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Intraoperative electrochemotherapy of colorectal liver metastases: A prospective phase II study

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ABSTRACT

Background and objectives: A previous pilot study proved the feasibility, safety and efficacy of electrochemotherapy in the treatment of colorectal liver metastases. The aim of this study was to evaluate long-term effectiveness and safety of electrochemotherapy in the treatment of unresectable colorectal liver metastases.

Patients and methods: In this prospective phase II study, patients with metachronous colorectal liver metastases were included. In all patients, at least one metastasis was unresectable due to its central location or a too-small future remnant liver volume. Patients were treated by electrochemotherapy using intravenously administered bleomycin during open surgery. Treated were 84 metastases in 39 patients. Local tumor control, progression-free survival and overall survival were evaluated.

Results: The objective response was 75% (63% CR, 12% PR). The median duration of the response was 20.8 months for metastases in CR and 9.8 months for metastases in PR. The therapy was significantly more effective for metastases smaller than 3 cm in diameter than for larger ones. There was no difference in response according to the metastatic location, *i.e.,* metastases in central *vs.* peripheral locations. Progression-free survival was better in patients who responded well to electrochemotherapy compared to those metastases that had a partial response or progressive disease. However, there was no difference in overall survival, with a median of 29.0 months.

Conclusions: Electrochemotherapy has proven to be safe and effective in the treatment of colorectal liver metastases, with a durable response. It provides local tumor control that enables patients with unresectable metastases to receive further treatments.

1. Introduction

Colorectal liver metastases are diagnosed in approximately 25%–30% of patients after or during the diagnosis of primary tumors [1,2]. The best management of resectable metastases is surgery [3]. Radiofrequency ablation, microwave ablation and stereotactic body radiotherapy (SBRT) are alternative local treatments for unresectable metastases [4]. Recently, electroporation-based approaches, irreversible electroporation and electrochemotherapy, have been

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introduced into the treatment of liver tumors, including colorectal liver metastases [5–8].

Electrochemotherapy was previously shown to be a feasible and safe treatment for unresectable colorectal liver metastases as well as an effective treatment for unresectable hepatocellular carcinoma not suitable for other local ablative methods [6,9,10]. These two pilot studies provided evidence that electrochemotherapy is a treatment option for other ablative techniques. Electrochemotherapy is specifically suitable for the treatment of colorectal liver metastases located in the vicinity of the major hepatic vessels that are not resectable and not suitable for radiofrequency ablation or microwave ablation due to the heat sink effect [6,9]. The safety of electrochemotherapy in the treatment of metastases located near large liver vessels was also proven in a normal porcine liver model, and no side effects due to electroporation or electrochemotherapy of the vessels were observed [11].

After the first pilot study on 16 patients [6,9], additional patients were recruited in this phase II study. The aim of the study was to evaluate the long-term safety and effectiveness of electrochemotherapy in the treatment of unresectable colorectal liver metastases. Here, we report on 39 patients with the same inclusion and exclusion criteria as in the first pilot study [6,9]. This study confirms the previous data but with longer patient follow-up and reports on the probability of local tumor control and progression-free survival. All the data presented demonstrate the feasibility, safety and effectiveness of electrochemotherapy in the treatment of colorectal liver metastases during open surgery. With further development of the technology, a percutaneous approach in the treatment of liver metastases with electrochemotherapy will become more competitive with other ablative techniques.

2. Patients and methods

2.1. Study design

The study was a prospective, phase II study conducted at the Institute of Oncology Ljubljana, University Medical Centre Ljubljana and University Medical Centre Maribor, Slovenia. Regulatory approval from the Institutional Board, as well as from the National Medical Ethics Committee (#45/09/08 and #108/10/12), was obtained. The study was registered at ClinicalTrials.gov with numbers NCT01264952 and NCT02352259. Informed consent was obtained from all patients included in the trial. Patients were presented at multidisciplinary team meetings consisting of a surgeon, radiologist, medical oncologist and radiation oncologist. Electrochemotherapy was performed according to the standard operating procedures for the treatment of cutaneous tumors and the associated modifications for the treatment of liver tumors [5,12]. The main objectives of this study were to determine the effectiveness and safety of electrochemotherapy in the treatment of colorectal liver metastases with the primary outcome to determine the long-term local tumor control. As secondary outcome, toxicity was determined according to the Common Terminology Criteria for Adverse Events (CTCAE) ver. 5.0, and the response rate was measured according to the mRECIST criteria [13]. As a stopping rule, clear evidence of harmful side effects (as determined by CTCAE) and no evidence of treatment benefit, were set.

