Slovenian Medical Journal

Irreversible electroporation as a novel method for soft tissue ablation: review and challenges in clinical practice

Ireverzibilna elektroporacija kot metoda ablacije mehkih tkiv: pregled in izzivi pri uporabi v kliničnem okolju

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Abstract

Irreversible electroporation has been evaluated as a novel method for ablation of various soft tissues for the last fifteen years. This method presents an alternative to the established thermal ablation methods due to its predominantly non-thermal mechanism of cell kill. It is currently mostly used for treating patients in whom the application of thermal ablation is contraindicated due to risk of thermal damage to sensitive nearby structures, or when the presence of heat sinks reduces ablation efficacy. The main medical application of irreversible electroporation has until recently been ablation of deep seated tumours, e.g. in the liver, prostate and kidney, however, in the last few years its potential for the treatment of various arrhythmias has sparked great interest. Since irreversible electroporation is still a relatively new method, there is a lack of standardized treatment protocols and planning procedures for use in clinical setting. Numerical modelling has proven to be an indispensable tool in investigating and designing electroporation-based treatments and preparing patient-specific treatment plans. In this paper the most recent developments in clinical use of irreversible electroporation ablation are summarized and its major advantages as well as challenges and possible drawbacks in introducing this novel ablation method into clinical routine are highlighted.

Izvleček

Koncept ireverzibilne elektroporacije kot samostojne ablacijske metode so prvič predstavili pred petnajstimi leti. Ireverzibilna elektroporacija je alternativna metoda uveljavljenim termičnim ablacijskim metodam, saj mehanizem uničevanja celic ni odvisen od dviga temperature. Zaradi netermičnega načina delovanja se zaenkrat uporablja predvsem v primerih, pri katerih uporaba termične ablacije ni mogoča zaradi nevarnosti, da se poškodujejo bližnje občutljive anatomske strukture ali se učinkovitost ablacije zmanjša zaradi odvajanja toplote (t. i. *heat sink* učinek). Trenutno se ireverzibilna elektroporacija v medicini uporablja predvsem za odstranjevanje globlje ležečih tumorjev, na primer v jetrih, prostati in ledvicah. V zadnjih letih je veliko zanimanja vzbudila tudi uporaba metode v srcu, in sicer za zdravljenje različnih motenj srčnega ritma. Ker je ablacija z ireverzibilno elektroporacijo sorazmerno nova tehnologija, še vedno ni standardnih protokolov zdravljenja in postopkov za načrtovanje zdravljenja. Numerične metode so nepogrešljivo orodje pri preučevanju pojava elektroporacije in pri pripravi bolnikom prilagojenih načrtov zdravljenja. Prispevek pregledno prikazuje dosedanjo uporabo ireverzibilne elektroporacije v kliničnem okolju, povzema prednosti in osvetljuje glavne probleme pri uvajanju te obetavne ablacijske metode v klinično prakso.

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Key words:
irreversible electroporation; pulsed electric fields; ablation methods; minimally invasive treatments; treatment planning; numerical modelling

Ključne besede:
ireverzibilna elektroporacija; pulzirajoča električna polja; metode ablacije; minimalno invazivno zdravljenje; načrtovanje zdravljenja; numerično modeliranje

Received: 21. 2. 2020
Accepted: 20. 7. 2020

Cite as/Citirajte kot: Cindrič H, Kos B, Miklavčič D. Irreversible electroporation as a novel method for soft tissue ablation: review and challenges in clinical practice. Zdrav Vestn. 2021;90(1–2):38–53.

DOI: https://doi.org/10.6016/ZdravVestn.2954

1 Introduction

1.1 On electroporation

Electroporation (also called electropermeabilization) is a phenomenon by which we temporarily change the structure of the cell membrane with short high-voltage electric pulses. Under the influence of an electric field membrane lipids are redistributed, so-called hydrophilic pores appear and chemical reactions on lipids and transport proteins occur, which increases permeability and allows passage to various substances which otherwise couldn't pass the membrane or do so only with difficulty (1-4). With the appropriate choice of parameters of electric pulses, i.e. the number and duration of pulses, the dynamics of pulse delivery and the amplitude of the applied voltage, electroporation can be reversible or irreversible. In reversible electroporation, the cell membrane returns to its original state relatively quickly, so the long-term ability of the cell to divide and function is not impaired by the procedure. With irreversible electroporation, however, the cell loses functionality and dies (a process similar to apoptosis) due to exposure to the field (4-7).

