Original Article

Does hyperthermic povidone-iodine lavage increase the apoptotic rate of residual cancer cell in patients with malignant pleural mesothelioma?—a prospective pilot study

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Background: Malignant pleural mesothelioma (MPM) is an incurable, late presenting primary cancer, conferring a survival of 8–14 months. Different intrapleural treatments have been tested as part of a multimodality approach to treat a select group of patients with limited disease, increasing survival. Recently, povidone-iodine has been shown to induce apoptosis in microscopic tumour cells *in vitro*, with no reported complications. This is the first *in vivo* study assessing the apoptotic rate caused by intraoperative hyperthermic betadine lavage using routine immunohistochemistry combined with transmission electron microscopy (TEM).

Methods: We included surgically fit patients aged >18, undergoing minimally invasive video-assisted thoracoscopic surgery (VATS) pleural biopsy between December 2016 and February 2018, for confirmed or presumed pleural malignancy. Parietal pleural biopsies were obtained at 7.5, 15 and 30 minutes after hyperthermic betadine lavage, and compared to pre-lavage biopsy samples, for apoptotic changes. Viable tumour samples underwent histological, immunohistochemical and ultrastructural analysis as well as TEM for features of apoptosis.

Results: N=6. Median age was 76 years. Median overall survival was 26.7 months. There was no statistical impact on survival of side of disease (left *vs.* right). There was no significant difference in expressions of markers of apoptotic index pre and post betadine treatment upon immunohistochemical analysis. There was no discernible effect on morphological features of apoptosis seen with betadine treatment, on TEM analysis. No side effects were identified post betadine lavage.

Conclusions: Although hyperthermic betadine lavage is a safe antiseptic solution with no toxicity when performed intraoperatively, it confers no effect on apoptotic rate or necrosis. It is therefore unlikely that hyperthermic betadine lavage will have an impact on reducing the microscopic residual disease after pleurectomy decortication and enhancing survival.

Keywords: Malignant pleural mesothelioma (MPM); betadine lavage; apoptosis; transmission electron microscopy (TEM)

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Introduction

Malignant pleural mesothelioma (MPM) is an incurable primary pleural cancer related to asbestos exposure, usually presenting at a late stage, and conferring a poor prognosis of typically 8–14 months (1). A selected group of patients with limited disease may benefit from a multimodality approach including chemotherapy and surgery with an increased median overall survival of 22 months (2).

In order to improve survival, over the years, different intrapleural treatments have been tested to improve local control and survival. The aim of intrapleural treatment was to reduce the volume of microscopic disease left after cytoreduction surgery. Intraoperative chemotherapy showed poor results with increased rates of atrial fibrillation (3). Photodynamic therapy showed interesting preliminary results with a median survival of 41.2 months in epithelial tumours however only 15.1 months progression-free survival (4). Lang-Lazdunski has reported the use of hyperthermic povidone-iodine lavage at the end of pleurectomy decortication with good preliminary results (5). Povidone-iodine is an antiseptic solution widely used in general and thoracic surgery for decades to prevent infection, with no side effects reported to date. Povidone-iodine has even been shown to increase cell death in mesothelioma cancer cells (6). This has led to the subsequent incorporation of povidone-iodine in treatment as an additive to oncological thoracic resection at the time of surgery, with the aim of reducing infection risk and actively inducing apoptosis of the residual microscopic tumour cells. In this series the overall survival was over 20 months. This technique was found to be safe with no reported complications.

Despite its use, there is little published scientific evidence on impact on apoptosis in patients with cancer. The effect and apoptotic rate of povidone-iodine have only been precisely quantified *in vitro*. However, the quantification of the apoptotic rate induced in humans at the time of surgery has only been done through routine analysis of pathology specimens without analysis of ultrastructural changes.

In this prospective pilot study, we aim to assess the apoptotic rate caused by the povidone iodine using routine immunohistochemistry combined with electron microscopy. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-282/rc).