2.2. Patients

From May 2011 to November 2018, 39 patients with 84 lesions were enrolled in this trial based on the inclusion and exclusion criteria as previously described [13]. All included patients were AJCC stage IV, with metastatic disease limited to the liver only. Five patients had not receive any treatment prior to electrochemotherapy; all other patients were treated at least with systemic chemotherapy, most of them in combination with targeted therapy (bevacizumab or cetuximab), and some of them also received other local treatment modalities (Table 1). Included patients presented with at least one unresectable liver metastasis that either demanded excessive resection or was untreatable by standard thermal ablative therapies due to its close proximity to major blood vessels. Electrochemotherapy was offered to the patients as the only treatment option. Based on the relation of the metastases to the major blood vessels, they were assigned as “central” or “peripheral”. The term “central” was used for metastases located in the vicinity of surrounding major vessels, therefore not amenable for surgical resection or radiofrequency ablation. The term “peripheral” was used for metastases located away from the major vessels, which were treated in addition to central ones in the same electrochemotherapy session. The patient characteristics and location of the metastases are presented in Table 1.

2.3. Treatment procedure

All patients in the study were treated during open surgery. The electrodes used for electric pulse delivery were either long needle electrodes (variable geometry) or hexagonal electrodes with fixed geometry [14]. The choice of electrode used was dependent on the location of the lesion. Electrodes with variable geometry, *i.e.,* long needle electrodes, were used for deep-seated tumors located more than 3 cm below the surface of the liver. The hexagonal electrodes

Table 1
Patient, tumor and electrochemotherapy characteristics.

Characteristics	Patients	Percentage
Sex		
Male	28	72%
Female	11	28%
Age (years)		
Median	63.1	
Range	35–81	
Previous treatment		
None	5	12.8%
Chemotherapy only	3	7.7%
Chemotherapy + Targeted therapy (TT)	19	48.7%
Chemotherapy + TT + Surgery	4	10.2%
Chemotherapy + TT + Radiotherapy	5	12.8%
Chemotherapy + Radiotherapy	1	2.6%
Chemotherapy + TT + Surgery + RFA	1	2.6%
Chemotherapy + TT + Radiotherapy + RFA	1	2.6%
Performance status ECOG		
0–1	29	
2	8	
3	2	
Number of metastases treated		
Total	84	
Average per patient	2.1	
Range	1–7	
Tumor size		
Average	2.0 cm	
Range	0.3–6.0 cm	
Location		
Segment I	0	
Segment II	6	
Segment III	5	
Segment IV	22	
Segment V	11	
Segment VI	11	
Segment VII	10	
Segment VIII	19	
Type of electrodes used in electrochemotherapy (ECT)		
Fixed geometry	59	70.2%
Variable geometry	25	29.8%

RFA - radiofrequency ablation.

were used for more superficial tumors that had their deepest margins less than 3 cm from the liver surface (Table 1).

Intraoperative ultrasound was used to identify the lesions and aid in the positioning of the electrodes into and around the tumor. The long needle electrodes were positioned according to the pre-treatment plan prepared individually for each patient and his or her specific metastases using previously developed procedures [15–17]. Plans were developed based on computed tomography and/or magnetic resonance scans obtained less than 30 days prior to treatment. Target lesions were segmented. A gradient-based optimization algorithm was used to optimize the voltage between each electrode pair to ensure full coverage of the tumor above the reversible electroporation threshold (400 V/cm) and minimize the volume of affected healthy liver parenchyma above the irreversible electroporation threshold (700 V/cm), while also keeping the predicted currents below 50 A, which is the hardware limit of the pulse generator [17,18].