Reversible and irreversible electroporation each offer possibilities in numerous fields – from medicine to biotechnology to food and wood processing (5,8,9). The most interest is elicited by its use in medicine (10). Reversible electroporation is interesting mainly from the point of view of the introduction of various molecules into cells, where one of the applications is electrochemotherapy, in which reversible electroporation of the tumor enables the transfer of some chemotherapeutics (bleomycin and cisplatin) into cancer cells, significantly increasing their cytotoxicity (11-14). In contrast to electrochemotherapy, in which we wish to preserve the cell membrane, we strive for direct destruction of cells with an electric field with irreversible electroporation. Irreversible electroporation (IRE) is used mainly for ablation of various tumors (5,6,15-19), and lately for cardiac ablation for treatment of arrhythmias (20-23).

1.2 Irreversible electroporation in medicine

The concept of irreversible electroporation as a standalone method of tissue ablation was first introduced by Davalos et al. in 2005 (7). Using a mathematical model they showed that irreversible electroporation allows for the destruction of target tissue without tissue heating and thus without thermal damage, in contrast to other established ablation methods. The non-thermal mechanism of cell destruction quickly aroused considerable interest in research groups, which was followed by a number of *in vitro* studies and *in vivo* studies on animal models. Later research showed that a thermal component is still present directly adjacent to electrodes, a consequence of high current density and

increased electrical conductivity of tissues due to electroporation (24-26). Despite this, thermal damage causes only a small fraction of cell deaths when the procedure is used properly, as the primary mechanism of this new method is still mostly non-thermal (26).

The first clinical studies on IRE ablation were published in 2010 – in the prostate (27) and the kidneys (29), which focused above all on the safety of the procedure. Numerous clinical trials followed in the following years, focused on ablating deep-seated tumor in various organs – the prostate (27,29-33), kidneys (28,34,35), liver (36-42), pancreas (43-46) and lungs (the latter so far unsuccessfully) (47,48). In recent years, in addition to its use in oncology, the use of IRE in the heart has been investigated, namely for the isolation of pulmonary veins in the treatment of atrial fibrillation (AF) (20-22,49) and for the ablation of Purkinje fibers in the treatment of ventricular fibrillation (23).

Ablation with irreversible electroporation has many advantages over established thermal methods, which enables its use in cases when thermal damage to the surrounding tissues is not acceptable, e.g. in the immediate vicinity of the bile duct, or when ablation with thermal ablation techniques cannot be reliably performed , for example due to the proximity of larger blood vessels (10,19,26,36,41,50-52). As the method is independent of temperature, it is not sensitive to the heat sink effect in proximity to blood vessels, which is a common problem with thermal ablation methods. An important property of irreversible electroporation is also that it destabilizes only the membranes of living cells, while the remaining structures and proteins in the intercellular space remain intact, which improves the integrity of damaged tissue, reduces scarring and allows for faster tissue regeneration (53). Involvement of the immune system also plays an important role in the antitumor effect of irreversible electroporation. A fraction of cells in the immediate vicinity of the electrodes die due to thermal damage (necrosis), which stimulates the local immune response and thus accelerates the removal of cancer cells (54,55). Thermal techniques such as radiofrequency and microwave ablation and cryoablation are still routinely used for soft tissue ablation, but interest in irreversible electroporation ablation is also growing.

2 Technical aspects of ablation with irreversible electroporation

With irreversible electroporation we strive for complete destruction of cells in the target tissue. Ablation effectiveness is directly connected to the strength of the local electric field in the tissue and the duration of exposure to the field (Figure 1).

The strength of the electric field is mainly influenced by pulse amplitude, but it is also dependent on electrode dimensions (electrode diameter and length), the distance between paired electrodes and on (electric and thermal) properties of biological tissue. Additionally, the level of tissue electroporation is also influenced by the dynamics of pulse delivery (pulse duration and pulse delivery rate). Determining the optimal parameters for irreversible electroporation is the subject of intensive research, as the pulse parameters differ between different target tissues/organs.

