Surg Endosc (2002) 16: 1170–1174
DOI: 10.1007/s00464-001-9226-z

SURGICAL ENDOSCOPY
and Other Interventional Techniques

Impact of pneumoperitoneum on tumor growth

Results of an experiment on two ovarian carcinoma models

F. Lécuru,[1,2] A. Agostini,[1] S. Camatte,[1] F. Robin,[1] M. Aggerbeck,[2] J. P. Jaïs,[3] F. Vilde,[4] R. Taurelle[1]

[1] Department of Gynecologic Surgery, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France
[2] INSERM U-490, Université René Descartes, 45 rue de Saint Pères 75270 Paris Cedex, France
[3] Departement de Biostatistiques, Hôpital Necker, rue de Sèvrès, 75015 Paris, France
[4] Service d' Anatomie et de Cytologie Pathologiques, Hôpital Boucicaut, 78 rue de la Convention, 75015 Paris, France

Received: 2 April 2000/Accepted in final form: 6 December 2001/Online publication: 9 April 2002

Abstract
Aim of the study: To compare intraperitoneal tumor growth after CO_2 laparoscopy (L), gasless laparoscopy (GL), midline laparotomy (ML), and general anesthesia (GA) as a control.
Materials and methods: A prospective randomized trial was carried out in nude rats. A carcinomatosis was obtained by intraperitoneal injection of either one of the two human ovarian cancer cell lines IGR-OV1 or NIH:OVCAR-3. Rats secondly underwent randomly different kind of procedures: CO_2 L (8 mmHg, 60 min), GL (traction by a balloon for 60 min), ML (bowel removed and let on a mesh for 60 min), or GA. The rats were finally killed 10 or 35 days after surgery (respectively in IGR-OV1, or NIH:OVCAR-3 models). Tumor growth was assessed by the weight of the omental metastasis and MIB1 immunostaining. Peritoneal dissemination as well as abdominal wall metastases were assessed by pathological examination. Statistical analysis used the chi-square test (or Fisher exact test) and Bonferroni method for multiple comparison between groups.
Results: Fifteen rats were included in each group. Mean omental weight was significantly increased after surgery (3.1 to 5.6 g), when compared to control (2.4 g), but no significant difference was recorded between the three surgical accesses. MIB1 immunostaining was poor in the PNP group (37%), whereas it was higher after midline laparotomy (51%), but the difference was not significant ($p = 0.07$). Similarly, no significant variation was recorded in the NIH:OVCAR-3 model for omental weight or MIB1 staining. CO_2 pneumoperitoneum significantly increased right diaphragmatic dome involvement in the NIH:OVCAR-3 model. Abdominal wall metastases were significantly more frequent after surgery when compared to the control group, but no significant difference could be demonstrated between surgical groups in each model.
Conclusion: In these solid tumor models, CO_2 pneumoperitoneum had no deleterious effect on tumor growth when compared to gasless laparoscopy or midline laparotomy.

Key words: Laparoscopy — Tumor growth — Peritoneal carcinomatosis — Gasless laparoscopy — Randomized trial

A strong debate has arisen since laparoscopy was introduced in oncologic surgery. Many case reports have been published, presenting evidence of potential adverse effects of CO_2 pneumoperitoneum (PNP) in cancerous patients [5]. It is now admitted that an inappropriate laparoscopic treatment of early ovarian cancers can provoke port-site metastases and can worsen the stage of the disease [14]. Numerous animal experiments have also demonstrated that CO_2 PNP enhanced cell seeding and increased the occurrence of port-site metastases [16, 19].

Effects of surgery on tumor growth is also an old debate [18]. It has been demonstrated that surgery could enhance tumor growth and metastasis development [4]. Laparoscopy could be less deleterious, since it gives rise to a lower surgical trauma when compared to laparotomy. Conversely, laparoscopy creates a particular peritoneal ambience which could have deleterious effects on tumor growth [1, 20]. Some experiments have addressed the problem of implantation of tumors after different surgical approaches. Conversely, influence of laparoscopy on preexisting tumor growth is more obscure.

