorax to detect small air leaks not immediately obvious at the bedside. By clamping the chest drain for several hours, followed by a chest radiograph, a minor air leak may be detected, avoiding the need for later chest drain reinsertion. In the ACCP Delphi consensus statement about half the consensus group supported clamping and half did not, and this seems similar to the UK spread of opinion. Drain clamping is therefore not generally recommended for safety reasons, but is acceptable under the supervision of nursing staff who are trained in the management of chest drains and who have instructions to unclamp the chest drain in the event of any clinical deterioration. Patients with a clamped chest drain inserted for pneumothorax should not leave the specialist ward area.

There have been reports of re-expansion pulmonary oedema following rapid evacuation of large pleural effusions as well as in association with spontaneous pneumothorax. This has been reported to be fatal in some cases (up to 20% of subjects in one series of 53 cases). In the case of spontaneous pneumothorax this is a rare complication with no cases of re-expansion pulmonary oedema reported in two large studies of 400 and 375 patients, respectively. It is usually associated with delayed diagnosis and therefore awareness of its potential occurrence is sufficient.

Milder symptoms suggestive of re-expansion oedema are common after large volume thoracentesis in pleural effusion, with patients experiencing discomfort and cough. It has been suggested that the tube be clamped for 1 hour after draining 1 litre. While there is no evidence for actual amounts, good practice suggests that no more than about 1.5 litres should be drained at one time, or drainage should be slowed to about 500 ml per hour.

14.2 Closed system drainage

- All chest tubes should be connected to a single flow drainage system e.g. under water seal bottle or flutter valve. [C]
- Use of a flutter valve system allows earlier mobilisation and the potential for earlier discharge of patients with chest drains.

The chest tube is then attached to a drainage system which only allows one direction of flow. This is usually the closed underwater seal bottle in which a tube is placed under water at a depth of approximately 3 cm with a side vent which allows escape of air, or it may be connected to a suction pump. This enables the operator to see air bubble out as the lung re-expands in the case of pneumothorax or fluid evacuation rate in empyemas, pleural effusions, or haemothorax. The continuation of bubbling suggests a continued visceral pleural air leak, although it may also occur in patients on suction when the drain is partly out of the thorax and one of the tube holes is open to the air. The respiratory swing in the fluid in the chest tube is useful for assessing tube patency and confirms the position of the tube in the pleural cavity. The disadvantages of the underwater seal system include obligatory, inpatient management, difficulty of patient mobilisation, and the risk of knocking over the bottle.
The use of integral Heimlich flutter valves has been advocated in patients with pneumothoraces, especially as they permit ambulatory or even outpatient management which has been associated with a 85–95% success rate. In 176 cases of pneumothorax treated with small chest tubes and a Heimlich flutter valve there were only eight failures (hospital admissions for problems with tube function or placement). The mean length of inpatient stay has been quoted at 5 hours with a thoracic vent and 144 hours with an underwater seal, with a cost saving US$5660. Case reports of incorrect use (wrong direction of flow) of such valves have been described, however, with tension pneumothorax as a result. Flutter valves cannot be used with fluid drainage as they tend to become blocked. However, in the UK a similar short hospital stay is achieved by initial aspiration of pneumothoraces (see guidelines on pneumothorax, page ii39).

The use of a drainage bag with an incorporated flutter valve and vented outlet has been successfully used postoperatively. A randomised trial of 119 cases following elective thoracotomy compared the use of an underwater seal with the flutter bag and found no difference in drainage volumes, requirement for suction, or complications with the added advantage of earlier mobilisation with drainage bags.

In cases of malignant pleural effusion drainage a closed system using a drainage bag or aspiration via a three way tap has been described to aid palliation and outpatient management.

One report of a modified urinary collecting bag for prolonged underwater chest drainage has been described for use with empyemas, bronchopulmonary fistula, and pneumothorax associated with emphysema with no complications in the 12 patients studied.

14.3 Suction

- When chest drain suction is required, a high volume/low pressure system should be used. [C]
- When suction is required, the patient must be nursed by appropriately trained staff. [C]

The use of high volume/low pressure suction pumps has been advocated in cases of non-resolving pneumothorax or following chemical pleurodesis, but there is no evidence to support its routine use in the initial treatment of spontaneous pneumothorax. If suction is required, this may be performed via the underwater seal at a level of 10–20 cm H₂O. A high volume pump (e.g. Vernon-Thompson) is required to cope with a large leak. A low volume pump (e.g. Roberts pump) is inappropriate as it is unable to cope with the rapid flow, thereby effecting a situation similar to clamping and risking formation of a tension pneumothorax. A wall suction adaptor may also be effective, although chest drains must not be connected directly to the high negative pressure available from wall suction.