Methods

Patient recruitment

Between December 2016 and February 2018, surgically fit patients aged >18, undergoing minimally invasive video-assisted thoracoscopic surgery (VATS) pleural biopsy for confirmed or presumed pleural malignancy were recruited prospectively into the study. Patients were excluded if they had benign pleural disease (including empyema, tuberculosis, benign pleural disease), if they had neoadjuvant chemotherapy, and if allergic to or intolerant of iodine. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study has been approved by the NHS Health Research Authority (REC reference 16/LO/1081) and informed consent was taken from all individual participants.

Surgical protocol

For each patient, at the start of the operation, pleural fluid was routinely drained from the chest cavity and the sample sent for cytology analysis to determine the presence of any abnormal cells in the sample. Simultaneously, a similar sample of 20 mLs of pleural fluid was collected for flow cytometry analysis to determine the cell morphology of the fluid. A povidone-iodine lavage was then performed in all patients with a total of 5 litres lavage fluid (made up in a ratio of 1:4 povidone-iodine to 0.9% saline) heated to 41 ℃ The lavage fluid was left in the pleural cavity for 5 minutes before being removed using a standard Yankeur suction catheter as previously reported by our group (7). At the end of the povidone-iodine wash, a second sample of the pleural/wash fluid was taken for cytology analysis.

During surgery, 3 biopsies involving both superficial and deep layers of the parietal pleura were performed: at the beginning of the case before hyperthermic povidone-iodine lavage, at 7.5 minutes and at 15 minutes after hyperthermic povidone-iodine lavage, and at 30 minutes after pleural hyperthermic povidone-iodine lavage. The hyperthemic lavage time was double compared to the study published by Lang-Lazdunski *et al.* (7) where the lavage lasted

15 minutes. The biopsy samples measured approximately 2 mm in diameter.

Histological, immunohistochemical and ultrastructural analysis

Hematoxylin and eosin (H&E) stained slides from the pleural specimens were reviewed on all samples, and only the slides with sufficient amount of tumour were selected for further study. The diagnosis of malignant mesothelioma was confirmed according to the 2015 World Health Organisation classification of pleural tumours (8). There were 11 cases of epithelioid type and one case of biphasic type.

Blocks of the selected cases were cut at 3 µm in adhesive slides and stained with Bcl-2 (mouse, clone 124, 1:100, Agilent, Germany), Ki67 (mouse, clone MIB1, 1:400, Agilent, Germany) and p53 (mouse, clone DO-1, 1:100, Leica Biosystems, Germany).

The optimization and validation protocol for all 3 antibodies were performed on Leica Bond III platform (Leica Biosystems). For Bcl-2 and p53, a citrate-based solution (ER1, pH 6, Leica Biosystems) was used to perform antigen retrieval for 30 minutes. For Ki67, the heat-induced epitope retrieval was performed with an Ethylenediamine Tetra-acetic Acid (EDTA) based solution (ER2, pH 9, Leica Biosystems) for 30 minutes. Slides were incubated in primary antibody for 25 minutes after antigen retrieval. The secondary detection was performed by using the Bond Polymer Detection (Leica Biosystems).

A standard Olympus BX41 microscope was used to identify tumour 'hot spots' in Bcl-2, p53 and Ki67 staining. The percentage of positive tumour cell staining was counted and reported as a percentage.

Apoptotic index was assessed by counting apoptotic cells in 1,000 tumour cells, and reported as a percentage.

For transmission electron microscopy (TEM) analysis, samples were removed from the patient and immediately immersed in 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and stored overnight at 4 degrees. The following day, samples were washed twice with 0.1 M sodium cacodylate buffer before being post-fixed at 4 ℃ with 1% (w/v) osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 1.5 hours. Samples were then dehydrated through a graded ethanol series, before infiltration with TAAB epoxy resin and polymerization at 70 ℃ for 24 hours.

The electron microscopy was used to analyse structural changes in tumour cells due to an increased apoptotic rate. Ultra-thin sections (50–70 nm) were prepared using a Leica UC7 ultramicrotome (Leica microsystems, Austria), mounted on grids and contrasted using UranyLess 22409 and Lead Citrate 22410 (Electron Microscopy Sciences, USA). Samples were examined on a JEOL 1400Flash transmission microscope (JEOL, Japan) operated at 120 kV and images were acquired with a FLASH 2k×2k CCD camera (JEOL, Japan).