An intravenous bolus of bleomycin (15,000 IU/m^2, Bleomycin medac, Medac, Hamburg, Germany) was given to the patient after intraoperative ultrasound confirmed the correct electrode placement. Eight minutes after bleomycin injection, electric pulses were delivered by Cliniporator®*VITAE* (IGEA SpA, Carpi, Italy). Trains of 8 electric pulses (each pulse 100 μs long) were delivered to each pair of electrodes consecutively (ranging between 6 and 13 pairs for electrodes with variable geometry and 12 pairs for those with hexagonal geometry). Treatment was performed in an optimal window for electrochemotherapy of 8–40 min after the intravenous injection of bleomycin as described in an updated SOP [12]. All pulses were synchronized with the absolute refractory period of the heart to prevent the electrical pulses from being delivered during the vulnerable ventricle period [5].

2.4. Safety assessment

Adverse events were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The ECG was monitored continuously during the surgical procedure.

2.5. Efficacy assessment based on radiology

Liver metastases treated in the study were assessed before and after electrochemotherapy by contrast-enhanced computed tomography (CECT) or with magnetic resonance imaging (MRI) using a specific hepatocyte contrast agent (gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid—Gd-EOB-DTPA, Primovist, Bayer, Berlin, Germany). The treatment response was evaluated by CECT or MRI using the mRECIST criteria [13]. The first evaluation occurred after a median of 34 days, and the second after a median of 123 days.

2.6. Statistical analysis

All data were entered into a Microsoft Access 2010 database, which was used for all calculations except for statistical analysis, which was performed with GraphPad Software (La Jolla, CA, USA). The log-rank (Mantel-Cox) test was performed on the Kaplan-Meier estimates. A chi-squared test was used for the statistical comparison of response according to tumor location. A two-tailed P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Demographics, feasibility and safety

The clinical features and treatment characteristics of the 39 patients with 84 metastases are presented in Table 1. In this prospective phase II study, patients with metachronous colorectal liver metastases were included. In all patients, at least one metastasis was unresectable due to the central location of the tumor or a too-small future remnant liver volume. If peripheral metastases were present in the same patient, they were also treated with electrochemotherapy during the same session. The majority of patients were previously treated with chemotherapy and/or targeted therapy. The patients were in good performance status, with the majority having an ECOG status of 0–1.

Electrochemotherapy was performed during open surgery using bleomycin administered intravenously and electrodes having fixed geometry (59 metastases) or variable geometry (25 metastases). The average number of metastases per patient treated with electrochemotherapy was 2.1, ranging from 1 to 7 metastases. The average diameter of the treated metastases was 2.0 (range 0.3–6.0 cm), 44.0% of which were located centrally and 56.0% peripherally (Table 2). The centrally located metastases were larger (average 2.3 cm in diameter) than those located peripherally (average 1.7 cm in diameter).

The treatment procedure was feasible and safe in all patients, demonstrating no immediate or delayed electrochemotherapy-related adverse events (Tables 1 and 2), although some patients had performance status 3. However, there were some general surgical complications (ileus, abscesses, ascites, arrhythmias, pleural effusion, and biliary leaks), which were not related to the electrochemotherapy itself.

3.2. Response to treatment

The response of the 84 electrochemotherapy-treated metastases according to the mRECIST criteria was 63% CR and 12% PR, *i.e.*, an objective response rate 75%. A relatively small percentage of the treated metastases did not respond to electrochemotherapy (2% SD, 23% PD). The median observation time of the patients was 330 days.

The response per patient was 44.0% CR, 15.0% PR, 2.5% SD and 38.5% PD. In the patients who had two or more metastases treated, a lower complete response rate per patient was observed (44.0%) due to the partial or lack of response of some metastases (Table 2).

The median duration of the response of the CR metastases was 20.8 months (7.4-ongoing) (Fig. 1A) and was significantly longer than that of the PR metastases ($p < 0.0001$). The PR metastases progressed much faster, with a median duration of the response of 9.8 months (7.9–19.3), compared to CR metastases.

The average diameter of the treated metastases was 2.0 cm (range 0.3–6 cm). Of these, 16.7% were larger and 83.3% were equal to or smaller than 3 cm in diameter. The response of the smaller metastases (up to 3 cm in diameter) was significantly better ($p = 0.035$) compared to the larger metastases (larger than 3 cm). The larger metastases were treated either with variable geometry (9 metastases) or fixed geometry electrodes (5 metastases). The complete response rate did not differ whether electrodes with fixed (33.3% CR) or variable geometry (40.0% CR) were used ($p > 0.99$).