In the management of pleural infection, the use of suction is less clear. Most studies are observational and have used suction applied via the chest tube after flushing to prevent blockage and have reported success, but this has not been compared with cases without suction. This is discussed further in the guideline on pleural infection (page ii18).

There is no evidence that briefly disconnecting a drain from suction used for spontaneous pneumothorax or pleural effusion is disadvantageous. Therefore, as long as adequate instruction is given to patient, portering and nursing staff with regard to keeping the underwater seal bottle below the level of the chest, it is acceptable to stop suction for short periods such as for radiography.

14.4 Ward instructions

- Patients with chest tubes should be managed on specialist wards by staff who are trained in chest drain management. [C]

Audit points

- The presence and use of an appropriate nursing chest drain observation chart should be noted.
- The frequency of chest drain complications should be recorded.
- The use of premedication and analgesics and patient pain scores relating to chest drain insertion should be recorded.
- The duration of chest tube drainage should be recorded.
- A chest radiograph should be performed after insertion of a chest drain. [C]

Patients should be managed on a ward familiar with chest tubes. Instruction to and appropriate training of the nursing staff is imperative. If an underwater seal is used, instructions must be given to keep the bottle below the insertion site at all times, to keep it upright, and to ensure that adequate water is in the system to cover the end of the tube. Daily reassessment of the amount of drainage/bubbling and the presence of respiratory swing should be documented, preferably on a dedicated chest drain chart. Instruction with regard to chest drain clamping must be given and recorded.

Patients should be encouraged to take responsibility for their chest tube and drainage system. They should be taught to keep the underwater seal bottle below the level of their chest and to report any problems such as pulling on the drain insertion site. Educational material (e.g. leaflets) should be available on the ward for patients and nursing staff.

A chest radiograph should be performed to assess tube position, exclude complications such as pneumothorax or surgical emphysema, and assess the success of the procedure in the volume of fluid drainage or pneumothorax resolution. Concern has previously been expressed in cases where the tube enters the lung fissure. In a study of 66 patients with chest tubes inserted for acute chest trauma, 58% of which were located within a pulmonary fissure, no difference in outcome was seen between these cases and those in whom the tube was located outside the fissures.

14.5 Removal of the chest tube

- In cases of pneumothorax, the chest tube should not be clamped at the time of its removal. [B]

In cases of pneumothorax, there is no evidence that clamping a chest drain at the time of its removal is beneficial.

The chest tube should be removed either while the patient performs Valsalva’s manoeuvre or during expiration with a brisk firm movement while an assistant ties the previously placed closure suture. The timing of removal is dependent on the original reason for insertion and clinical progress (see guidelines for management of pneumothorax (page ii39), malignant pleural effusions (page ii29), and pleural infections (page ii18)).

In the case of pneumothorax, the drain should not usually be removed until bubbling has ceased and chest radiography demonstrates lung reinflation. Clamping of the drain before removal is generally unnecessary. In one study the removal of chest tubes after discontinuation suction was compared with the removal after a period of disconnection from suction to an underwater seal. No significant difference was seen between these two methods with only two of 80 cases (2.5%) requiring reininsertion of a chest tube.

15 PATIENTS REQUIRING ASSISTED VENTILATION

During the insertion of a chest tube in a patient on a high pressure ventilator (especially with positive end expiratory pressure (PEEP)), it is essential to disconnect from the ventilator at the time of insertion to avoid the potentially serious
complication of lung penetration, although as long as blunt dissection is carried out and no sharp instruments are used, this risk is reduced.

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Phosphodiesterase 4 inhibition decreases MUC5AC expression induced by epidermal growth factor in human airway epithelial cells

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Background: A common pathological feature of chronic inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) is mucus hypersecretion. MUC5AC is the predominant mucin gene expressed in healthy airways and is increased in asthmatic and COPD patients. Recent clinical trials indicate that phosphodiesterase type 4 (PDE4) inhibitors may have therapeutic value for COPD and asthma. However, their direct effects on mucin expression have been scarcely investigated.