Statistical analysis

All patients were followed up until December 2021. Differences in apoptotic rates in the pleural biopsies before and after the povidone-iodine lavage were analysed using Chi-squared and unpaired Student's *t*-tests. Clinical data are expressed as appropriate as counts or mean ± standard deviations. Statistical significance was determined at a P value of <0.05. Statistical tests were performed with SPSS software package.

Results

A prospective case control pilot study was conducted and 17 patients were enrolled in the study, 5 were excluded due to no evidence of mesothelioma on final pleural biopsy results. Out of 12 patients, only 6 patients had enough viable tumour in the biopsy to perform immunohistochemistry and electron microscopy analysis. Tumours with high percentages of fibrotic/stromal components were excluded.

In the study population, the median age was 76 years old (range, 70–85), there were 2 female and 10 male patients (*Table 1*). Seven patients were exposed to asbestos and 9 patients had a previous smoking history. One patient presented with clinical stage I, 6 with stage II mesothelioma and the remaining patients with stage III. Right sided disease was predominant (10 cases), 11 patients were diagnosed with epithelioid mesothelioma and only one with biphasic mesothelioma. Nine patients passed away during the study follow-up and 3 are still alive, 2 with evidence of recurrence. Median overall survival (OS) was 26.7 months (95% CI: 12.4–40.9 months). Six underwent multimodality treatment including surgery (radical pleurectomy decortication) and chemotherapy and 6 had chemotherapy only according to the standard regimen of platinum- based chemotherapy and pemetrexed for 6 cycles. In the multimodality treatment group, two patients are still alive with a median OS of 37 months (95% CI: 32.3–41.7 months). In the palliative chemotherapy

Table 1 Demographic and operative data of patients included in the study

Patient	Age, years (sex)	Smoking status	Asbestos exposure	ECOG performance status (9)	Type of surgery	Radical surgery	OS, months	Status D/A
1	79 (M)	Ex smoker	Y	1	Pleural decortication	Y	35.0	D
2	71 (M)	Ex smoker	Y	1	VATS pleural biopsy and pleurectomy	N	2.3	D
3	76 (M)	Ex smoker	N	1	Pleurectomy decortication	Y	49.0	A
4	73 (M)	Ex smoker	Y	1	Pleurectomy decortication	Y	6.8	D
5	76 (M)	Ex smoker	Y	2	VATS pleural biopsy and pleurectomy	N	1.9	D
6	78 (F)	Ex smoker	N	1	Pleurectomy decortication	Y	37.0	D

ECOG performance status (9). Performance status: 1= restricted in physically strenuous activity, but ambulatory and able to carry out work of a light and sedentary nature (e.g., light house work, office work); 2= ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours. ECOG, Eastern Cooperative Oncology Group; OS, overall survival; VATS, video-assisted thoracoscopic surgery; D/A, dead or alive.

group, only one patient is still alive with a median OS of 17.6 months (95% CI: 0–46.7) (P=0.164).

In the multimodality treatment group, there was one patient only with biphasic mesothelioma, 1 patient was staged as stage I, 2 patients were staged as stage II, 3 as stage III. In the chemotherapy group, they were all epithelioid and 2 patients were staged as stage II and 4 as stage III.

There was no statistical impact on survival of side of disease (right *vs.* left), R status (R1 *vs.* R2) and stage (II *vs.* III): 26.7 *vs.* 26.6 months (P=0.451), 37 *vs.* 17.6 months (P=0.164) and 26.7 (95% CI: 16–36.7) *vs.* 6.8 (95% CI: 0–16) months (P=0.231) respectively.

Samples from six cases contained sufficient amount of tumour for immunohistochemistry study. There was no significant difference in expressions of any of the markers or apoptotic index in the samples between pre- and post-treatment. Median rate of p53 expression before and after hypothermic lavage was 80% *vs.* 92.5%, Bcl2 0% *vs.* 10% and MIB1 10% *vs.* 10% (P= not significant). There was no evidence of betadine-induced apoptosis macroscopically.

