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# Evaluating the Effect of Micropump© Position, Internal Pressure and Doxorubicin Dosage on Efficacy of Pressurized Intra-peritoneal Aerosol Chemotherapy (PIPAC) in an *Ex Vivo* Model

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**Abstract.** *Background/Aim: Pressurized intraperitoneal aerosol chemotherapy (PIPAC) is a novel clinical approach to the treatment of peritoneal carcinomatosis. A well-established, not anatomic ex vivo PIPAC model was used to investigate the influence of changes in internal pressure, distance of the Micropump© (MIP) to the distributing surface and the drug concentration on the penetration depth of doxorubicin in the target tissue. Materials and Methods: Doxorubicin was aerosolized in an ex vivo PIPAC model using a hermetic container system mimicking the abdominal cavity. Fresh post-mortem swine peritoneum was cut into proportional samples. Tissue specimens were spatially placed at 4 different spots within the box: $P_1$, on the distributing surface of the box, directly opposite to MIP; $P_2$, on the side wall of the box; $P_3$, on the ceiling of the box; $P_4$, on the distributing surface with a partial cover. Impact of changes in the following parameters were analyzed and compared with clinically established values (CEVs) at our center: pressure (CEV=12 mmHg), distance of the MIP from the distributing surface (CEV=8 cm) and doxorubicin concentration (CEV=3 mg/50 ml). In-tissue doxorubicin penetration depth was measured using fluorescence microscopy on frozen thin sections. Results: Tissue positioning in the box had a significant impact on drug penetration after PIPAC with CEV. Under CEV conditions, the highest drug penetration depth was observed in the tissue placed on the distributing surface directly opposite to the MIP ($P_1$: 351 μm, $P_2$: 77 μm, $P_3$: 66 μm, $P_4$: 34 μm). A closer positioning of the MIP lead to a significantly higher mean depth penetration of doxorubicin in the P1 in contrast to other samples in which a reduced drug penetration was observed (1 cm vs. 8 cm distance from MIP to the distributing surface, $P_1$ at 1 cm: 469 μm vs. $P_1$ at 8 cm: 351 μm, $p<0.0001$; $P_2$ at 1 cm: 25 μm vs. $P_2$ at 8 cm: 77 μm, $p<0.0001$; $P_3$ at 1 cm: 21 μm vs. $P_3$ at 8 cm: 66 μm, $p<0.001$; $P_4$ at 1 cm: 13 μm vs. $P_4$ at 8 cm: 39 μm, $p=0.021$). Higher doxorubicin concentrations led to a highly significant increase of drug penetration in P1 (1 cm vs. 8 cm, $p<0.0001$), but only a little significant increase in other samples. An increase of internal pressure did not show a significant increase in penetration depth of doxorubicin. Conclusion: Our ex vivo data suggest that a higher pressure does not increase the penetration deepness of doxorubicin. Higher drug dosage and a closer positioning of the MIP toward the target lead to a higher penetration of doxorubicin within the samples. A more homogeneous penetration within all targets cannot be achieved by changing drug concentration, position of the nozzle or pressure increase.*

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Key Words: Local drug distribution, pressurized intra-peritoneal aerosol chemotherapy (PIPAC), peritoneal carcinomatosis, doxorubicin.

Peritoneal carcinomatosis (PC) is a common manifestation of several tumor diseases with a poor prognosis (1, 2). Pressurized intra-peritoneal aerosol chemotherapy (PIPAC) has been recently reported as a new approach to PC. Now, after more

than 1,000 PIPAC procedures at our center with promising results (unpublished data), we intent to improve the treatment technique and procedures. The role of changes in the clinically established values (CEVs) in this therapy regime is unknown. These CEVs have been established based on the experiences and empiric procedures derived from intraperitoneal chemotherapies (IP). Clinically established values are: capnoperitoneum at 12 mmHg (3), the Micropump© (MIP) at a distance of 8 cm to the peritoneal surface of the small intestine and the drug-containing solution (in case of doxorubicin, 3 mg/50 ml). This study was performed to evaluate the effect of changes of these parameters and to see if they have to be optimized to increase the local drug penetration and improve the local outcome. Pressure is assumed to play a key role in the efficacy of PIPAC. Theoretically, a higher pressure might increase the overall penetration of the cytotoxic agent into tumor cells (4-6) through generation of an artificial pressure gradient, which counterbalances the intra-tumoral interstitial fluid pressure, an obstacle in systemic therapy of cancer (7-8). Nevertheless, data on the practical application and factors affecting the drug penetration during PIPAC for patients with PC are rare. A previous study (9) showed an unequal special drug distribution of PIPAC in the *ex vivo* model. In this work, we are reporting a well-established, not anatomic *ex vivo* PIPAC model used to investigate the influence of internal pressure, distance of the MIP to the target tissue and the drug concentration on the penetration depth of doxorubicin in the target tissues.