Methods: MUC5AC mRNA and protein expression were examined in cultured human airway epithelial cells (A549) and in human isolated bronchial tissue stimulated with epidermal growth factor (EGF; 25 ng/ml). MUC5AC mRNA was measured by real time RT-PCR and MUC5AC protein by EUSA (cell lysates and tissue homogenates). Western blotting (tissue homogenates) and immunohistochemistry.

Results: EGF increased MUC5AC mRNA and protein expression in A549 cells. PDE4 inhibitors produced a concentration dependent inhibition of the EGF induced MUC5AC mRNA and protein expression with potency values (−log IC50): rolipram (−7.5) > cilomilast (−6.5) > cilomilast (−5.5). Roflimilast also inhibited the EGF induced expression of phosphorysine proteins, EGF receptor, and phospho-p38- and p44/p42-MAPK measured by Western blot analysis in A549 cells. In human isolated bronchus, EGF induced MUC5AC mRNA and protein expression was inhibited by rolipram (1 μM) as well as the MUC5AC positive staining shown by immunohistochemistry.

Conclusion: Selective PDE4 inhibition is effective in decreasing EGF induced MUC5AC expression in human airway epithelial cells. This effect may contribute to the clinical efficacy of this new drug category in mucus hypersecretory diseases.
in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% endotoxin-free fetal calf serum (FCS), 10 mM HEPES, L-glutamine (4 mM), and standard antimicrobials.

Human lung tissue was obtained from patients (five men, one woman) of mean age 59 years (range 48–69) who had undergone surgery for lung carcinoma as previously outlined.\(^{13}\) Experiments were approved by the local ethics committee and informed consent was obtained. At the time containing 10% endotoxin-free fetal calf serum (FCS), in Roswell Park Memorial Institute (RPMI) 1640 medium with EGF (5–50 ng/ml). The selected EGF concentration and selected in further experiments. Also, 25 ng/ml EGF was 24 hours for MUC5AC mRNA and at 24 hours for MUC5AC expression in response to EGF stimulation was determined at 0.5, 1, 3, 12, and 24 hours. In inhibition studies A549 cells and human bronchus were pretreated with drugs or their vehicles for 15 minutes before stimulation with EGF and remained until termination of experiments. When used, antagonists were added 15 minutes before the corresponding drug and remained for the rest of the experiment.

### Mucin MUC5AC expression

The mucin MUC5AC mRNA transcripts were measured by real time quantitative RT-PCR as previously described.\(^ {13}\) The method used for obtaining quantitative data of relative gene expression, the comparative Ct (\(\Delta\Delta Ct\)) method, was as described by the manufacturer (PE-ABI PRISM 7700 Sequence Detection System; Perkin-Elmer Applied Biosystems, Perkin-Elmer Corporation, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was chosen as the endogenous control gene. Total RNA was extracted using TriPure isolation reagent (Roche, IN, USA). The PCR primers and probes for human MUC5AC and human GAPDH were designed using the Primer Express (PE-ABI PRISM 7700) method, was as described by the manufacturer (PE-ABI PRISM 7700 Sequence Detection System; Perkin-Elmer Applied Biosystems, Perkin-Elmer Corporation, CA, USA).

### Experimental protocol

In preliminary experiments with A549 cells the MUC5AC expression in response to EGF stimulation was determined at 3, 12, 18 and 24 hours. Peak responses were observed at 18–24 hours for MUC5AC mRNA and at 24 hours for MUC5AC protein; an incubation time of 24 hours was therefore selected in further experiments. Also, 25 ng/ml EGF was selected as a near maximal response from pilot experiments with EGF (5–50 ng/ml). The selected EGF concentration and time of observation are within the values reported by others in cultured airway epithelial cells.\(^ {16} \) For human isolated bronchus, MUC5AC responses to EGF stimulation were studied at 0.5, 1, 3, 12 and 24 hours. In inhibition studies A549 cells and human bronchus were pretreated with drugs or their vehicles for 15 minutes before stimulation with EGF and remained until termination of experiments. When used, antagonists were added 15 minutes before the corresponding drug and remained for the rest of the experiment.

### Table 1

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</table>

bp, base pairs.

