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The Addition of 4% Oxygen to the CO_2 Pneumoperitoneum Does Not Decrease Dramatically Port Site Metastases

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ABSTRACT **Study Objective:** Port site metastases (PSM) after laparoscopic surgery for advanced-stage ovarian carcinoma are a cause of concern, but the pathophysiology is unknown. Because CO_2 pneumoperitoneum was recently demonstrated to be a cofactor in adhesion formation and tumor implantation in a laparoscopic mouse model, and because both could be prevented by the addition of 4% oxygen to the CO_2 pneumoperitoneum, we wanted to test the hypothesis that PSM could be related to tumor cell hypoxia during CO_2 pneumoperitoneum.

Design: A randomized controlled pilot trial to compare the incidence of PSM in women undergoing laparoscopy with a pure CO_2 pneumoperitoneum in comparison with women with CO_2 pneumoperitoneum with the addition of 4% oxygen (Canadian Task Force classification C).

Setting: University Hospital Gasthuisberg, Leuven, Belgium.

Patients: Since January 1, 2007, 22 consecutive women undergoing laparoscopy for suspected ovarian cancer with subsequent debulking laparotomy were included.

Interventions: Diagnostic laparoscopy with 100% CO_2 versus laparoscopy with addition of 4% oxygen.

Measurements and Main Results: In the control group, 9 (47%) PSM found in 19 port sites were excised. In the CO_2+oxygen group, a similar incidence was found, that is, 8 (50%) PSM in 16 port sites. The incidence of PSM was higher in small women ($p < .018$) and in high-grade malignancies. The pathophysiology of PSM is unknown, but besides direct wound contamination, aerosolization of tumor cells and gas leaks have been suggested together with a causal relationship with the CO_2 pneumoperitoneum. Tumor cell hypoxia probably is not an important mechanism because PSM were not prevented by adding small amounts of oxygen to the CO_2 pneumoperitoneum.

Conclusion: The hypothesis of tumor cell hypoxia by the CO_2 pneumoperitoneum as a mechanism for PSM could not be confirmed. Journal of Minimally Invasive Gynecology (2008) 15, 700–703 © 2008 AAGL. All rights reserved.

Keywords: Port site metastases; Laparoscopy; Oxygen

Port site metastases (PSM) are a frequent complication of laparoscopy for malignant conditions [1], especially in advanced ovarian cancer, with an incidence of 17% PSM [2]. The risk of clinical PSM was independent of the type of tumor, tumor grade, and presence of ascites. Different mechanisms have been suggested, such as direct wound contamination during extraction of the malignant specimen through the laparoscopic port, aerosolization of exfoliated tumor cells, gas leaks known as the ''chimney effect,'' and a lowered immune response because of the carbon dioxide [3–22]. Nevertheless, concern remains that laparoscopic surgery, and more specifically CO_2 pneumoperitoneum, might be a predisposing factor for PSM.

Over the years, CO_2 pneumoperitoneum has been identified in a laparoscopic mouse model as a cofactor in adhesion formation through mesothelial hypoxia, an effect that could be prevented by adding 4% oxygen to the CO_2 pneumoperitoneum, thus restoring a physiological partial O_2 tension of approximately 30 mm Hg. This hypoxic effect is pressure and duration dependent and is associated with retraction and bulging of mesothelial cells, thus directly exposing the extracellular matrix. A similar effect was observed for the number of tumor cells implanted after intraperitoneal injection of syngeneic dispersed renal adenocarcinoma or colon-carcinoma cells. This effect could also be prevented by

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adding small amounts of oxygen to the CO_2 pneumoperitoneum [23–25] and Bastidas (unpublished data).

Hypoxia is known to activate the hypoxia induced factor (HIF) cascade leading to enhanced expression of angiogenic factors as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). Also, several other processes such as PGC_1-α [26] are activated. The overall effect can be enhanced cell growth instead of inhibition. Also during CO_2 pneumoperitoneum, this HIF-angiogenesis cascade is activated, as was demonstrated by the absence of peritoneal adhesion in HIF knockout mice, and after treatment with monoclonal antibodies against VEGF and against HIF [27]. We therefore wanted to investigate whether in the human, the addition of 4% oxygen to the CO_2 pneumoperitoneum could reduce the incidence of PSM, by preventing the tumor cell hypoxia caused by CO_2 pneumoperitoneum.

Materials and Methods

Patients

All patients presenting with an ovarian mass suspected to be malignant were included (n = 35). Of these 22 fulfilled the criteria of malignancy with the necessity of a second laparotomy; 13 women were excluded because 2 had an endometrial cancer, 2 did not have a malignancy, and in 9 no primary or interval debulking was performed.

Trial

An exploratory prospective randomized controlled trial was initiated with either pure CO_2 or 96% CO_2 + 4% oxygen, with the Thermoflator Plus for insufflation (Karl Storz, Tuttlingen, Germany). Randomization was done with unmarked and sealed envelopes in block randomization to be opened at the time of the laparoscopy. Pressure was standardized at exactly 15 mm Hg. At subsequent debulking, the laparoscopic ports were excised and examined by the pathologist for PSM. PSM was defined as the presence of at least 1 microscopic cluster of tumor cells observed in a single microscopic slide made from the port site. Body mass index, type of tumor, stage according to the classification of the International Federation of Gynecology and Obstetrics, and duration of laparoscopy were used for subgroup analysis.