## Materials and Methods

*Micropump© (MIP).* The MIP (Reger Medizintechnik, Rottweil, Germany) consists of a high pressure injector, a high pressure connecting line, a connecting port at the shaft of the nozzle and a nozzle head with an opening of 200 μm. Using this device, the drug is delivered with a pressure of up to eight bars. Doxorubicin was filled in a sterile plastic syringe and applied at the injector head of the high pressure injector (Injektron 82 M; MedTron, Saarbrücken, Germany) and, then, connected to the connecting port of the nozzle *via* a high pressure line (High Pressure Injection Line with Male/Female Luer lock 120 cm, 1,200 psi; Smith Medical, Hranice, Czech Republic).

*Ex vivo PIPAC model.* The experiments were performed in an *ex vivo* model on commercially available tissue samples. No approval of the local board on animal care was required. The statement of the animal safety representative of the Ruhr-University Bochum on our application for approval of experiments was that experiments with *post-mortem* pigs are excluded from the ''Protection of Animal Act'' (TierSchG). Fresh *post-mortem* swine peritoneum was cut into equi-proportional samples (3×3×0.5 cm). A similar *ex vivo* PIPAC model has been previously described (10-11). Briefly, a commercially hermetic sealable plastic box with a total volume of 3.5 l, mimicking the abdominal cavity, was used. In the center of the top cover of the plastic box, 10- and 5-mm trocars (Kii®Balloon Blunt Tip System; Applied Medical, Rancho Santa Margarita, CA, USA) were placed. The nozzle of the MIP and a temperature/humidity sensor probe (XA 1000; Lufft Mess- und Regeltechnik GmbH, Fellbach, Germany) were inserted and placed into the trocars. The plastic box was situated in a water bath (Typ 3043; Köttermann, Häningsen, Germany) and kept at constant temperature of 36˚C during the whole procedure (Figure 1).

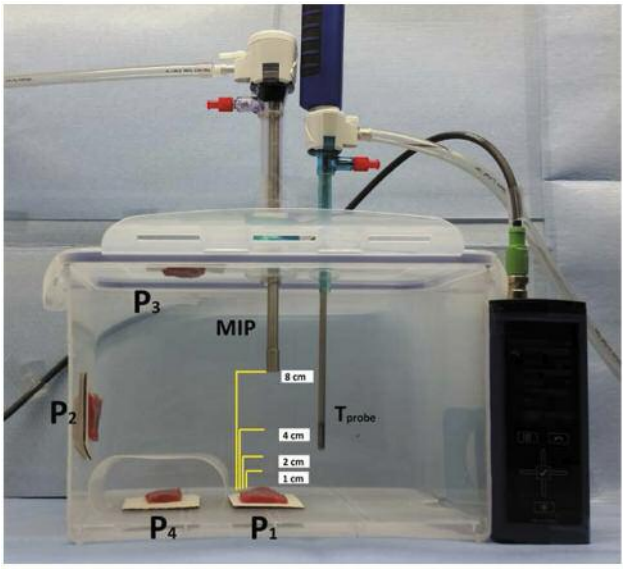


Figure 1. *Laparoscopy-like ex vivo experiment with fresh swine peritoneum to investigate the spatial distribution pattern of aerosolized doxorubicin during pressurized intraperitoneal aerosol chemotherapy. $T_{probe}$: Temperature and humidity probe. MIP: Micropump. Positions of the nozzle to the bottom at A (1 cm), B (2 cm), C (4 cm), D (8 cm). Peritoneum of the swine at different positions. $P_1$: Spray jet. $P_2$: Wall. $P_3$: Top. $P_4$: Bottom covered.*