2.1 Ablation of deep-seated tumors

For ablation of deep-seated tumors long monopolar needle electrodes are used to deliver high-voltage electrical pulses. The

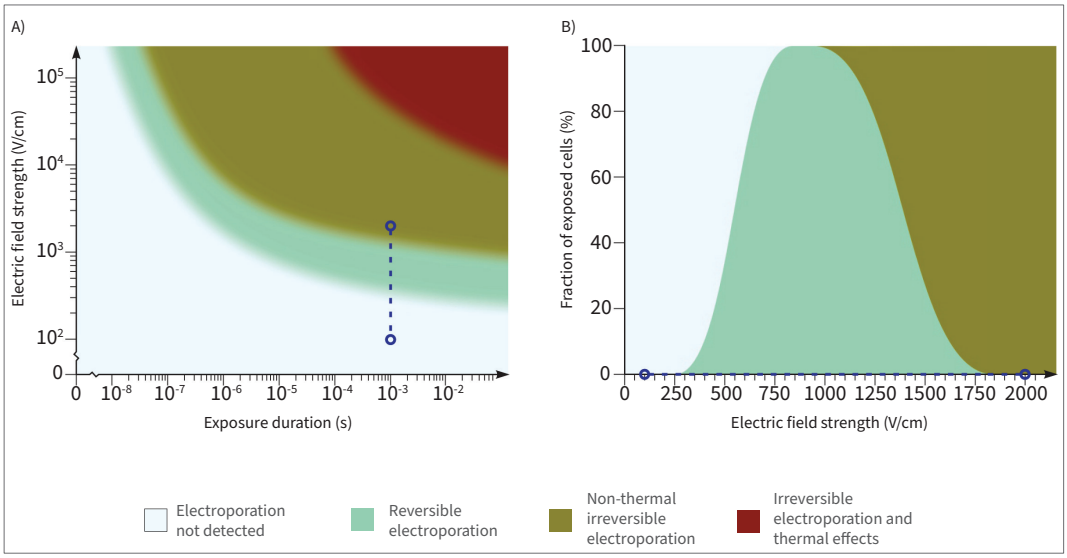


Figure 1: A) Reversible electroporation, irreversible electroporation and thermal effects of electroporation depend on both the strength of the electric field and the time of exposure (depending on the duration and number of applied electric pulses). In tissue, the same effect can be achieved with a shorter exposure time and a higher strength of the electric field, or with a longer exposure time and a lower strength of the electric field. B) With the selected duration of electric pulses (example for 1-millisecond pulses - dashed line on panels A and B), the fraction of reversibly and irreversibly electroporated cells increases with the strength of the electric field. The figure is summarized from Yarmush ML, et al (8).

number and placement of electrodes depend on the size of the tumor. The use of two to six electrodes is typical, which are placed as parallel as possible to each other and are arranged around the tumor. If necessary, one or more electrodes may also be located inside the tumor. The electrodes have an adjustable length, but in practice a length of 2 cm is most commonly used as longer electrodes result in excessive electric currents. Electric pulses are applied to individual pairs of electrodes so the entire target volume, i.e. the tumor volume with a safety margin, is successively covered. The safety margin width depends on the type of tumor and usually extends 5-10 mm from the tumor borders. In the case of larger tumors, the electrodes can also be retracted (15-20 mm) during therapy, thus covering the target volume in segments. Parameters of electric pulses and delivery protocols differ between studies, but a train of 70-100 electric pulses per electrode pair is most commonly used, and the duration of individual pulses in the train is typically 90 μs (50–100 μs) (18,37,45). To determine the amplitude, the ratio between the voltage and the distance between paired electrodes is mostly used, but it varies from study to study, as it also depends on the type of target tissue. Published *in vivo* studies show the use of 1000–2500 V/cm ratios (18).

