A best evidence topic in thoracic surgery was written according to a structured protocol. The question addressed was: ‘is water washout more effective than normal saline washout after lobectomy in preventing local recurrence?’ Altogether more than 48 papers were found using the reported search, of which nine represented the best evidence to answer the clinical question. The authors, journal, date, country of publication, patient group studied, study type, relevant outcomes and results of these papers are tabulated. Tumour cell ‘spillage’ after cancer resection is linked to a worse prognosis, so washout to minimize contamination is an established surgical technique. While the mechanical effects of lavage are well validated, the differential cytocidal effects of water versus saline as irrigation fluids are not. There are currently no studies addressing this issue in the thoracic surgery setting, after lung cancer lobectomy. However, the majority of relevant papers describe the use of basic in vitro methods and animal models to produce data that can conceivably be extrapolated to the clinical question in hand. The number of studies is small, and some have technical limitations. While two of the better-designed experiments suggest that water exerts a superior cytocidal effect on tumour cells, data from other studies are somewhat unimpressive, with two studies reporting that water washout controls tumour growth to a lesser extent than saline. This, together with the complete paucity of clinical trials on the subject, leads us to conclude that water is unlikely to represent a superior irrigation fluid in lung cancer patients after lobectomy.

Keywords: Water • Saline • Washout • Lobectomy • Cancer

INTRODUCTION
A best evidence topic was constructed according to a structured protocol. This is fully described in the ICVTS [1].

THREE-PART QUESTION
In [lung cancer patients undergoing lobectomy], is [water superior to normal saline] in [preventing recurrence]?

CLINICAL SCENARIO
When assisting in theatre, you notice that after lobectomy in lung cancer patients your consultant performs the washout of the chest cavity with water, not normal saline. Apparently, this is a time-honoured tradition because water is more likely to lyse free tumour cells due to its hypotonicity. Although the theory sounds right, you are unsure whether there is evidence to support this practice and resolve to check the literature.

SEARCH STRATEGY
Medline 1948 to September week 4 2011 using OVID interface. (Lobectomy.mp OR Lung resection.mp OR exp Pneumonectomy/ OR exp Surgery/) AND (exp therapeutic irrigation/ OR Irrigation.mp OR washout.mp) AND (cancer.mp. or exp Neoplasms/ OR Tumour.mp).

SEARCH OUTCOME
Forty-eight papers were found using the reported search. From these, nine papers were identified that provided the best evidence to answer the question. These are presented in Table 1.

RESULTS
Park et al.’s in vitro study compared the cytotoxic effects of different irrigation fluids. The authors validated tryptan blue as an accurate marker of breast cancer cell viability, then showed that water reduced the number of viable tumour cells compared...
Table 1: Best evidence papers