The aim of this study was to compare tumor growth after different surgical approaches. We designed a pro-

Correspondence to: F. Lécuru

spective randomized controlled study, in rats. We have developed two ovarian carcinoma models in rats (xenografts), which have radically different evolutions [12]. The IGR-OV1 model is characterized by a short survival after IP (mean survival < 20 days), giving finally a voluminous solid omental metastasis, with numerous little peritoneal nodules. We have demonstrated that the weight of the omental cake was proportional to the time frame following IP [12]. On the other hand, the NIH:OVCAR-3 model progresses slowly (mean survival after IP:60 days). The final presentation gives numerous peritoneal nodules, with abundant ascites containing numerous viable neoplastic cells. It seemed interesting to compare effects of laparoscopy with two different models.

Materials and methods

Our models were developed with two human ovarian carcinoma cell lines injected in nude rats (rnu/rnu) (Charles Rivers Deutschland, Sulzfdeld, Germany). IGR-OV1 and NIH:OVCAR-3 cells have been previously described and were obtained from IDRS (Institut de Recherche Servier, Suresnes, France) [2, 10]. Lines were maintained in RPMI 1640 medium with 10% fetal calf serum (virus and mycoplasm screened) and 5% glutamine (INSERM U 490, Pr Ph Beaune, Paris, France). Cells were harvested before passage 12 and counted after viability assessment by trypan blue exclusion. Then, 27×10^6 IGR-OV1 cells or 36×10^6 NIH:OVCAR-3 cells were injected intraperitoneally (IP) in 28-days-old female nude rats. Natural progression of those two models has been previously fully described [12].

Rats were operated on day 7 after the IP in the IGR-OV1 model and on day 14 in the NIH:OVCAR-3 model. Animals were given a number at their admission to the laboratory (conserved in a sealed envelope). Allocation to surgical groups was performed by the zootechnician who randomly chose envelopes. Surgical steps were as follows. CO_2 laparoscopy (L): A standard CO_2 pneumoperitoneum was created via a 2.1 mm Veress needle inserted through the umbilicus (Veress needle and insufflator: Olympus, SCOP, Antony, France). The pneumoperitoneum was maintained at 8 mm Hg for 60 min. Skin was sutured using resorbable suture (VICRYL 000, Ethicon, Neuilly sur Seine, France). Gasless laparoscopy (GL): The balloon of a No. 18 Foley catheter was used to stretch the anterior abdominal wall to a tension in proportion with the animal's weight. The procedure lasted for 1 h. The abdominal wall was then sutured in two layers with a resorbable suture (VICRYL 000, Ethicon, Neuilly sur Seine, France). Laparotomy (ML): Bowels were removed out of the abdomen through a xyphopubic midline laparotomy and left on a mesh for 1 h. The abdominal wall was sutured in two layers (one running suture for peritoneum and muscles and one running suture for the skin) (VICRYL 000, Ethicon, Neuilly sur Seine, France). Control (GA): Rats were submitted to a general anesthesia for 1 h (subcutaneous injection of ketamine (160 mg/kg) and chlorepromazine (2 mg/kg)). All surgical interventions were carried out in aseptic conditions. The time interval between IP and surgery had been determined in a previous experiment. It allowed us to perform the surgical step when a microscopic peritoneal carcinomatosis was already present [12].

Postoperatively, animals were kept under specific pathogen-free conditions in accord with European standards. A standard laboratory diet (A-04, rats–mice, UAR, Epinay sur Orge, France) and autoclaved water were available ad libitum. Rats were systematically sacrificed 10 days after surgery in the IGR-OV1 model and after 35 days in the NIH:OVCAR-3 model. The anterior abdominal wall was resected by the zootechnician. Then, necropsy was carried out by the surgeon, who was blind to the kind of surgery previously performed. The abdominal cavity was first inspected. Then, the omentum (or the omental cake) was resected and weighted. We particularly looked for neoplastic nodules on gross examination in six different sites: pelvis, right and left colic gutters, right and left diaphragmatic domes, and intestinal mesentry. The six sites were then resected for pathological examination. Specimens were fixed in 10% formalin. Serial sections were studied on light microscopy (hematoxylin, eosin, and safran (HES). The pathologist was blind to the kind of surgery performed.

Immunostaining was performed on formation fixed paraffin embedded tissue sections using Ki67 Antigen Iopath+, clone MIB1 (Immunotech SA, Marseille, France). The 4 μm thick sections underwent deparaffinization in xylene and rehydration in decreasing grades of ethanol (100% to 70%). Endogenous peroxidase activity was blocked by a 10 min treatment with 3% hydrogen peroxide. Sections were then incubated with mouse monoclonal antibodies for 30 min at room temperature. We used microwaving of tissue sections for unmasking Ki67. The immunostaining was achieved with diaminobenzidine as chromogen. Slides were counterstained with haematoxylin. MIB1 staining was then assessed in 200 cells picked up in an area that was determined by the pathologist to be representative.