The voltage-to-distance ratio (V/d ratio with a unit of measurement V/cm), which is used to determine the voltage at the electrodes, is often confused in literature with the electric field threshold, required for irreversible electroporation of the target tissue. The electric field in a tissue depends on the electrical properties and structure of the tissue. Tissue (and therefore electrical conductivity) is essentially nonhomogeneous, and in addition, conductivity changes dynamically during the procedure due to electroporation and also due

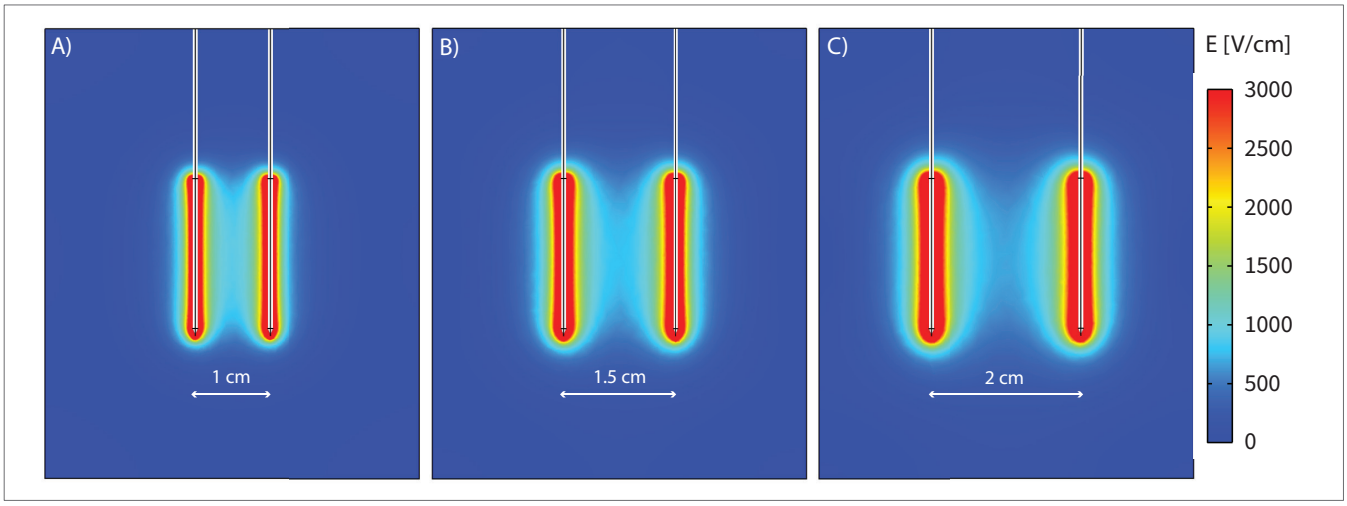


Figure 2: Example of electric field distribution in homogeneous tissue at different distances between needle electrodes (1 cm, 1.5 cm and 2 cm) and at the same voltage-to-distance ratio V/d = 1500 V/cm. For better visibility, the color scale is limited to a range of up to 3000 V/cm. The electric field is not homogeneous and is very high in the immediate vicinity of the electrodes (even up to 10,000 V/cm), but decreases rapidly with the distance from the electrodes and is between 500 and 1000 V/cm in the middle between the electrodes. The distribution of the electric field also depends on the placement of the electrodes. At the same V/d ratio, the field in the middle between the electrodes is almost homogeneous at a distance of 1 cm (A), whereas it becomes increasingly nonhomogeneous with increasing distance (B, C).

to heating (56,57). In fact, the electric field near the electrodes is very high (even up to 10000 V/cm) due to high current density, while with increasing distance from the electrodes it decreases drastically and is lower than the selected V/d ratio in most of the target volume (Figure 2). Therefore, high values are used to determine the voltage at the electrodes, e.g. 1500 V/cm, although the electric field threshold at which irreversible electroporation occurs in tissue is in fact only around 500-700 V/cm (depending on the type of tissue and the number of pulses supplied).

Electrode position planning and placement is still mostly done manually. The interventional radiologist determines the positions and trajectories for electrode insertion on the basis of the patient's pre-intervention imaging. Electrode insertion is then performed with the help of contrast-enhanced ultrasound, for example at ablation in the prostate, or with the help or interventional CT imaging, for example in treatment of liver or pancreatic tumors. The final electrode positions are then inserted into the *NanoKnife* device, which displays the schematic shape of the ablation zone according to the selected electric field parameters (voltage-to-distance ratio at the electrodes). The needle electrodes are long and thin (diameter 0.8–1.2 mm) and often bend when inserted. Therefore, it is extremely difficult to achieve a completely parallel layout.