For MUC5AC, reverse transcription of RNA to generate cDNA was performed with Taqman RT reagents (ref. N808-0233; Applied Biosystems, NJ, USA) and the PCR was performed with Taqman Universal PCR Master Mix (ref. 4304437; Applied Biosystems). The specificity of PCR primers was tested under normal PCR conditions and the products of the reaction were electrophoresed into a 2.5% NuSieve\(^ {4304437}\) agarose gel (BMA, Rockland, ME, USA). One single band with the expected molecular size was observed for MUC5AC and GAPDH. For the validation of the \(\Delta\Delta Ct\) method, the Ct values for target (MUC5AC) and reference (GAPDH) genes were measured at different input amounts of total RNA (2.34–300 ng). \(\Delta\Delta Ct\) values (target vs reference) were then plotted against log total RNA and the absolute value of the slope was found to be 0.008 (i.e. \(<0.1\)), indicating similar efficiency of the two systems.

### Table 1

<table>
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<th>Gene</th>
<th>Primers and probes</th>
<th>Sequence</th>
<th>Product size (bp)</th>
<th>GenBank accession no</th>
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<td>U60711</td>
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<tr>
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<td>TaqMan probe</td>
<td>5’-CAAGCTTCCGCTTCACGCC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bp, base pairs.

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5AC and has no cross reactivity with other mucins). After 1 hour the plates were washed with PBS and then incubated with 100 µl horseradish peroxidase-goat anti-mouse IgG conjugated (1:10 000). The colour reaction was developed with TMB peroxidase solution (Sigma) and stopped with 1 M H2SO4. Absorbance was read at 450 nm.

In addition, Western blot analysis of MUC5AC was carried out in human bronchial homogenates as previously.

**Figure 1** Relative quantitation of MUC5AC mRNA and protein levels in A549 cells unstimulated (control) or stimulated with epidermal growth factor (EGF; 25 ng/ml, 24 hours incubation) in the absence or presence of selective inhibitors of EGF receptor tyrosine kinase activity (tyrphostin A46 and AG1478; upper panels) or a selective phosphodiesterase 4 inhibitor (roflumilast; lower panels). Incubation with DMSO (0.1% v/v) was without significant effect on MUC5AC expression in the absence and presence of EGF (upper panel). The EGF induced increase in MUC5AC expression was abolished by pre-incubation with EGF receptor tyrosine kinase inhibitors (A46 100 µM or AG1478 3 µM) or roflumilast (1 µM). MUC5AC mRNA was determined using real time RT-PCR by the ΔΔCt method; columns show the fold increase in expression of MUC5AC relative to GAPDH values as mean (SE) of the 2-ΔΔCt values of three independent experiments. MUC5AC protein was determined by enzyme linked immunosorbent assay (ELISA); columns show the fold increase from control levels as mean (SE) values of three independent experiments. *p<0.05 v control; †p<0.05 v EGF.

**Figure 2** Concentration-response curves for inhibition by the selective PDE4 inhibitors roflumilast, cilomilast and rolipram of the epidermal growth factor (25 ng/ml; 24 hours incubation) induced expression of MUC5AC mRNA (left panel) and protein (right panel) in A549 cells. MUC5AC mRNA and protein were determined as indicated in fig 1. Points are mean (SE) values of three to five independent experiments. The corresponding IC50 value for each PDE4 inhibitor is shown in the Results section.

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reported.\(^1\) In brief, aliquots of supernatants from 13,000 g centrifugation of the tissue homogenate containing 25 μg total protein were suspended in SDS sample buffer and boiled for 5 minutes. Proteins were separated by SDS-PAGE electrophoresis in 8% acrylamide-bisacrylamide (80:1). The resulting gel was equilibrated in the transfer buffer: 25 mM Tris-HCl, 192 mM glycine, and 20% (v/v) methanol, pH 8.3. The proteins were then transferred electrophoretically to nitrocellulose membranes which were incubated with 5% fat-free skimmed milk in phosphate buffered saline (PBS) containing 0.5% BSA and 0.05% Tween 20 for 1 hour, and incubated with mAb to MUC5AC (clone 45M1, 1:500, NeoMarkers) for 2 hours at room temperature. Bound antibody was visualised according to standard protocols for the avidin-biotin-alkaline phosphatase complex method (ABC kit; Vector Laboratories, Burlingame, CA, USA).