<table>
<thead>
<tr>
<th>Author, date, country, study type</th>
<th>Study group</th>
<th>Key outcomes</th>
<th>Key results</th>
<th>Comments</th>
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<tr>
<td>Kosuga et al., 2011, Japan [2], Uncontrolled in vitro (human patients) study</td>
<td>Three human oesophageal SCC (ESCC) cell lines: exposed to distilled water</td>
<td>Cell morphology changes, Cell volume changes</td>
<td>Cell swelling then rupture within 3 min. Overall cell volume post-hypotonic shock was smaller than pre-hypotonic shock volume, implying cell fragmentation. No surviving cells 48 h after 10-min exposure to water in two of the cell lines; the third was more resistant to cytotoxic effects and 30-min exposure was needed to kill all cells.</td>
<td>The most recent study and the only one to focus on pleural lavage. No controls were used, and most values are displayed graphically rather than stated. Although the clinical component appears to be an afterthought, it refutes Huguet et al.’s notion that intrapleural/peritoneal secretions raise the osmolality of lavage fluid such that tumour cell lysis is unlikely to occur. This may encourage the use of pleural lavage in clinical practice, pending higher-quality evidence.</td>
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<td>Ito et al., 2011, USA [3], Controlled in vitro and in vivo (animal peritoneal lavage model) study</td>
<td>Five patients undergoing surgery for ESCC: received pleural lavage with distilled water</td>
<td>Osmolarity of pleural lavage fluid during surgery</td>
<td>The cell line studies in this investigation did not include normal saline as a control. Fortunately, the murine wound model compared water, saline and povidone-iodine—despite stating that povidone-iodine and water exert cytotoxic effects compared with saline, the authors fail to present specific values.</td>
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<td>Hah et al., 2011, South Korea [4], Controlled in vitro and in vivo (animal wound model) study</td>
<td>Syngeneic ‘SCC’ cell line: exposed to distilled water and povidone-iodine (different concentrations and exposure times)</td>
<td>In vitro tumour growth: analysis of survival fraction after 72 h</td>
<td>100% water: tumour growth almost completely inhibited after 5 min exposure. 100% povidone-iodine: tumour growth completely inhibited after 30 s exposure.</td>
<td>The most recent study and the only one to focus on pleural lavage. No controls were used, and most values are displayed graphically rather than stated. Although the clinical component appears to be an afterthought, it refutes Huguet et al.’s notion that intrapleural/peritoneal secretions raise the osmolality of lavage fluid such that tumour cell lysis is unlikely to occur. This may encourage the use of pleural lavage in clinical practice, pending higher-quality evidence.</td>
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<td>Lin et al., 2006, China [5], Controlled follow-up study (level III)</td>
<td>32 patients with spontaneous ruptured ‘hepatocellular carcinoma’ undergoing curative liver resection: 13 patients received post-operative peritoneal lavage with distilled water, 19 did not</td>
<td>Number of patients with tumour recurrence, Mean disease-free time, Survival time</td>
<td>Lavage: 2 patients No lavage: 11 patients. Lavage: 3.59 ± 0.60 years No lavage: 2.05 ± 0.74 years (P = 0.045). Survival time P = 0.0158</td>
<td>This clinical study effectively compared the benefits of lavage with no lavage in terms of survival time post-liver resection for ruptured hepatocellular carcinoma. The differential effects of water versus normal saline as irrigation fluid are not investigated.</td>
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### Table 1: Continued