We compared the weight of the omental cake, as well as the number of sites histologically involved within the peritoneal cavity, the rate of abdominal wall metastases, and the frequency of MIB1 marked cells in the four experimental groups. We used a chi-squared test (or Fisher exact test when appropriate) for nominal variables; p-value adjustments by the Bonferroni method in a step-down fashion were used for multiple comparisons between groups (procedure MULTTEST — SAS Institute Inc). A p value of 0.05 was considered significant. The protocol was approved by the Committee on Animal Research of Faculté de Médecine Necker Enfants Malades and the Veterinary Inspection of the French Agriculture Ministry.

Results

Animals who failed to respond to tumoral seeding or that deceased after surgery were excluded from the analysis. Final populations were as follows: IGR-OV1: GA = 14, L = 15, GL = 15, ML = 13; NIH:OVCAR-3: GA = 13, L = 14, GL = 13, ML = 12. Animal characteristics were similar between groups at the surgical step (Table 1). The mean omental weight at the time of sacrifice is given in Table 2. In the IGR-OV1 model, mean weight of omental cakes ranged from 2.4 g (control) to 5.6 g (CO_2 L). Omentum of the control group were significantly lighter when compared to those obtained from gasless laparoscopy, midline laparotomy, and CO_2 pneumoperitoneum ($p < 0.05$, $p < 0.005$, and $p < 0.0001$, respectively). However, there was no significant difference between gasless laparoscopy, midline laparotomy, and CO_2 pneumoperitoneum.

In the NIH:OVCAR-3 model, mean omental weights did not significantly differ between groups ($p = 0.7$). Omentums were heavier after GL than in control animals, but the difference was not significant.

For MIB 1 immunostaining, specimen issued from the PNP group stained poorly (37%), whereas those arising from animals operated on by laparotomy expressed the highest staining potency (51%), but the difference was not significant ($p = 0.07$) (Table 3). In the NIH:OVCAR-3 model, the poorest staining potency was observed in the gasless group, whereas the highest potency was observed in the control group; however, the overall comparison showed no significant difference ($p = 0.6$) (Table 3). The spread of the tumor within the peritoneal cavity is given in Table 4. In the NIH:OVCAR-3 model, the right diaphragmatic dome was significantly more frequently involved by tumoral locations after CO_2 PNP. No other preferential metastatic site has been recorded in this model. In the IGR-OV1 model, no significant variation was recorded between groups ($p = 0.4$). Abdominal wall metastasis rates are given in

Table 1. Mean rat weight at surgery

	GA	L	GL	ML	*p*
IGR-OV1	110.1 (22.0)	120.1 (8.2)	116.4 (15.2)	118.5 (22.1)	0.9
NIH:OVCAR-3	118.6 (14.4)	122.7 (21.3)	124.0 (21.7)	126.4 (19.5)	0.7

Weights in grams are given in mean (standard deviation)
GA, control; L, CO_2 laparoscopy; GL, gasless laparoscopy; ML, midline laparotomy

Table 2. Mean omental weight at sacrifice

	GA	L	GL	ML	*p*
IGR-OV1	2.4 (1.3)	5.6 (2.4)	4.2 (2.1)	3.1 (1.2)	<0.05
NIH:OVCAR-3	13.4 (7.5)	15.3 (12.1)	20.2 (10.1)	13.5 (9.3)	0.7

Weights in grams are given in mean (standard deviation)
GA, control; L, CO_2 laparoscopy; GL, gasless laparoscopy; ML, midline laparotomy

Table 3. MIB1 immunostaining (percentage of marked cells/200 counted cells)

	GA	L	GL	ML	*p*
IGR-OV1	41	37	42	51	0.07
NIH:OVCAR-3	56	49	46	52	0.6

GA, control; L, CO_2 laparoscopy; GL, gasless laparoscopy; ML, midline laparotomy

Table 5. No metastasis has been observed in the control group, and surgery significantly increased wound involvement for all surgical groups ($p < 0.05$).