For MUC5AC immunocytochemical analysis of human bronchus, specimens were fixed, cut into sections, stained with haematoxylin-eosin and periodic acid-Schiff (PAS) reagent (to visualise goblet cells), and incubated with mouse monoclonal antibody to MUC5AC (clone 45M1, 1:100; NeoMarkers, Fremont, CA) as previously reported.\(^1\)

**Western blotting of EGFR, phospho-p38 MAPK, phospho-p44/42 MAPK and phosphotyrosine**

A549 cells were prepared for Western blot analysis as indicated above, and preparations were incubated with either EGFR mouse mAb (Ab-12, cocktail R19/48, NeoMarkers, CA, USA), phospho-p38 MAPK (Thr180/Tyr182) mAb (28B10; Cell Signaling Technology, Beverly, MA, USA), phospho-p44/42 MAPK (Thr202/Tyr204) mAb (20G11; Cell Signaling Technology, Beverly, MA, USA), phospho-p44/42 MAPK (Thr202/Tyr204) mAb (20G11; Cell Signaling Technology, Beverly, MA, USA), phospho-p44/42 MAPK (Thr202/Tyr204) mAb (20G11; Cell Signaling Technology, Beverly, MA, USA), phospho-p44/42 MAPK (Thr202/Tyr204) mAb (20G11; Cell Signaling Technology, Beverly, MA, USA), or anti-phosphotyrosine mAb (clone PY20; ICN Biomedical Inc, Aurora, OH, USA) according to the manufacturers’ instructions. Expression of EGFR and phosphotyrosine was measured at 24 hours of EGF exposure and expression of phospho-p38 MAPK and phospho-p44/42 MAPK at 5, 15, 30 and 60 minutes of EGF (25 ng/ml) exposure. According to the supplier information, these mAbs are highly selective and do not appreciably cross react with the corresponding confounding targets.

**Measurement of cAMP accumulation**

Formation of cAMP was measured as previously outlined.\(^2\) Cultured A549 cells were exposed to EGF or vehicle in the absence or presence of roflumilast for the indicated times, and the cAMP content was quantified using an enzyme immunoassay kit according to the assay protocol provided by the manufacturer (RPN225; Amersham Life Sciences, UK).

**Cytotoxicity assessment**

To exclude the presence of non-selective detrimental effects of the compounds studied, the percentage of lactate...
Statistical analysis
Data are expressed as mean (SE) of n experiments. In concentration-response experiments the \(-\log\) inhibitory concentration 50% (IC50) was calculated by non-linear regression to express compound potency (GraphPad Software Inc, San Diego, USA). Statistical analysis was carried out by analysis of variance followed by appropriate post hoc tests including Bonferroni correction. Significance was accepted as p<0.05.

RESULTS
Cytotoxicity studies and drug vehicle effects
None of the compounds at their maximal concentrations used showed any significant cytotoxicity (values for LDH release were below 5%).

DMSO (0.1% v/v) did not alter the MUC5AC mRNA and protein expression in the absence and presence of EGF 25 ng/ml (fig 1).

Effect of PDE4 inhibition on EGF induced MUC5AC expression and EGFR signalling cascade in A549 cells
EGF (25 ng/ml; 24 hours incubation) increased MUC5AC gene expression and protein production in A549 cells (fig 1). This finding was confirmed by immunocytochemical staining for MUC5AC (not shown). The dependency of this response on the tyrosine kinase activity of the EGFR was confirmed by inhibition of the EGF induced increase in MUC5AC mRNA
PDE4 inhibition and MUC5AC expression in airway epithelial cells

Figure 7 Time course of the relative expression of MUC5AC mRNA and protein in human isolated bronchus. The peak expression for MUC5AC mRNA was observed 1 hour after stimulation with EGF, thus preceding the peak expression of MUC5AC protein at 3 hours. MUC5AC mRNA was determined using real time RT-PCR by the ΔΔCt method; points show the fold increase in expression of MUC5AC relative to GAPDH values as mean (SE) of the 2--DDCt values. MUC5AC protein was determined in tissue by ELISA; points are mean (SE) of bronchial tissues. Data were obtained from three to five different patients. *p<0.05 vs basal values.

Figure 8 Relative quantitation of MUC5AC mRNA (upper panel) and protein levels (middle panel) in human bronchus unstimulated (control) or stimulated with epidermal growth factor (EGF; 25 ng/ml) in the absence or presence of roflumilast (ROF, 1 μM). Exposure time was 1 hour for MUC5AC mRNA determination and 3 hours for MUC5AC protein measurements. The EGF induced increase in MUC5AC expression was abolished by roflumilast. MUC5AC mRNA was determined using real time RT-PCR by the ΔΔCt method; columns show the fold increase in expression of MUC5AC relative to GAPDH values as mean (SE) of the 2--DDCt values of three independent experiments. MUC5AC protein was determined by enzyme linked immunosorbent assay (ELISA); columns show the fold increase from control levels as mean (SE) values of three independent experiments. *p<0.05 vs control; †p<0.05 vs EGF. The lower panel shows MUC5AC protein in human bronchus determined by Western blotting with anti-MUC5AC monoclonal antibody. A representative experiment of three independent experiments is shown for the same experimental groups (C, control; E, EGF; R+E, roflumilast-EGF). Molecular weight marker is shown on the left (213 kDa). The immunostained bands of high molecular weight were abolished these EGF induced responses (fig 3). The functional requirement for p38 MAPK and for p44/42 MAPK in the EGF induced augmentation of MUC5AC mRNA was shown by using their respective selective inhibitors SB202190 (10 μM) and PD98059 (10 μM) was selected for additional experiments.