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<tr>
<td><strong>Huguet et al., 2004, UK [7]</strong>, Controlled in vitro study and uncontrolled in vivo (human patients) assay</td>
<td>Five patients immediately post-‘colorectal cancer’ resection surgery: received five sequential 1000-ml peritoneal lavages. Each aliquot left to equilibrate for 1 min with agitation. Sample of fluid taken before aspiration and administration of next aliquot. Two animal colorectal cancer cell lines: exposed to water or saline (or betadine or culture medium)</td>
<td>Osmolality of lavage water</td>
<td>50 mM after first lavage; decreased with each subsequent lavage, reaching plateau of 10 mM after third lavage. Water: 100% cell lysis within 12 min. Saline: 0% cell lysis within 36 min. Linear regression analysis—water more toxic than saline (P &lt; 0.0001). Betadine: 100% cell lysis not achieved within 40 min. Linear regression analysis—water more toxic than betadine (P &lt; 0.0001). ‘Lavage solution’ (10 mM): 100% cell lysis within 32 min. Linear regression analysis—lavage solution’ more toxic than saline (P &lt; 0.0001) and betadine (P &lt; 0.0008) but less toxic than water (P &lt; 0.0001)</td>
<td>This oft-cited study sought to comment on the utility of peritoneal lavage as it was being performed by surgeons at the time. The authors found that three sequential lavages were required to dilute lavage fluid to an osmolality of 10 mM, since it becomes contaminated with intra-peritoneal detritus upon introduction into the cavity. More significantly, the authors found that at this minimum osmolality, more than 30 min are required for lavage fluid to achieve 100% tumour cell lysis in vitro. This suggests that current lavage techniques are not in fact efficacious.</td>
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<td><strong>Allegretto et al., 2001, USA [8]</strong>, Controlled in vivo (animal wound model) study</td>
<td>40 non-obese diabetic/SCID mice: surgical wounds created and inoculated with human ‘SCC’ cells. Ten mice had incisions closed immediately (controls), 10 had water irrigation, 10 had saline irrigation and 10 had 1 mM gemcitabine irrigation</td>
<td>Percentage of mice with palpable tumour growth</td>
<td>Day 17: ‘control’ ~70%, ‘water’ ~15% (P &lt; 0.0005), ‘saline’ ~0% (P &lt; 0.0001), ‘gemcitabine’ ~0%. Day 56: ‘control’ ~80%, ‘water’ ~75%, ‘saline’ ~40% (P &lt; 0.01), ‘gemcitabine’ ~35% (P &lt; 0.04)</td>
<td>This wound model study is inferior to that by Ito et al., as the outcome measure is relatively subjective. However, adequate controls were used and the authors presented data and significance values in a transparent manner. Interestingly, results indicate that saline is superior to water for long-term control of tumour growth.</td>
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<td><strong>Morris and Scholten, 1996, USA [9]</strong>, Controlled in vivo (animal model) study</td>
<td>36 nude mice: injected intraperitoneally with human ‘ovarian cancer’ cells. Intraperitoneal lavage administered with saline or water. Mice were followed up until they reached moribund status or until 60 days post-injection</td>
<td>Percentage of mice with clinically detectable tumour growth at 30 days</td>
<td>Water: 55%, saline: 89% (P &lt; 0.03)</td>
<td>This peritoneal lavage model study again uses ‘soft’ outcome measures to compare water with saline lavage in an ovarian carcinoma setting. Data for tumour recurrence at 30 days are in favour of water over saline, but the other longer-term outcomes suggest that saline is in fact significantly superior to water in controlling the sequelae of tumour spillage.</td>
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<td><strong>Swietzer et al., 1993, USA [10]</strong>, Controlled in vivo (animal wound model) study</td>
<td>C57BL/6 mice: surgical wounds created and inoculated with syngeneic B16-F10 ‘melanoma’ cells. Mice received saline irrigation, water irrigation or no irrigation</td>
<td>Tumour weight at dissection (g)</td>
<td>Water: 0.0 ± 0.0. Saline: 0.007 ± 0.015. No irrigation: 0.017 ± 0.012 (P &lt; 0.03)</td>
<td>This well-controlled study investigated the effects of saline versus water versus no irrigation. The authors also incorporated experiments to test the effects of: 1. Timelag between tumour inoculation and lavage 2. Duration of incubation with lavage fluid 3. Concentration of tumour cells per unit volume of inoculating fluid. There were no significant differences reported for any of the above factors, with the exception that irrigation with either fluid was beneficial in the ‘immediate irrigation’ setting after contamination with a low-concentration inoculum.</td>
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with phosphate-buffered saline ($P < 0.01$). Normal saline was omitted from investigation.

At the other end of the spectrum, Lin et al.'s clinical trial measured recurrence after liver resection for ruptured hepatocellular carcinoma. Peritoneal lavage with water (recurrence in 15%) was superior to no peritoneal lavage (recurrence in 58%). Normal saline again did not feature, rendering it impossible to separate factors related to lavage per se from factors caused by osmotic absorption.

Swetzer et al. seeded a murine wound model with melanoma cells and looked at effects of water versus saline irrigation, time lag to irrigation, duration of irrigation and tumour cell concentration of the inoculum. Overall, there were no outcome differences between mice receiving water, saline, or no irrigation ($P > 0.73$), suggesting that neither the mechanical action of lavage nor the hypotonic effect of water alter tumour growth.

Allegretto et al. used a similar murine model to assess three irrigation fluids (water, saline, gemcitabine) in the head and neck cancer setting. Mice with non-irrigated wounds served as controls. All fluids initially delayed the development of squamous cell carcinomas (SCCs)—by day 24, 70% of controls had developed tumours versus 15% of the ‘water’ group ($P < 0.0005$), 0% of the ‘saline’ group ($P < 0.0001$) and 0% of the ‘gemcitabine’ group ($P < 0.0001$). By day 56, incidence of recurrence in the ‘water’ group was similar to that of controls. In contrast, saline ($P < 0.01$) and gemcitabine ($P < 0.004$) improved tumour control compared with water irrigation or no irrigation.