Discussion

The impact of laparoscopy on tumor growth or dissemination is now a major subject of concern, since laparoscopy is increasingly used for the staging and the treatment of cancers. Some aspects have been largely evaluated by experimental studies, such as port-site metastasis or cell dissemination after IP inoculation during a pneumoperitoneum. On the other hand, impact of laparoscopy on preexisting tumor growth is less well evaluated.

We have used two ovarian carcinoma models which have radically different evolutions, because we felt that differences could be observed because of their different biological characteristics.

In the IGR-OV1 model, intraperitoneal tumor weight was increased after surgery, but was similar among gasless laparoscopy, laparotomy, and CO_2 pneumoperitoneum. This result is in contrast with most published papers, which reported that laparotomy was significantly more deleterious than CO_2 laparoscopy. It has been reported that laparotomy had the worst influence on final tumor volume or weight, and that air pneumoperitoneum, CO_2 pneumoperitoneum, and helium pneumoperitoneum had decreasing impacts [3, 9, 11]. In fact, most of these experiments have been conducted with immunocompetent models and demonstrated the major detrimental effect of laparotomy on postoperative immunity [4]. The use of spontaneous T immunodeficient models tones down the interference of surgery on postoperative immunity and thus on tumor growth [8]. Our experiment has focused on the direct effect of the surgical ambience on cell proliferation. It demonstrates that CO_2 pneumoperitoneum, despite the creation of a very particular ambience with a decreased temperature, microcirculation alterations, and peritoneal acidosis [23], had no impact on tumor growth. Results of MIB1 immunostaining were similar with those of tumor weight. There was no significant difference between groups, giving evidence that PNP had no impact on this parameter. In fact, specimens for MIB1 staining were processed several days after surgery, and this procedure gives information on cell proliferation on the day of sacrifice. Postoperative local immunity as well as peritoneal response to trauma probably plays an important role in neoplastic cell settling and growth [7, 17, 24, 25].

Surprisingly, the results of gasless laparoscopy were not better than those of CO_2 pneumoperitoneum. We used a model of GL that is quite close to reality (balloon with a strong traction). This system was probably more aggressive than those used in previous experiments and may have deleterious effects on the peritoneum. For MIB1 staining, gasless laparoscopy was also in an intermediate position between CO_2 laparoscopy and laparotomy, suggesting that level of local trauma could linked to the length of the peritoneal incision [22]. However, our results question the enthusiastic reports that indicated that gasless laparoscopy was oncologically safer than CO_2 laparoscopy [16].

The NIH:OVCAR-3 model showed no significant difference in the final omentum weight as well as in MIB1 immunostaining. Despite different biologic patterns of each line, surgical results were similar. Finally, we used human ovarian cancer xenografts which could have different biologic properties from those of colon adenocarcinoma or melanoma cell lines which have been predominantly used in previous studies [22, 25].

Other experiments addressed the question of subcutaneous tumor growth. They reported a more detrimental effect of laparotomy when compared to

Table 4. Dissemination of the tumor within the peritoneal cavity for the IGR-OV1 and NIH:OVCAR-3 models

	16R-OV1 model				
	GA	L	GL	ML	*p*
R diaphragmatic dom	100%	100%	83%	83%	0.4
L diaphragmatic dom	85%	100%	67%	100%	0.2
R paracolic gutter	100%	100%	67%	83%	0.1
L paracolic gutter	100%	100%	67%	83%	0.1
Pelvis	85%	100%	83%	67%	0.4
Intestinal mesentery	100%	100%	83%	85%	0.5
NIH:OVCAR-3 model					
R diaphragmatic dom	100%	92%	89%	50%	0.04
L diaphragmatic dom	85%	92%	89%	67%	0.5
R paracolic gutter	71%	92%	89%	50%	0.1
L paracolic gutter	71%	92%	89%	67%	0.4
Pelvis	67%	91%	89%	67%	0.4
Intestinal mesentery	100%	91%	89%	100%	0.7

GA, control; L, CO_2 laparoscopy; GL, gasless laparoscopy; ML, midline laparotomy; R, right; L, left

Table 5. Wound metastasis rate

	GA	L	GL	ML	*p*
IGR-OV1	0%	75%	50%	50%	< 0.05
NIH:OVCAR-3	0%	81.8%	60%	100	< 0.01

GA, control; L, CO_2 laparoscopy; GL, gasless laparoscopy; ML, midline laparotomy

laparoscopy [1]. Tumors were heavier and proliferating cell nuclear antigen assay showed significantly higher indexes [13]. But, in such models, tumors were not directly exposed to the surgical ambience and could have been influenced by metabolic consequences of pneumoperitoneum. All those experiments were carried out on small animals breathing spontaneously during surgery, so metabolic changes interacting with tumor growth could have occurred.