Addition of EGF (25 ng/ml; 24 hours incubation) to A549 cells resulted in the phosphorylation of the tyrosine residues of different intracellular proteins and the augmented expression of the EGFR, as shown by Western blot analysis of cell lysates with the corresponding specific antibodies (fig 3). Expression of phospho-p38 MAPK and phospho-p44/42 MAPK reached peak values after 15 minutes of exposure to EGF (25 ng/ml). Treatment with roflumilast (1 μM) abolished these EGF induced responses (fig 3). The functional requirement for p38 MAPK and for p44/42 MAPK in the EGF induced augmentation of MUC5AC mRNA was shown by using their respective selective inhibitors SB202190 and PD98059 (10 μM). Addition of EGF (25 ng/ml; 24 hours incubation) to A549 cells resulted in the phosphorylation of the tyrosine residues of different intracellular proteins and the augmented expression of the EGFR, as shown by Western blot analysis of cell lysates with the corresponding specific antibodies (fig 3). Expression of phospho-p38 MAPK and phospho-p44/42 MAPK reached peak values after 15 minutes of exposure to EGF (25 ng/ml). Treatment with roflumilast (1 μM) abolished these EGF induced responses (fig 3). The functional requirement for p38 MAPK and for p44/42 MAPK in the EGF induced augmentation of MUC5AC mRNA was shown by using their respective selective inhibitors SB202190 and PD98059 (10 μM).

**Relationship between inhibition of EGF induced MUC5AC expression by PDE4 inhibitors and the cAMP/PKA pathway in A549 cells**

We then examined whether the inhibitory effect of roflumilast on the overexpression of MUC5AC promoted by EGF was related to its ability to inhibit PDE4, thus increasing cAMP and subsequently activating PKA. EGF alone failed to alter the cellular content of cAMP significantly. Roflumilast (1 μM) produced an early (peak at 5 minutes) and transient increase in the cAMP content of A549 cells (fig 5). The inhibitory effect of roflumilast on the EGF induced MUC5AC response was reversed in the presence of H-89 (5 μM), an inhibitor of PKA,24 thus reinforcing the view of a mechanism of action for roflumilast related to the cAMP/PKA pathway (fig 6).

To establish the ability of the cAMP/PKA pathway to interfere with the EGF induced overexpression of MUC5AC we showed that forskolin (10 μM), a direct activator of...
adenylyl cyclase,\textsuperscript{24} db-cAMP (100 μM), a membrane permeable analogue of cAMP,\textsuperscript{25} and Sp-5,6-DCl-cBIMPS (100 μM), an activator of PKA—while not altering the control level of MUC5AC expression—were impeding the enhanced expression of MUC5AC elicited by EGF (fig 6).

Effect of PDE4 inhibition on EGF induced MUC5AC expression in human isolated bronchus

Since A549 cells are a cancer cell line, the results obtained with these cells may differ from responses of normal airway epithelium. Additional experiments were therefore performed using human isolated bronchial tissue. In this preparation EGF (25 ng/ml) augmented the MUC5AC mRNA and protein expression with peak values reached at 1 hour and 3 hours after EGF exposure, respectively (fig 7). These effects of EGF were suppressed in the presence of tyrphostin A46 (not shown). Roflumilast (1 μM) prevented the EGF induced overexpression of MUC5AC (fig 8).

Immunohistochemistry experiments showed that MUC5AC immunoreactivity was localised in goblet cells that were stained with PAS (fig 9). The MUC5AC positive staining in airway epithelium was increased in EGF exposed preparations, and this augmentation was reduced in roflumilast treated tissues.