*Probe positioning ($P_1$-$P_4$).* The tissue specimens of peritoneum (from German land race pigs), each measuring 3.0×3.0×0.5 cm, were placed at 4 different positions of the plastic box. ($P_1$) on the bottom in direct extension of the axis of the micropump nozzle in the core of the aerosol jet, ($P_2$) on the bottom at the margin of the aerosol jet with a bilaterally open plastic cover to mimic anatomic barriers in the abdomen, ($P_3$) on the side wall and ($P_4$) on the inner side of the top of the cover (Figure 1). The plastic box was then tightly sealed and a constant $CO_2$ capnoperitoneum of 12 mm Hg (Olympus UHI-3; Olympus Australia, Notting Hill, Australia) was established throughout the whole PIPAC procedure. Doxorubicin (doxorubicin hydrochloride, purchased from Teva® Pharmachemie B.V., Haarlem, The Netherlands), 3.5 mg in 50 ml NaCl 0.9% at room temperature (23˚C), was aerosolized with a flow rate of 30 ml/min. After the aerosol phase, the tissue specimens were exposed for another 30 minutes to aerosolized doxorubicin (exposure phase). The CEV for PIPAC at our center are as follows: pressure=12 mmHg, distance of the MIP from the distributing surface=8cm and doxorubicin concentration=3 mg/50 ml.

*MIP distance to the distributing surface.* In the first experiment, the MIP was positioned at 1 cm, 2 cm, 4 cm and 8 cm of the sample right in front of it (the distributing surface). The other two CEVs were kept constant.

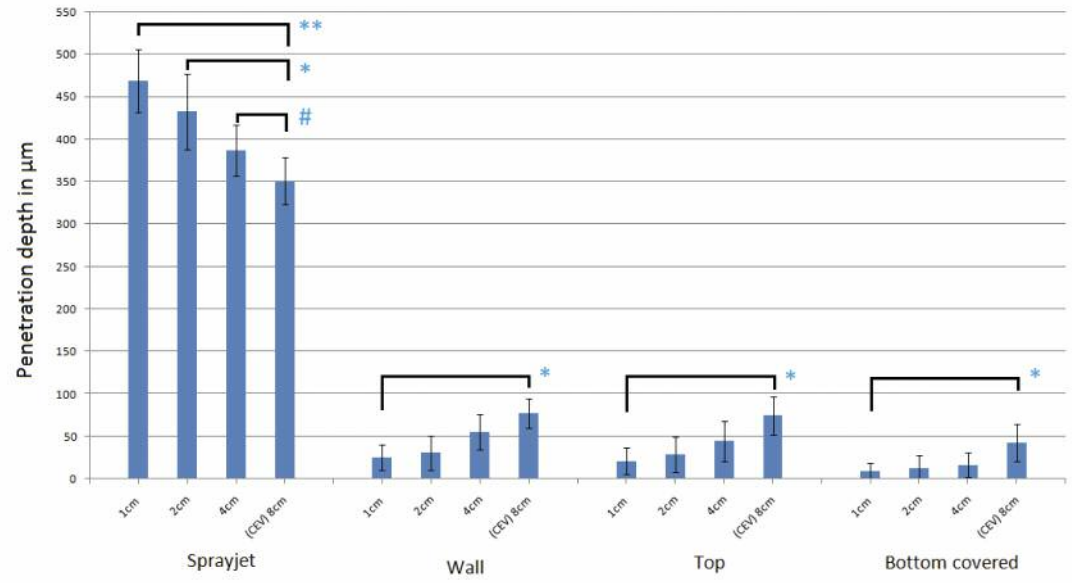


Figure 2. *Doxorubicin penetration at different positioning of the nozzle of the MIP to the bottom. 1cm, 2 cm, 4 cm 8 cm at different targets. CEV, clinically established values; #p>0.05, *p<0.01, **p<0.001.*