We then used transmission electron microscopy (TEM) to assess any early alteration due to an apoptotic process induced by betadine. A range of cell morphologies were observed *Figure 1*, but we could not detect a discernible effect of the treatment in TEM images of cells from the 3 patients, with respect to typical morphological features of apoptosis such as nuclear condensation, blebbing, and the presence of phagolysosomes (10).

Discussion

This is the first reported *in vivo* study in mesothelioma patients, assessing the apoptotic effects of povidone-iodine in MPM. Due to the poor outcome that mesothelioma confers even in patients treated with multimodality treatment, intrapleural treatments such as chemotherapy (11,12), photodynamic therapy (4) and hyperthermic betadine lavage (5) have been considered in attempt to improve survival. Povidone-iodine is a safe antiseptic solution used routinely for intraperitoneal and intrathoracic lavage with no established side effects (13). Our patients, as also previously reported, treated with betadine did not display any side effects and had a median OS of 37 months. These results are comparable to a median OS of 25.6 months following intrapleural chemotherapy (14) and 20.8 months following radiotherapy (15).

Interestingly, a recent murine study demonstrated lower expressions of colonic and hepatic malignancy tumour markers using flow cytometry, following the administration of povidone-iodine (16). However, a recent *in vitro* study in rats showed concerns over the safety of using povidone-iodine as a peritoneal lavage in the context of colorectal cancer, through increased metabolic acidosis and compromising of the integrity of the mucosal barrier (17). In thymic carcinoma, povidone-iodine has been shown to induce rapid cell death through cellular fixation (18).

The use of povidone-iodine in inducing apoptosis has largely been described in, *in vitro* studies, however povidone-iodine has also been described as a safe alternative in chemical pleurodesis in the context of malignant pleural effusions, with results comparable to cisplatin (12), gemcitabine (14), bleomycin (19,20), tetracycline (21), and talc (22,23). *In vitro* studies have shown povidone-iodine to have no effect on apoptosis