The number of metastases was evenly distributed between the central (44%) and peripheral locations (56%). The response between the locations was not significantly different; specifically, the CR was 62.2% *vs.* 63.8%, respectively (Table 2). Furthermore, the local metastasis control rate did not differ between the two groups ($p = 0.22$) (Fig. 1C).

The response of tumors to electrochemotherapy was also

Table 2
Toxicity and treatment outcomes.

Characteristics	Pts./Events/Percentage	
Toxicity (CTCAE grade)		
Electrochemotherapy (ECT)-related	0	
Non ECT-related within 24 h	8 (20.5%)	
Non ECT-related after 24 h	9 (23.0%)	
Duration of hospitalization		
7 days or less	13	
7–14 days	15	
More than 14 days	11	
Response to ECT/tumor (mRECIST v1.1)		
Number of metastases	84	
Complete Response (CR)	53 (63.0%)	
Partial Response (PR)	10 (12.0%)	
Stable Disease (SD)	2 (2.0%)	
Progressive Disease (PD)	19 (23.0%)	
Response to ECT/patient (mRECIST v1.1)		
Number of patients	39	
Complete Response (CR)	17 (44.0%)	
Partial Response (PR)	6 (15.0%)	
Stable Disease (SD)	1 (2.5%)	
Progressive Disease (PD)	15 (38.5%)	
Response according to tumor location	Central	Peripheral
Number of metastases	37 (44.0%)	47 (56.0%)
Complete Response (CR)	23 (62.2%)	30 (63.8%)
Partial Response (PR)	2 (5.4%)	8 (17.0%)
Stable Disease (SD)	1 (2.7%)	1 (2.1%)
Progressive Disease (PD)	11 (29.7%)	8 (17.0%)
Response according to the metastases size	≤3 cm diameter	>3 cm diameter
Number of metastases	70 (83.3%)	14 (16.7%)
Complete Response (CR)	48 (68.6%)	5 (35.7%)
Partial Response (PR)	8 (11.4%)	2 (14.3%)
Stable Disease (SD)	1 (1.4%)	1 (7.1%)
Progressive Disease (PD)	13 (18.6%)	6 (42.9%)

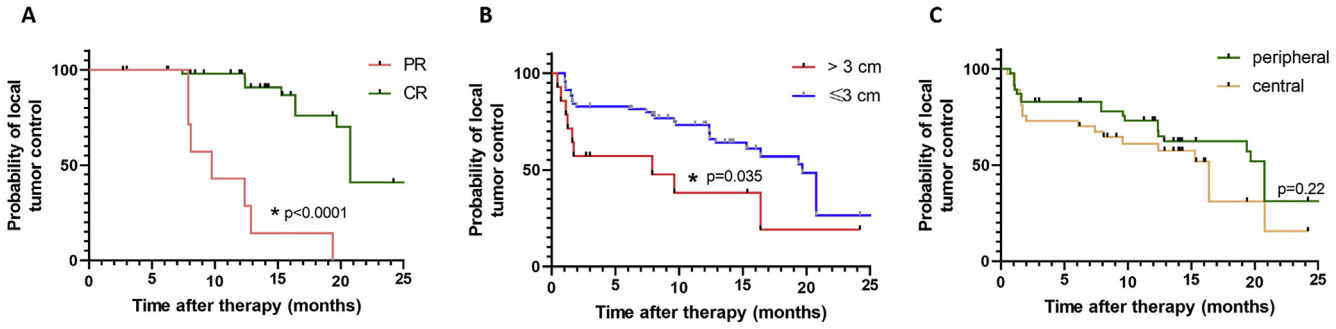

Fig. 1. (A) Local tumor (metastasis) control over time. The duration of metastasis response was calculated for patients with CR and PR, with censored patients marked. (B) Response of electrochemotherapy-treated metastases according to their size, either larger or smaller than 3 cm in diameter. (C) The response of electrochemotherapy-treated metastases according to their location; "central location" is considered to be in the vicinity of major blood vessels, and "peripheral location" is considered distal from major hepatic vessels.

reflected in the progression-free survival of the patients. Patients who had a good response to electrochemotherapy also had significantly slower progression of the diseases locally or systemically ($p = 0.0016$) than patients with PD (Fig. 2A).