Statistics

This trial was not powered to detect minor decreases in PSM. Indeed considering the 17% incidence of PSM, 192 women would be needed to detect a 50% reduction. Therefore an interim analysis after 1 year was performed to evaluate continuation. Analysis was done with the SAS system (SAS Institute Inc., Cary, NC), with the use of a Spearman r correlation.

Results

As expected, both groups were comparable for demographic data as length, weight, body mass index, duration of surgery, type of tumor, and pathologic condition (Table 1). No major complications such as bowel perforations, bowel fistula, major bleedings, or infections were observed.

Table 1
Comparison of the two groups

Parameter	Oxygen-group (n = 11)	Control (n = 11)
Mean height (cm) (±SD, 95% CI)	164 (±6.5,151–166)	167 (±7.5, 152–182)
Mean weight (kg) (±SD, 95% CI)	72 (±19.0, 35–74)	67 (±13.7, 40–94)
BMI (kg/m^2) (±SD, 95% CI)	26.7 (±6.5, 14–29)	24.0 (±4.3, 16–32)
Median duration of laparoscopy (range)	65 min (50–90)	72 min (45–135)
Median L-D interval (range)	12 (6–22)	18 (3–34)
Disease		
Serous papillary carcinoma	8	10
Carcinosarcoma	0	1
Adenocarcinoma	1	0
Other	2	0
Organ with carcinoma		
Ovary	10	9
Tube	0	2
Colon	1	0
FIGO Stage		
1-2	0	2
3-4	11	9
Primary debulking	10	11

BMI = body mass index; FIGO = International Federation of Gynecology and Obstetrics; L-D interval = median time in days between the laparoscopy and the subsequent debulking.

The interval between diagnostic laparoscopy and initiation of therapy (primairy debulking or neoadjuvant chemotherapy) was 18 days for the control group (n = 11) and 12 days for the study group (n = 11). In the control group 19 port sites were excised, and 9 (47%) showed microscopic PSM. In the oxygen group 16 port sites were excised, and 8 (50%) had PSM. In both groups 5 women had PSM (45%). PSM were not higher in the 12-mm umbilical port than in the 5 mm ports. PSM were not higher in ports through which specimens or biopsy specimens had been extracted.

PSM correlated with the tumor grade ($p < .05$) and negatively with height ($p < .018$), but not with body mass index. Also after correcting for these variables, oxygen addition did not have any effect on the incidence of PSM.

Discussion

The overall incidence of PSM in this trial, that is, 47%, is much higher than reported previously. This might be related to the study design, which was prospective in contrast with previous retrospective studies, probably with a risk of underreporting. As predictive factors, we found a correlation with tumor grade, something not observed before. Although this seems not to be surprising, it should, however, be interpreted with caution, given the risk of spurious correlations in a small series. Factors such as type and stage of tumor, duration of

laparoscopy, and primary debulking therapy or neoadjuvant chemotherapy were not predictive for PSM. Stratification for randomization obviously is imposible because these factors will only be known after laparoscopy.

The addition of 4% oxygen to the CO_2 pneumoperitoneum unfortunately did not dramatically affect the incidence of PSM. Given the strong beneficial effects of adding 4% oxygen observed in mouse models, we wanted to test the hypothesis of a reduced incidence of PSM through a direct effect on the tumor cells exposed to the pneumoperitoneum, or by an indirect effect through effects on peritoneal fluid such as an increase of angeogenic factors, as was demonstrated for adhesion formation. We fully realize that the trial was not powered to detect minor differences, but given the absence of any effect and the high prohibitive number of patients necessary to detect even differences of 50%, we did not consider continuation of the trial indicated. The pathophysiology of PSM is poorly understood. With this trial we may at least conclude that the CO_2 used for the pneumoperitoneum is not causally related, given the absence of effect of adding 4% oxygen. This trial also did not confirm the hypotheis of direct soiling from ports through which tumors had been extracted. Aerosolization and gas leaks, known as ''the chimney effect,'' also could not be supported, because we did not observe a higher incidence of PSM in thin women (correlation with body mass index not significant) in whom the risk of gas leaks is probably higher. Also, the negative correlation with height could be related to gas leaks, although spurious correlations cannot be ruled out given the borderline significance and the small series. It needs to be emphasized that in this trial insufflation pressure was strictly standardized, whereas in retrospective studies the insufflation pressure might have been increased in obese women.

Debate remains regarding whether macroscopic PSM affect survival given the controversial data reporting no effect and decreased survival rates [28]. Whether microscopic PSM affect survival is unknown, and this series obviously is too small to answer the question. A fortiori, the effect of adding oxygen to the pneumoperitoneum on patient survival in advanced-stage ovarian cancer cannot be excluded today. In BALB/c mice, we observed a significant decrease in number and size of metastasis after injection of 2 types of syngeneic tumors, an effect that we interpreted as a consequence of the decreased trauma on the mesothelial cell lining. Because, with the exception of the effect of the peritoneum, a direct effect on tumor cells cannot be excluded, the addition of oxygen still might affect tumor cell implantation and growth in the abdominal cavity and patient survival. In this study we did not look at this aspect because this is very subjective and would require far more inclusions. In conclusion, this is a negative trial that nevertheless seems useful, given the strong effect of adding 4% oxygen to the CO_2 pneumoperitoneum on tumor implantation observed in an experimental mouse model and on adhesion formation.

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