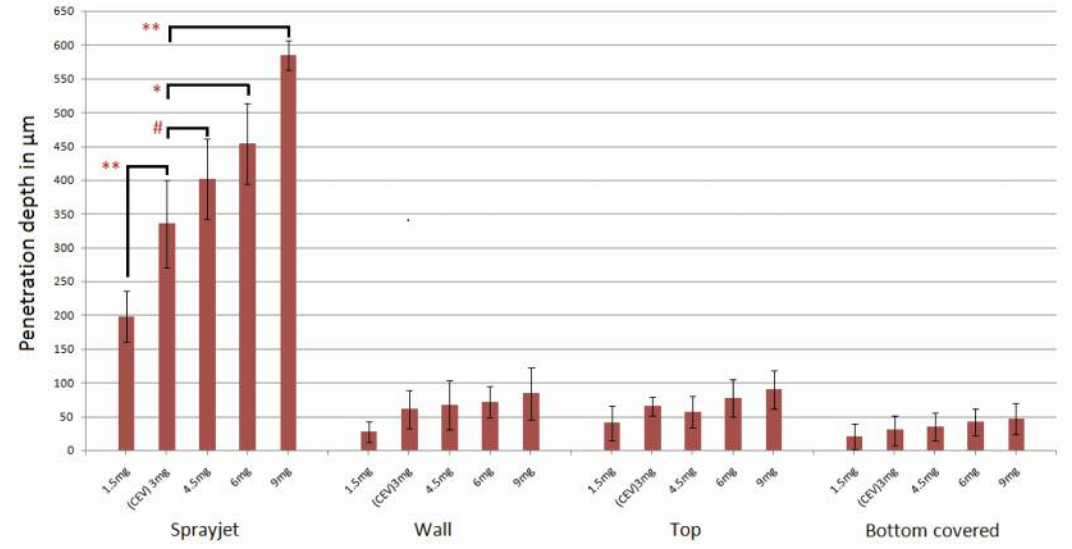


Figure 3. *Doxorubicin penetration with higher doxorubicin concentrations (1.5 mg, 3 mg, 4.5 mg, 6 mg, 9 mg) at different targets. CEV, clinically established values; #p>0.05 *p<0.05 **p<0.0001.*

*Different doxorubicin concentrations*. In the second experiment, the impact of changes in the different doxorubicin concentrations (1.5 mg/50 ml, 3 mg/50 ml, 4.5 mg/50 ml, 6 mg/50 ml and 9 mg/50 ml) were evaluated, whereas the other two CEVs were kept constant (pressure=12 mmHg, distance of MIP=8 cm).

*Changes in the pressure*. In the third experiment, the $CO_2$ capnoperitoneum was varied using pressures of 0 mmHg, 12 mmHg and 20 mmHg. The other two CEVs were kept constant (concentration=3 mg/50 ml, distance of MIP=8 cm).

Impact of changes in the parameters were analyzed and compared with the CEVs.

*Detection of doxorubicin penetration using fluorescence microscopy.* All tissue samples were rinsed with sterile NaCl 0.9% solution in order to eliminate superficial, no bound cytostatics and immediately

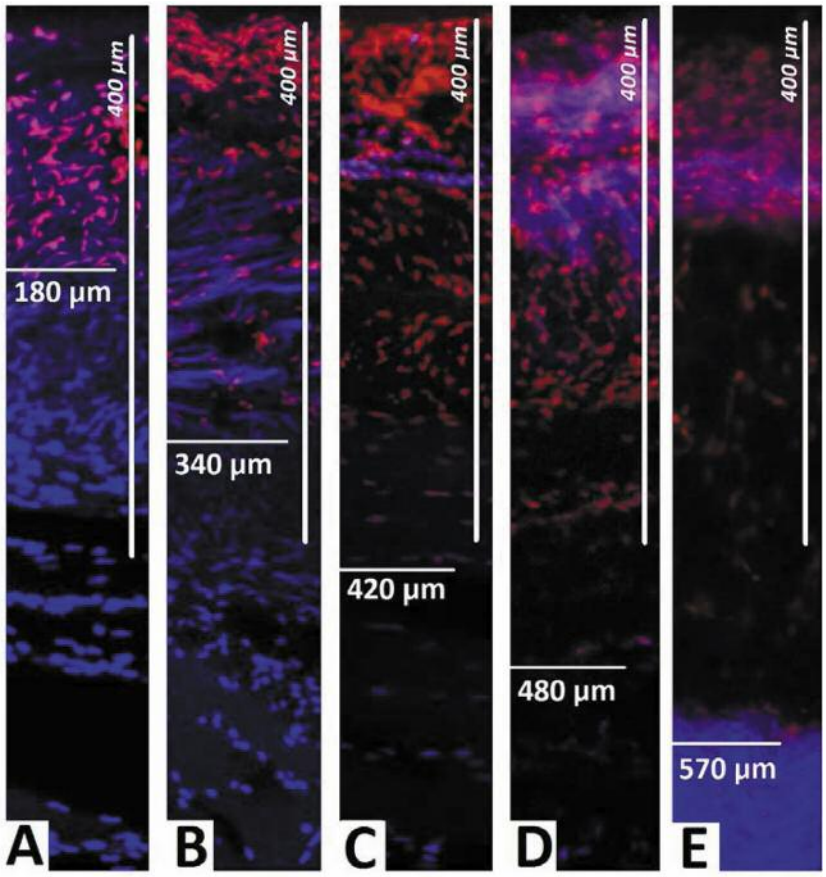


Figure 4. *Fluorescence microscopy of representative penetration depth of doxorubicin into fresh peritoneal tissue samples of German Land race pigs. Nuclei (blue) were stained with 4',6-diamidino-2-phenylindole (DAPI). Doxorubicin concentration (mg/50 ml). Left side to right: A=1.5 mg/50 ml, B=3 mg/50 ml, C= 4.5mg/50 ml, D=6 mg/50 ml, E=9 mg/50 ml.*