This result was corroborated by Morris and Scholten using their peritoneal lavage murine model of ovarian tumour control. Although short-term data suggested that water controls recurrence better than saline (55 versus 89%, $P = 0.03$), longer-term outcomes suggested that water exerts a smaller effect than saline (ascites: $P = 0.003$, tumour score at dissection: $P = 0.05$, survival: $P = 0.002$). However, most endpoints were subject to variable interpretation.

Some papers attempt to correlate the differential effects of water and saline between in vitro and in vivo settings. Like Allegretto’s group, Hah et al. used an animal wound model to investigate tumoricidal effects on SCC cells. Preliminary work with cell lines confirmed water’s lytic activity, but saline was not used as a control. In vivo experiments showed that water had a small effect on tumour growth, while saline had none.

In Huguet et al.’s appealing study, the authors navigate seamlessly between clinical and experimental settings. They first showed that peritoneal secretions contaminate lavage water after colorectal cancer resection, producing a minimum osmolality of 10 mM. With this in mind, they incubated colorectal cancer cells with water, saline, Betadine and simulated ‘lavage solution’ (osmolality 10 mM). Water was most cytotoxic, followed by ‘lavage solution’ and then Betadine. Saline exerted no significant lytic effect compared with water ($P < 0.0001$). The authors concluded that the efficacy of ‘oncological’ lavage is likely to be reduced by intraperitoneal secretions, recommending sequential lavages and an incubation period of 32 min to maximize cell lysis. They conceded the impracticality of this in the operating theatre.

Ito et al.’s elegant investigation correlated in vitro and animal model data to show that the lytic properties of water translate into superiority over saline in improving the sequelae of colorectal cancer cell spillage at laparotomy. A highlight of this study is its use of magnetic resonance imaging to provide an objective and quantitative outcome measure (tumour volume in ‘water’ mice $316 \pm 181 \text{mm}^3$ versus ‘saline’ mice $1477 \pm 181 \text{mm}^3$, $P < 0.05$). The authors also reported significant differences in peritoneal tumour burden, comorbidities and mouse survival.

Kosuga et al.’s recent work stands alone in its focus on cytotoxic effects of hypotonic shock in pleural rather than peritoneal lavage, albeit using oesophageal SCC cells. In the in vitro experiments, the authors employed video microscopy and flow cytometry to demonstrate morphological cell changes and loss of cell viability. However, the paper presents mainly graphical data, suffering from lack of clarity on actual values. Furthermore, while solutions of graded hypotonic osmolalities are used to assay cytotoxic effects, isotonic fluid such as normal saline is not used as a control. Finally, the authors performed intra-operative pleural lavage on several oesophageal cancer patients, attempting to debunk the notion that its clinical utility may be undermined by contamination with intraperitoneal secretions and cells. They reported only a small increase in lavage fluid osmolality of $<10 \text{mOsmol/kgH}_2\text{O}$ and then showed that cancer cell rupture alone can elevate osmolality in vitro by $10 \pm 6.5 \text{mOsmol/kgH}_2\text{O}$. They concluded that osmolality of lavage fluid prior to washout has a more significant influence on cytotoxic effects than that after washout.

**CLINICAL BOTTOM LINE**

Tumour cell ‘spillage’ after cancer resection is linked to worse prognosis, so washout to minimize contamination is an established technique. While mechanical effects of lavage are well-validated, differential cytotoxic effects of water versus saline are not. Currently, no studies address this issue in the thoracic surgery setting. However, most papers use in vitro methods and animal models to produce data that can conceivably be
extrapolated to the clinical question in hand. There are few studies, some with technical limitations. While two of the better-designed experiments suggest that water exerts a superior cytotoxic effect on tumour cells, data from other studies are unimpressive, with two studies reporting that water washout controls tumour growth less effectively than saline. This, together with the complete paucity of clinical trials on the subject, leads us to conclude that water is unlikely to represent a superior irrigation fluid in lung cancer patients after lobectomy.

Conflict of interest: none declared.

REFERENCES