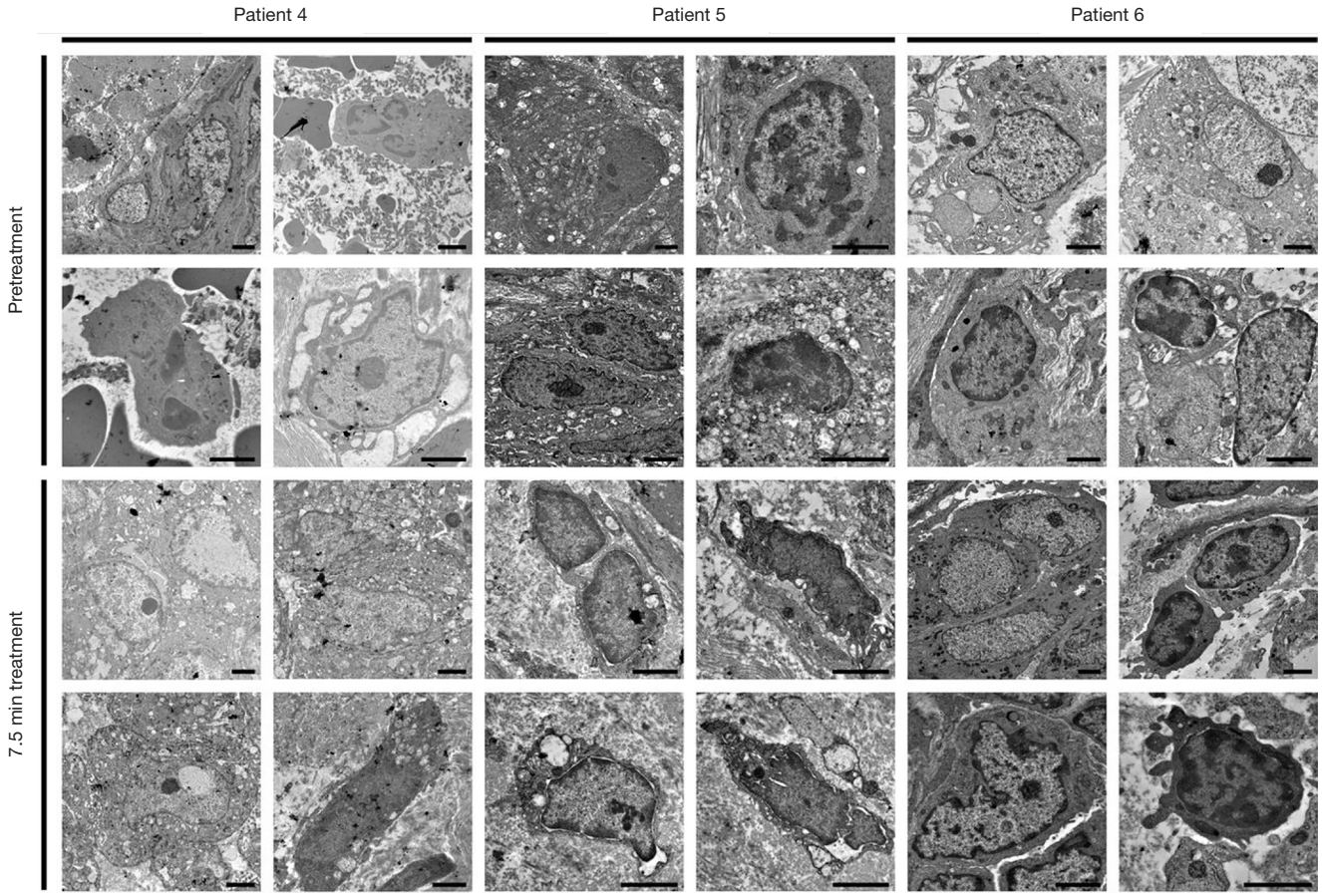

Figure 1 TEM images of pleural biopsy of viable mesothelioma before and after treatment with hyperthermia povidone-iodine solution. 4 images are shown for each condition. The images illustrate the range of morphologies observed, but should not be taken as representative of specific proportions. Bars, 2 µm. TEM, transmission electron microscopy.

proteins expressed on MPM cells, however it inhibits cell growth (6), and exhibits a necrotic rather than apoptotic mechanism of cell death (24).

In our study, the samples post hyperthermic betadine lavage was compared to each patient's baseline samples, thereby acting as their own control. We did not find any evidence of betadine-induced apoptosis and induced necrosis or ultrastructural cellular changes on either immunohistochemical analysis or TEM, or indeed macroscopically. Our results, which contradict findings of these *in vitro* studies (6,16,17,24) could highlight the variation in response to povidone-iodine of commercially available MPM cells compared to cells extracted from actual patients. This study can reflect the more complex interaction of the tumor and immune response in mesothelioma patients compared to *in vitro* or animal models, showing reduced effects in real patients.

Despite being a small pilot study, this is the first study which examines the response of *in vivo* human malignant pleural mesothelioma cells to hyperthermic betadine lavage. Our finding shows no post-operative betadine induced toxicity probably but also no impact on residual microscopic disease induced by povidone-iodine.

Our study has limitations which include the exposure time of mesothelioma cells *in vivo* to betadine, which, although the time was double compared to Lang-Lazdunski surgical series (7), 30 minutes may not be enough to cause any significant apoptotic effect and our study may confirm the need of longer lavage time. Also, only 6 out of 12 patients were available for full examination and this shows the difficulty in having good samples to be analyzed even with macroscopic tumor as target and shows how to target microscopic residual disease areas post resection and intra pleural treatment may not be possible.

Conclusions

In conclusion, this is a single-centre small pilot study, first ever attempted in real patients, which however shows no real effect on apoptotic rate and survival caused specifically by hyperthermic betadine lavage. In future, further larger studies are needed to test for prolonged intracavity hyperthermic lavage times to further evaluate a delayed apoptotic effect.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-282/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-282/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-282/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study has been approved by the NHS Health Research Authority (REC reference 16/LO/1081) and informed consent was taken from all individual participants.

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