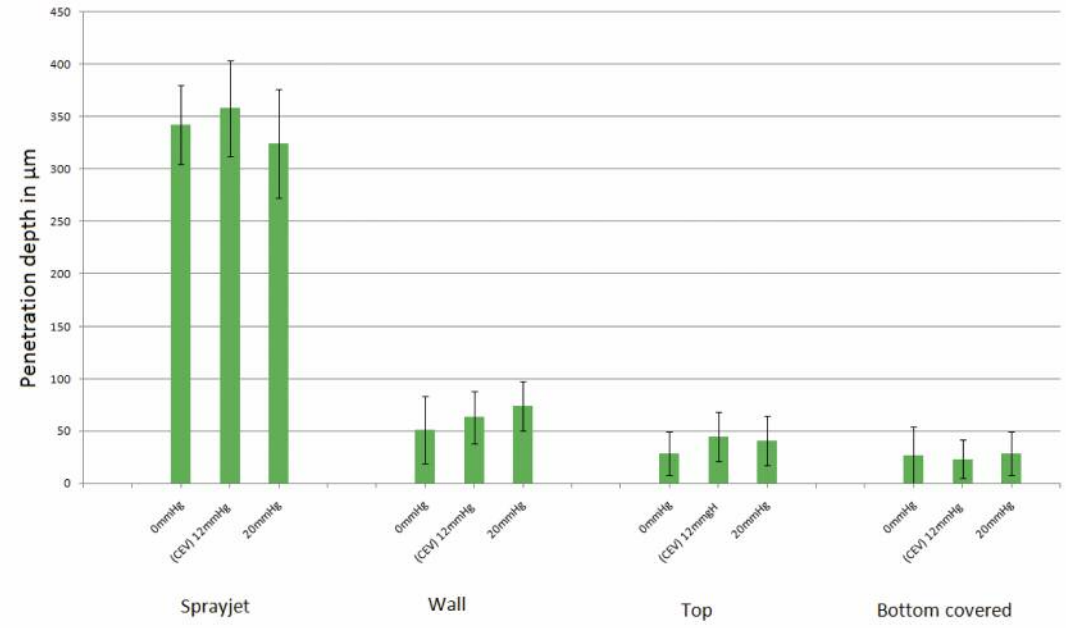


Figure 5. *Doxorubicin penetration at different targets at 0 mmHg, 12 mmHg and 20 mmHg pressure in the box. CEV, Clinically established values.*

frozen in liquid nitrogen. Cryosections (10 μm) were obtained and mounted with VectaShield containing 1.5 μg/ml 4',6-diamidino-2-phenylindole (DAPI) to stain nuclei. Penetration depth of doxorubicin was monitored using a Leica TCS SP8 (Leica Mikrosysteme GmbH, Wetzlar, Hessen, Germany) confocal laser scanning microscope. The distance between the luminal surface and the innermost positive staining for doxorubicin accumulation was measured and reported in micrometers.

*Statistical analyses*. Experiments were independently conducted three times for reproducibility. Tissue samples were subjected to doxorubicin penetration measurement. The statistical analyses were performed using Sigma Plot 12 (Systat Software Inc., San Jose, CA, USA). The Kruskal-Wallis one way analysis of variance on ranks was used to compare independent groups. A significant *p*-value was considered in case of $p<0.05$.

## Results

*Position of the MIP.* The mean depth of doxorubicin penetration was found to be significantly higher the closer the MIP was located towards the sample right in front of it at $P_1$, 1 cm: 469±36 μm (*vs*. CEV (8 cm), $p<0.0001$), 2 cm: 433±43 μm (*vs*. CEV (8 cm), $p<0.001$), 4 cm: 386±30 μm (*vs*. CEV (8 cm), $p=0.02$), 8 cm corresponds to the (CEV). This is in contrast to all other samples $P_{2-4}$ that have lower penetration rates as the MIP is brought closer to the sample at $P_1$ (Figure 2).