DISCUSSION

In this study we found that PDE4 inhibition abolished the EGF induced augmentation of MUC5AC mRNA and protein expression in cultured human airway epithelial cells and in human bronchial tissue in vitro. To our knowledge, this is the first report of a direct inhibitory effect on mucin production of PDE4 inhibitors, a new class of drugs with potential therapeutic interest in the treatment of COPD and asthma—diseases in which mucus hypersecretion is considered pathologically relevant.

EGF activates EGFR signalling cascade and MUC5AC expression in A549 cells

The EGFR signalling cascade is important for regulating MUC5AC mucin gene expression and protein production by airway epithelial cells,\textsuperscript{6} and both the EGFR and the MUC5AC expression are upregulated in chronic airway diseases such as asthma and COPD.\textsuperscript{13,17} The EGFR signalling pathway translates into increased MUC5AC expression, the activation produced by many different stimuli including oxidative stress, neutrophil elastase, tobacco smoke, bacterial and viral products, and inflammatory cytokines.\textsuperscript{17,18} In this study we have selected EGF, an endogenous ligand of the EGFR, as a direct activator of this pathway based on previous studies in cultured human airway epithelial NCI-H292 cells.\textsuperscript{18}

We confirmed that A549 cells have a constitutive expression of EGFR\textsuperscript{18} as shown by the faint band observed in Western blot analysis with anti-EGFR mAb in the control group (fig 3). The activation of the EGFR system results in an increase of about twofold in MUC5AC mRNA and protein expression as shown by ELISA data obtained after 24 hours of incubation with EGF. Immunocytochemistry of A549 cells confirmed this finding. The increase in MUC5AC mRNA and protein at 24 hours is within the time dependency shown in cultured human airway epithelial cells for MUC5AC.

Figure 9 Photomicrographs of representative histological sections from human bronchial tissue unstimulated (A, B, C) or stimulated with EGF (25 ng/ml) in the absence (D, E, F) or presence (G, H, I) of roflumilast (1 μM). Sections show haematoxylin-eosin (A, D, G) or periodic acid-Schiff (PAS; B, E, H) staining or immunohistochemical staining of MUC5AC (C, F, I). Mucin stores in goblet cells appear as purple staining (B, E, H). MUC5AC immunoreactivity was observed as brown staining in goblet cells (C, F, I). Ciliated cells showed no staining for MUC5AC. The sections demonstrate increased PAS and MUC5AC staining in the tissues exposed to EGF, and roflumilast prevented this augmentation. Original magnification ×400 (except panel E: ×250). Goblet cells are indicated by thick arrows and ciliated cells as thin arrows.
production elicited with various stimuli activating EGFR including EGF.\textsuperscript{6, 17 18} Consistent with the notion that the overexpression of MUC5AC is the consequence of the activation of the EGFR signalling cascade, we also found that preincubation with EGFR tyrosine kinase inhibitors prevented the EGF induced augmentation of the MUC5AC mRNA expression and protein production (fig 1). EGF therefore increases the protein-tyrosine kinase activity of its receptor and thereby activates other kinase cascades such as MAPKs including p38 and p44/42 MAPKs.\textsuperscript{27} As expected, we found an early activation of p38- and p44-42-MAPK as well as phosphorylation of tyrosine residues of different cell proteins and upregulation of the EGFR after exposure to EGF for 24 hours (fig 3). Furthermore, inhibition of p38- and p44/42-MAPKs with the selective inhibitors SB20202190 and PD98059 abrogated the EGF induced MUC5AC mRNA expression.

### PDE4 inhibitors suppress the EGF induced MUC5AC expression in A549 cells by activating the cAMP/PKA pathway

There is evidence to indicate that the functioning of the cAMP/PKA pathway is linked with that of the ERK/MAPK pathway. Thus, agents that increase the intracellular cAMP concentration block growth factor stimulated ERK activation in a number of cell types by inhibiting the activation of Raf proteins.\textsuperscript{13 30} In fact, PDE4 isoenzymes may provide a pivotal point for integrating cAMP and ERK signal transduction in cells.\textsuperscript{30} The known relevance of PDE4 isoenzyme activity in the regulation of cAMP levels in human airway epithelial cells, including A549 cells,\textsuperscript{11 12} prompted us to investigate the effects of monoselective PDE4 inhibitors on the EGF induced MUC5AC expression and related events occurring in A549 cells. We found that three different structurally unrelated PDE4 inhibitors—the archetypal PDE4 inhibitor rolipram and the second generation PDE4 inhibitors cilomilast and roflumilast—produced concentration dependent inhibitions of the EGF induced MUC5AC mRNA and protein expression. The potency order of their activities (expressed as $-\log IC_{50}$ values) was roflumilast ($-7.5$) > rolipram ($-6.5$) > cilomilast ($-5.5$). These differences in potencies are consistent with results obtained in other in vitro human cell systems, yet variation may exist depending on the stimulus and the cell type studied.\textsuperscript{30} Since rolumilast (1 μM) suppressed both MUC5AC mRNA and protein production in response to EGF, this concentration was selected for further studies.