The *NanoKnife* (AngioDynamics, Latham, New York, USA) is currently the only commercially available device for irreversible electroporation and dictates most protocols in clinical studies. The manufacturer recommends a V/d ratio of 1500 V/cm to determine the voltage amplitude at the electrode pair. The maximum voltage the *NanoKnife* can deliver is 3000 V, so we are limited to distances of up to 2 cm when placing the electrodes if we want to maintain a ratio of 1500 V/cm. Additionally, we are also limited by the maximum electric current the device can deliver. If the amplitude of the electric

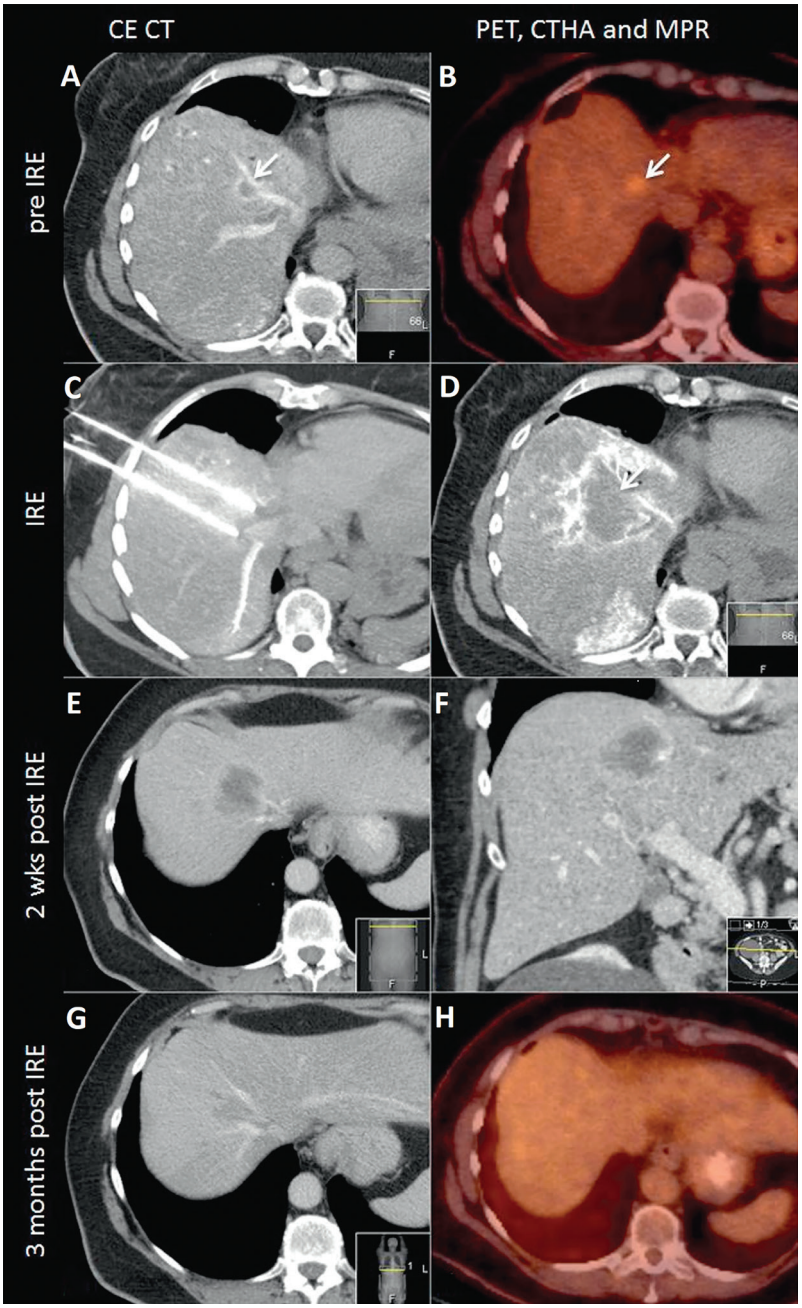


Figure 3: An example of a minimally invasive procedure – IRE ablation of colorectal metastases in the liver. A, B) Pre-interventional images show a tumor (arrow) near the hepatic and portal veins and the bile duct. C) Needle electrodes for electroporation are inserted percutaneously under CT guidance. D) The image taken immediately after the procedure shows a characteristic hypointense area of ablation with a hyperintense edge. E, F) The images show a shrinking of the ablation area two weeks after the procedure. G, H) Three months after the procedure, the area of ablation on a contrast enhanced CT is barely visible, and no metabolic activity is detected with a PET CT in the area of the lesion. The figure is summarized from Scheffer HJ, et al (19).