*Doxorubicin concentration*. The doxorubicin penetration increased in all probes with increasing higher doxorubicin concentrations. The highest increase of the penetration was documented in sample $P_1$. Other peripheral samples ($P_{2-4}$) showed only a slight increase. Tissue penetration for $P_1$ with doxorubicin concentration of 1.5 mg/50 ml, 3 mg/50 ml (CEV), 4.5 mg/50 ml, 6 mg/50 ml and 9 mg/50 ml was 198±38 μm, 336±34 μm, 401±39 μm, 454±60 μm and 585±22 μm, respectively (Figures 3 and 4).

*Effect of pressure*. An increase of pressure did not show a relevant increase of drug penetration into the tissue in all samples (Figure 5).

## Discussion

In spite of significant progress in systemic treatment of PC, outcomes of a considerable part of patients remain poor. Insufficient drug distribution in the tumor is one of the limitations of systemic therapy. (13). Thus, the novel PIPAC approach may be a promising new treatment for PC for the next decade. It offers hope to patients who had, in the past, not escaped from a terminal illness. PIPAC therapy has been introduced as a new approach to improve the treatment of advanced, multiresistant PC. Previous data obtained in animal experiments reveal a homogenous spatial methylene blue distribution pattern in the abdominal cavity after PIPAC-like procedures (14-16). Some authors have already reported that increasing the intraperitoneal pressure particularly enhanced the uptake of drugs into the tumors resulting in a higher local disposition (7, 17). In contrast, our study indicates that an increase of internal pressure does not affect drug penetration. However, optimizing of PIPAC applications remains necessary as recent findings demonstrated controversial results regarding the distribution patterns of the MIP and penetration patterns in the surrounding tissues (8). Changing the drug concentration or positioning of the MIP has revealed a strong impact on the sample in the spray jet ($P_1$) but, as observed, no significant effect on other samples. This effect might be used for the treatment of single tumor nodules on the peritoneum that could be directly targeted with the MIP. Our results demonstrate that PIPAC might need an optimization to ensure a homogeneous distribution of the drug inside the peritoneal cavity. There are several hypothetical and practical ways to improve the results of the treatments. Theoretically, a more homogenous drug distribution might be achieved by rotating the MIP during the injection phase. Furthermore, the MIP could be placed at the most possible outlying position to the tumor-bearing tissues for the application in combination with rotation of the MIP. This will ensure a wider and more equal distribution and sufficient penetration of the applied doxorubicin into difficult areas of access in the peritoneal cavity. The application device could be optimized as well. For example, adding several (rotating) heads and nozzles to the MIP might enable a more homogeneous distribution of the cytotoxic agent. These pre-clinical, as well as clinical aspects of PIPAC therapy, are currently under intense research at our center. However, the results of our study should be interpreted with caution as our experiments were performed in a *post-mortem* model. Although peritoneum is not a shock organ like heart, brain or liver, its response to PIPAC may differ in a living organism with regular blood circulation. In addition, one should take into consideration that cellular death after doxorubicin therapy is a sort of ''dirty death'' with release of toxic metabolites into the surrounding tissue. Therefore, higher local doxorubicin dosage or closer distance to peritoneum or bowel and higher tissue uptake might lead to higher local toxicity, such as perforation, ileus or local tissue necrosis. Presumably, with a higher tissue uptake of doxorubicin a higher systemic uptake of the drug might occur, which can lead to the incidence of known side-effects of systemic application of doxorubicin (18, 19). The total applied dose during PIPAC is approximately 10% of a usual systemic chemotherapy (13). Thus, even in the case of a 200% increase in doxorubicin dosage for PIPAC and full uptake of the drug in the circulatory system (*i.e*. 20% of a usual systemic chemotherapy dosage), life-threatening

adverse events are not expected. However, there is are clinical data reporting on the feasibility or efficacy of higher drug dosage or different positioning of the MIP for PIPAC currently. Any dose escalation for PIPAC should be performed in Phase I clinical studies. The toxicity of PIPAC should not be underestimated at any level.

## Conclusion

Our *ex vivo* data suggest that a higher pressure does not increase the penetration deepness of doxorubicin. Higher drug dosage and a closer positioning of the MIP toward the target lead to a higher penetration of doxorubicin within the samples. A more homogeneous penetration within all targets at the same time cannot be achieved by changing drug concentration, position of the nozzle or pressure increase. Essential changes in the application technique of PIPAC might be necessary to optimize the drug distribution and/or penetration depth and the resultant clinical outcomes of the patients. Further investigations are warranted to clarify the role of different parameters for better treatment results.

## Disclosure

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