Electrochemotherapy, regardless of being very or moderately effective, enabled the majority of patients to continue with systemic or local treatments. All the patients who had PD were heavily treated with systemic chemotherapy, including bevacizumab or cetuximab, and some also received other local treatments, such as surgery, stereotactic body radiation therapy (SBRT) or, in the case of one patient, additional electrochemotherapy for the liver metastases. Systemic treatment was added for patients with PR and SD as continuations of previously started systemic treatments. Patients with CR were given systemic treatment in the case of disease progression. In most cases, systemic chemotherapy in combination with cetuximab or bevacizumab was administered. A few patients did not receive any additional treatment (CR or PR). The overall survival of the patients did not differ between responders and nonresponders to electrochemotherapy ($p = 0.77$), with a median survival of 29.0 months.

4. Discussion

This study provides further evidence that electrochemotherapy has therapeutic potential for colorectal liver metastases that are unresectable due to the central location of the tumor or a too-small future remnant liver volume. Electrochemotherapy results in a durable response for treated metastases that were either located centrally or peripherally, enabling patients to be treated with other therapeutic options. The median overall survival of patients after electrochemotherapy was 29.0 months.

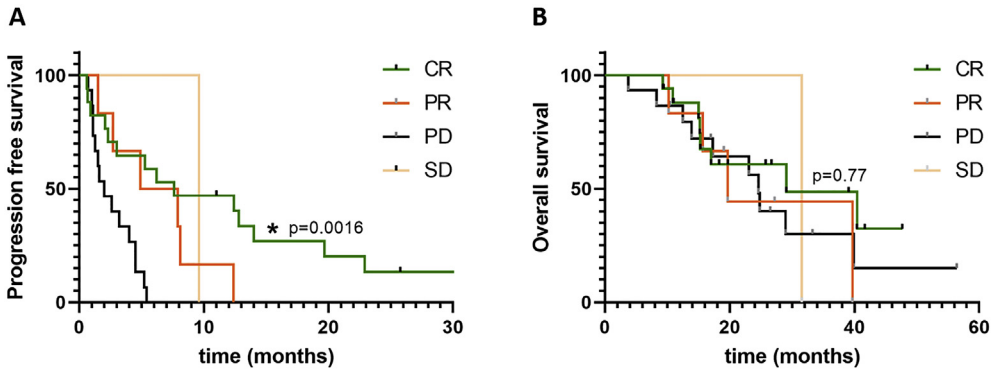

Fig. 2. (A) Progression-free survival and (B) overall survival of patients with colorectal liver metastasis treated with electrochemotherapy.

4.1. Safety aspects

The safety of electrochemotherapy has been demonstrated in several studies, including the two pilot studies on the treatment of colorectal liver metastases or hepatocellular carcinoma [6,9]. We confirmed the results of these studies, demonstrating no electrochemotherapy-related complications; however, there were some unrelated complications.

Forty-four percent of the metastases were found in central locations, those in the vicinity of the major hepatic vessels. These metastases were not resectable or were untreatable by standard thermal ablative methods; therefore, the patients were offered electrochemotherapy. No thromboses or other adverse events were recorded due to electrode puncture or delivery of electric pulses to those vessels. This safety aspect was also confirmed in a recent study on a porcine model, where electroporation or electrochemotherapy of major hepatic vessels was performed and no adverse events were recorded [11]. No side effects were recorded in the pilot study where hepatocellular carcinoma was treated by electrochemotherapy [6]. Similar findings are reported in irreversible electroporation studies, where the safety of the procedure was demonstrated in pig models and confirmed in clinical studies [19–21].

4.2. Effectiveness

The response of the treated metastases was lower than in the previous pilot study, where CR was 85% and PR 15%, although the patient population was similar and recruited with the same inclusion and exclusion criteria [9]. In the present study, we had a response rate of 75% with a CR rate of 63%. Per patient, the complete response rate was even lower (44%) because in some patients, not all treated metastases responded with CR. The reasons for the lower response rate are both technical and biological. The centrally located metastases that were not amenable for surgery or standard thermal ablative therapies were located in the proximity of the major hepatic vessels. These metastases responded equally well to electrochemotherapy as those metastases located peripherally, located away from the vessels. This indicates that electrochemotherapy is not prone to heat sink effect, as thermal ablative therapies are.