current in the pair of electrodes exceeds 50 A, the pulse delivery is automatically terminated. The voltage must then be lowered accordingly and the procedure repeated. High-voltage electric pulses can cause cardiac arrhythmia, so pulses are applied during the absolute refractory period of the ventricles (58,17). The pulse delivery is thus always synchronized with the patient's electrocardiogram, regardless of the selected delivery protocol. The *NanoKnife* delivers electric pulses in sequences of ten pulses, followed by a break during which the device is recharged. Electroporation pulses also cause strong muscle contractions, so complete pharmacological paralysis of the patient and general anesthesia are required. Ablation of deep-seated tumors by irreversible electroporation can be performed surgically or percutaneously. The percutaneous procedure is minimally invasive and greatly shortens the time of hospitalization and recovery of the patient, but it is technically much more demanding. It is extremely important that the needle electrodes are inserted exactly according to the pre-procedure plan. Electrode insertion can be performed manually under CT guidance or using navigation systems currently available for percutaneous treatments (59,61). Figure 3 shows an example of a minimally invasive procedure - IRE ablation of colorectal metastases in the liver.

2.2 Cardiac ablation

One of the most promising applications of irreversible electroporation is the pulmonary vein isolation for treatment of atrial fibrillation (AF). Catheter ablation of the atrial myocardium at the junction with the pulmonary veins is an established method for treatment of AF, with radiofrequency (RF) ablation and cryoablation being most commonly used. The main

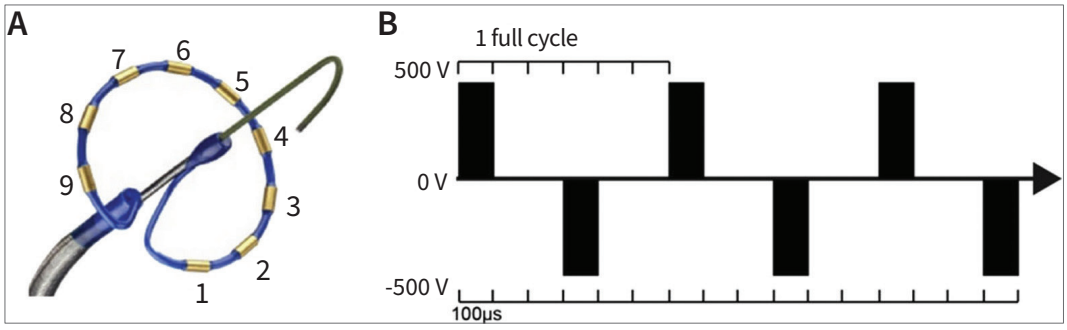


Figure 4: A) A circular catheter with numbered electrodes for pulmonary vein ablation; B) A schematic representation of three biphasic pulses for pulmonary vein ablation with a circular catheter. The figure is summarized from Stewart MT, et al (22).

weaknesses of RF ablation are limited control over the expansion of heat in tissue (and with it increased risk for damage to non-target tissue) and discontinuities in the ablation zone. The procedure is also lengthy and requires an experienced cardiologist or electrophysiologist as the RF electrode needs to be manually moved around the circumference of the pulmonary vein. Irreversible electroporation enables good control over the depth of the ablation zone and the success of ablation is not dependent on heat accumulation in tissue. Compared to RF ablation, the IRE procedure is much faster, as the pulse delivery takes only a few seconds after the placement of the circular catheter. With both thermal ablation techniques (RF and cryoablation) the target tissue for ablation is located outside the pulmonary veins to avoid scarring venous walls and with it the risk of pulmonary vein stenosis. Irreversible electroporation preserves the structure of the extracellular space and thus reduces tissue scarring. Therefore, it also allows ablation directly at the junction with the veins without the risk of stenosis (62). Catheter cardiac ablation by irreversible electroporation is currently in the development phase. Numerous preclinical studies and a first clinical study (20) have tested different forms of catheters, from balloon catheters (62) to different forms of catheters with bipolar electrodes and biphasic pulses (20). One of the more promising catheters is a circular catheter with nine electrodes (Figure 4) (22,49). Optimal electric pulse parameters are still the subject of research, but general conclusions suggest that a bipolar electrode configuration in the catheter (alternating positive and negative electrodes) and application of short biphasic pulses provide the best control over the size of the ablation zone and reduce the intensity of muscle contractions.