The inhibitory action of rolumilast appears to be exerted at different levels of the EGFR signalling cascade. Thus, we showed that rolumilast (1 μM) markedly inhibited the early phospho-p38 MAPK expression as well as the phosphorylation of tyrosine residues of proteins and the overexpression of EGFR in response to EGF stimulation measured at 24 hours EGF exposure.

The inhibitory effects of rolumilast on the EGFR cascade events leading to enhanced MUC5AC expression are probably related to the activation of the cAMP/PKA pathway since this selective PDE4 inhibitor elicited a transient early increase in cAMP levels in A549 cells, and its inhibitory effects on MUC5AC expression were reversed by preincubation with H-89, an inhibitor of PKA activity.\textsuperscript{24} Furthermore, forskolin (a direct activator of adenylyl cyclase),\textsuperscript{44} db-cAMP (a membrane permeant analogue of cAMP),\textsuperscript{45} and Sp-5,6-DCl-cBIMPS (a specific activator of PKA)\textsuperscript{46} prevented the enhanced expression of MUC5AC elicited by EGF (fig 6), thus supporting the notion that the activation of the cAMP/PKA pathway is effective in exerting an inhibitory influence on the EGFR cascade leading to MUC5AC expression in A549 cells.

### PDE4 inhibition attenuates EGF induced MUC5AC expression in human airways in vitro

The inhibitory effects resulting from PDE4 inhibition with roflumilast in cultured A549 cells may not necessarily be representative of the responses of the epithelial cells in the human airways. MUC5AC expression was therefore examined in human isolated bronchus, a preparation that has previously been shown to have a basal secretion of mucin MUC5AC produced principally by goblet cells.\textsuperscript{45} In the human airways in vitro, MUC5AC mRNA expression reached a peak at 1 hour after stimulation with EGF, while peak MUC5AC protein production in tissue and medium was observed at 3 hours (fig 7). This represents faster kinetics of MUC5AC expression than in cultured A549 cells, but we have not investigated the reason for this difference. Pretreatment with roflumilast (1 μM) markedly inhibited this augmented expression of MUC5AC induced by EGF activation, indicating that the direct inhibitory effects produced by this PDE4 inhibitor in cultured A549 cells are reproducible in intact airway epithelial cells. Immunohistochemical analysis of human bronchial tissues confirmed that EGF exposure resulted in an augmented expression of MUC5AC positive stained cells in airway epithelium and treatment with roflumilast effectively prevented this EGF induced overexpression of MUC5AC (fig 9).

In summary, the results of this study indicate that putative PDE4 inhibitors, in addition to their established inhibitory effects on the airway inflammatory cells,\textsuperscript{10} may also exert direct effects on human airway epithelial cells inhibiting the MUC5AC expression that follows the activation of the EGFR signalling cascade. These findings may be of added value to results from recent phase II/III clinical trials which suggest a therapeutic benefit for PDE4 inhibitors in mucus hypersecretory diseases such as COPD and asthma.\textsuperscript{46}

### ACKNOWLEDGEMENTS

The authors are indebted to the teams of the Services of Thoracic Surgery and Pathology of the University Clinic Hospital and ‘La Fe’ University Hospital of Valencia (Spain) for making the human lung tissue available to us, and to Altana Pharma for the gift of phosphodiesterase 4 inhibitors. The technical assistance of Pedro Santamaria and Dora Marti is also gratefully acknowledged.

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Correction

It has been brought to our attention that there is an error in figure 3 on page i55 of the Pleural Disease Guideline available at www.brit-thoracic.org.uk/docs/PleuralDiseaseChestDrain/pdf. Below is a corrected diagram illustrating the "safe triangle" for a chest drain. The publishers apologise for this error.
BTS guidelines for the insertion of a chest drain

D Laws, E Neville and J Duffy

Thorax 2003 58: ii53-ii59
doi: 10.1136/thorax.58.suppl_2.ii53

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