Technically, improvements could be made, predominantly by verification of the treated area. At the beginning of the study, we did not verify the treated area ultrasonographically, as we did in the later part of the study. Specifically, electroporation-induced changes, such as opacification and microbubbles, appeared in the treated area. These changes indicate adequate tumor coverage with the electric field, *i.e.,* whether the whole tumor mass was electroporated [22]. This enables verification of tumor coverage with the electric field that enables retreatment of areas that were not covered adequately. This is important specifically when electrodes with fixed geometry are used, and several applications of electroporation are needed to cover tumors larger than 2–3 cm in diameter. The use of longer electrodes with fixed geometry that enabled the treatment of deeper-seated tumors also improved the method by shortening the time of the procedure. In this study, there was an indication that electrodes with fixed geometry can provide equal effectiveness as electrodes with variable geometry in the control of tumors larger than 3 cm in diameter and that are centrally located. In addition, in our study, a significantly better response in tumors up to 3 cm in diameter was shown. This has already been documented in other studies using electrochemotherapy for the treatment of tumors and in studies using radiofrequency ablation and IRE [4,23]. Based on these findings, the introduction of percutaneous treatment using new electrodes with expandable tips is expected to open the possibility of a percutaneous approach in treating liver tumors with electrochemotherapy [14,24]. Some case reports have already described the feasibility of such an approach using long needle electrodes [25].

Biologically, two groups of tumors can be distinguished: those that had a CR and those that did not. The tumors with CR had a significantly longer time to progression, which could indicate a less aggressive biology in those tumors. This is assumed only if the treatment was performed without technical issues. Further biological characterization of these tumors is warranted. This result is similar to the delineation observed in tumors that originate in the left colon or rectum compared to those arising in the right [1].

Furthermore, those patients with CR and PR had less consecutive and less aggressive treatments than those who progressed shortly after electrochemotherapy. There was a trend toward better survival in complete responders, but this trend was not statistically significant. Comparison of the survival of these patients with that in published studies is difficult. To our knowledge, there is no comparable study with a similar cohort of patients. However, if we compare the survival of the patients in our study with the overall survival of the patients with nonresectable liver metastases where the median survival was 18 months, our group shows a much longer overall survival, with a median survival of 29 months, thus we believe that electrochemotherapy has an impact on survival of patients with unresectable liver metastases [3]. We have to emphasize that all those patients presented with unresectable metastases and numerous unsuccessful treatments. Therefore, electrochemotherapy reduced the tumor burden and enabled additional treatment options.

5. Conclusions

This study confirms the safety and provides further evidence on the effectiveness of electrochemotherapy in the treatment of colorectal liver metastases. It demonstrates a 75% response rate to electrochemotherapy for metastases of different sizes and locations treated either with variable or fixed geometry electrode arrays. Effective treatment provides long-term local tumor control as well as long progression-free survival. The patients had a median survival of 29 months. Electrochemotherapy, therefore, provides a treatment of choice for the reduction of unrespectable metastases or those not amenable to other ablative techniques, thus enabling further therapeutic options.

Declaration of competing interest

Damijan Miklavčič holds patents on electrochemotherapy that have been licensed to IGEA S. p.a (Carpi, Italy) and is also a consultant to various companies with an interest in electroporation-based technologies and treatments. The other authors have no competing interests.

CRediT authorship contribution statement

Ibrahim Edhemovic: Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Erik Brecelj:** Conceptualization, Investigation, Supervision, Writing - review & editing. **Maja Cemazar:** Conceptualization, Investigation, Supervision, Validation, Writing - original draft. **Nina Boc:** Data curation, Investigation, Methodology, Software, Writing - original draft. **Blaz Trotovsek:** Investigation, Methodology, Project administration, Writing - original draft. **Mihajlo Djokic:** Investigation, Methodology, Project administration, Writing - original draft. **Rok Dezman:** Investigation, Visualization, Validation. **Arpad Ivanecz:** Investigation, Validation, Writing - original draft. **Stojan Potrc:** Investigation, Validation, Writing - review & editing. **Masa Bosnjak:** Conceptualization, Data curation, Formal analysis, Writing - original draft. **Bostjan Markelc:** Formal analysis, Methodology. **Bor Kos:** Software, Validation, Writing - original draft. **Damijan Miklavcic:** Visualization, Writing - original draft. **Gorana Gasljevic:** Validation, Writing - original draft. **Gregor Sersa:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

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