Finally, intraperitoneal gas could play an important role. It has been demonstrated *in vitro* that helium or NO_2 had a lower impact on cell proliferation when compared to CO_2 [20], but *in vivo* studies have not confirmed this fact [6].

Tumor spread within the peritoneal cavity was similar for all surgical approaches, except right diaphragmatic dome in the NIH:OVCAR-3 model. Previous papers had reported an increased diffusion of the disease when IP inoculation was performed simultaneously with the PNP [16]. In fact, these experiments investigated early events after intraoperative rupture of a malignant cyst and studied impact of CO_2 PNP on cell adhesion and cell diffusion. Conversely, in our trial, carcinomatosis was already present at the time of surgery and was not dramatically modified by the surgical approach. Similar results have been observed after cecal tumor resection. Laparoscopy enhanced carcinomatosis in animals without serosal involvement (S-) at the time of surgery, but was without significant effect in S+ rats [15]. This brings into serious question treatment of stage I ovarian cancers or suspicious ovarian cysts by laparoscopy, because tumor spillage could alter the stage of the disease. It has been demonstrated that inappropriate laparoscopic treatment of stage I ovarian cancers with a delayed subsequent staging laparotomy significantly worsened the final stage of the disease [17]. On the other hand, assessment of advanced ovarian tumors or second look by laparoscopy appear less hazardous because disease is already disseminated in the peritoneum.

The increased involvement of the right diaphragmatic dome after CO_2 pneumoperitoneum in the NIH:OVCAR-3 model can be explained by the natural history of the model. Peritoneal dissemination of this gradually developing tumor could be boosted by PNP.

Our results on port-site metastases call for some comment. We observed that tumor seeding was frequently present after any kind of surgery. The difference was not statistically significant between the three surgical groups. However, there was only one laparoscopic port by animal in the L and GL groups. Our work was a simulation of what occurs at the laparoscope site, but cannot provide conclusions for operative trocars. Surprisingly, GL had worse results than expected [16]. Here again, a more accurate simulation of GL in our work than in previous trials could have promoted cell anchorage and proliferation. We would like to emphasize here that recent papers, which were not dedicated to port-site metastasis assessement, recorded no difference between laparoscopy and gasless laparoscopy [21].

Conclusion

In this experiment, pneumoperitoneum had no effect on tumor growth. The ambience created by laparoscopy with CO_2 does not seem to be more deleterious than laparotomy. The real benefit of gasless laparoscopy should be further assessed.

Acknowledgments. This work was supported by grants from "Ligue Nationale Centre le Cancer," Région Ile de France

References

1. Allendorf J, Bessler M, Horvath K, Marvin M, Laird D, Whelan R (1998) Increased tumor establishment and growth after open

vs laparoscopic bowel resection in mice. Surg Endosc 12: 1035–1038
2. Benard J, Da Silva J, De Blois MC, Boyer P, Duvillard P, Chiric E, Riou G (1985) Characterization of a human ovarian adenocarcinoma line IGROV1 in tissue culture and in nude mice. Cancer Res 45: 4970–4979
3. Bouvy N, Marquet R, Jeekel J, Bonjer H (1997) Laparoscopic surgery is associated with less tumor growth stimulation than conventional surgery: an experimental study. Br J Surg 84: 358–361
4. Da Costa ML, Redmond HP, Bouchier-Hayes D (1998) The effect of laparotomy and laparoscopy on the establishment of spontaneous tumor metastases. Surgery 124: 516–525
5. Dobronte Z, Wittmann T, Karacsony G (1978) Rapid development of malignant metastases in the abdominal wall after laparoscopy. Endoscopy 10: 127–130
6. Dorrance HR, Oien K, O'Dwyer PJ (1999) Effects of laparoscopy on intraperitoneal tumor growth and distant metastases in an animal model. Surgery 126: 35–40
7. Evrard S, Falkenrodt A, Park A, Tassetti V, Mutter D, Marescaux J (1997) Influence of CO_2 pneumoperitoneum on systemic and peritoneal cell-mediated immunity. World J Surg 21: 353–356
8. Festing M (1981) Athymic nude rats. In: Gershwin ME, Merchant B (eds) Immunological Defects in Laboratory Animals, vol 1, pp 267–283. Plenum Press, New York
9. Gutt CN, Riemer V, Kim ZG, Jacobi CA, Paolucci V, Lorenz M (1999) Impact of laparoscopic colonic resection on tumor growth and spread in an experimental model. Br J Surg 86: 1180–1184
10. Hamilton TC, Young RC, McKoy WM, Grozinger KR, Green JA, Chu EW, Whang-Peng J, Rogan AM, Green WR, Ozols RF (1983) Characterization of a human ovarian carcinoma cell line (NIH:OVCAR-3) with androgen and estrogen receptors. Cancer Res 43: 5379–5389
11. Jacobi C, Ordemann J, Böhm B, Zieren H, Volk H, Muller J (1997) The influence of laparotomy and laparoscopy on tumor growth in a rat model. Surg Endosc 11: 618–621
12. Lécuru F, Guilbaud N, Agostini A, Augereau C, Vildé F, Taurelle R (2001) Description of two new Human ovarian carcinoma models, in nude rats, suitable for laparoscopic experimentation. Surg Endosc 15: 1346–1352
13. Lee SW, Southall JC, Allendorf JD, Bessler M, Whelan RL (1999) Tumor proliferative index is higher in mice undergoing laparotomy vs. CO_2 pneumoperitoneum. Dis Colon Rectum 42: 477–481
14. Lehner M, Wenzl R, Heinzl H, Husslein P, Sevelda D (1998) Influence of delayed staging laparotomy after laparoscopic removal of ovarian masses later found malignant. Obstet Gynecol 92: 967–971
15. LeMoine MC, Navarro F, Burgel JS, Pellegrin A, Khiari A, Pourquier D, Fabre JM, Domergue J (1998) Experimental assessment of the risk of tumor recurrence after laparoscopic surgery. Surgery 123: 427–431
16. Mathew G, Watson D, Rofe A, Baigrie C, Ellis T, Jamieson G (1996) Wound metastases following laparoscopic and open surgery for abdominal cancer in a rat model. Br J Surg 83: 1087–1090
17. Mathew G, Watson D, Ellis T, Jamieson G, Rofe A (1999). The role of peritoneal immunity and the tumor-bearing state on the development of wound and peritoneal metastases after laparoscopy. Aust NZ J Surg 69: 14–18
18. Murthy S, Goldschmidt R, Rao L, Ammirati M, Buchmann T, Scanlon E (1989) The influence of surgical trauma on experimental metastasis. Cancer 64: 2035–2044
19. Neuhaus S, Watson D, Ellis T, Rowland R, Rofe A, Pike G, Mathew G, Jamieson G (1998) Wound metastasis after laparoscopy with different insufflation gases. Surgery 123: 579–583
20. Neuhaus S, Ellis T, Barrett M, Rofe A, Jamieson G, Watson D (1999) In vitro inhibition of tumor growth in a helium-rich environment: implications for laparoscopic surgery. Aust NZ J Surg 52–55
21. Paik P, Misawa T, Chiang M, Towson J, Im S, Ortega A, Beart Jr R (1998) Abdominal incision tumor implantation following pneumoperitoneum laparoscopic procedure vs. Standard open incision in a syngeneic rat model. Dis Colon Rectum 41: 419–422
22. Southall JC, Lee SW, Allendorf JD, Bessler M, Whelan RL (1998) Colon adenocarcinoma and B16 melanoma grow larger following laparotomy vs. pneumoperitoneum in a murine model. Dis Colon Rectum 41: 564–569
23. Taskin O, Buhur A, Birincioglu M, Burak F, Atmaca R, Yilmaz I, Wheeler JM (1998) The effects of duration of CO_2 insufflation and irrigation on peritoneal microcirculation assessed by free radical scavengers and total glutathion levels during operative laparoscopy. J Am Assoc Gynecol Laparosc 5: 129–133
24. Volz J, Köster S, Schaeff B, Paolucci V (1998) Laparoscopic surgery: the effects of insufflation gas on tumor induced lethality in nude mice. Am J Obstet Gynecol 178: 793–795
25. Volz J, Koster S, Spacek Z, Paweletz N (1999) The influence of pneumoperitoneum used in laparoscopic surgery on an intraabdominal tumor growth. Cancer 86: 770–774