2.3 Procedure planning

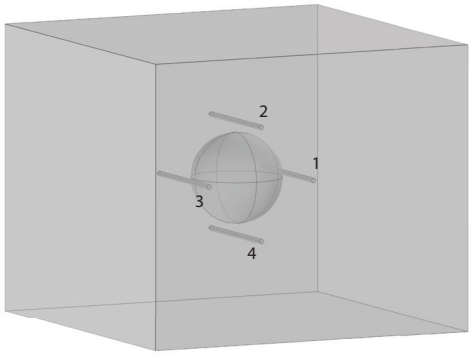
Technological advancement, especially with image-guided processes, now enables the use of minimally invasive procedures, which greatly shorten the duration of hospitalization and improve the patient's quality of life during recovery. With minimally invasive procedures, especially with deep-seated tumors, we are limited regarding electrode placement, as mechanical damage to critical anatomical structures must be avoided. The first step in treatment planning is to determine the insertion trajectory of the electrodes, following the manufacturer's instructions that the electrodes should be as parallel as possible to each other, as this is the only way to control the distribution and homogeneity

of the electric field in the tissue. In the second step, we must determine the optimal number and placement of electrodes and the optimal parameters of electric pulses by providing a sufficiently high electric field in the entire target volume of the tissue, without damaging nearby critical anatomical structures. Additionally, we are limited by the capabilities of clinically accessible pulse generators – with limited current and voltage.

Although the *NanoKnife* device allows for a schematic representation of the expected ablation zone according to the selected electrode placement and pulse parameters, it assumes that the tumor is perfectly round and does not take into account the local redistribution of the electric field due to biological tissue variability. In addition, the *NanoKnife* translates the three-dimensional placement of the electrodes into two dimensions, so the ablation zone is represented only in the plane perpendicular to the electrodes. The voltage at the electrodes is calculated automatically according to the desired voltage-to-distance ratio. The suitability of the calculated parameters is checked by measuring the current at the beginning of the procedure. Each pair of electrodes is first supplied with 10-20 test pulses. If the measured current is in the range of 20-30 A, the parameters are deemed suitable, so the delivery of the remaining pulses can follow. If the current value is higher or lower, the voltage or electrode placement must be adjusted accordingly.

The planning of IRE ablation based solely on electric current is not reliable, so in recent years software tools and applications for the preparation of comprehensive pre-intervention plans have been under active development. The preparation of a treatment plan is based on a numerical simulation of the procedure (37,63-65). The principle of modelling ablation with irreversible electroporation is presented on a simplified theoretical model of liver tumor (Figure 5 / A). The model consists of two tissues, namely the liver parenchyma and a spherical tumor with a diameter of 16 mm. Four needle electrodes are arranged around the tumor in a square configuration, and the maximum distance between the electrodes is 20 mm. The electrodes together form six active ablation pairs. The parameters of the electric pulses in the model are selected in accordance with the recommendations of the manufacturer of the *NanoKnife* device, namely 100 pulses per electrode pair for 90 μs and the ratio between the voltage and the distance between the electrodes of 1500 V/cm (Figure 5 / B). The dynamic of the pulse delivery is as follows: electric pulses are delivered with a frequency of 1 Hz in a train of 10 pulses, and there is a 3-second pause between individual trains, which is the time needed to charge the pulse generator. With a simplified model, we can quickly check the suitability of selected pulse parameters and electrode placement. For each active electrode pair, the spatial distribution of the electric field is calculated separately, and the contributions of individual pairs are finally combined to give the final distribution of the electric field or, in other words, the tissue coverage (Figure 5 / C). The threshold for irreversible electroporation of the tumor was 600 V/cm for the selected model parameters (37). By analyzing the contribution of individual pairs of electrodes to the total coverage of the target volume, we can optimize the ablation parameters. In Figures 5 / D-I we see that with the first four pairs we cover 75% of the tumor volume, and with the fifth pair we already achieve 100% coverage. In the model, in addition to the distribution of the electric field, we also calculate the heating of the target tissue due to Joule heating. Figure

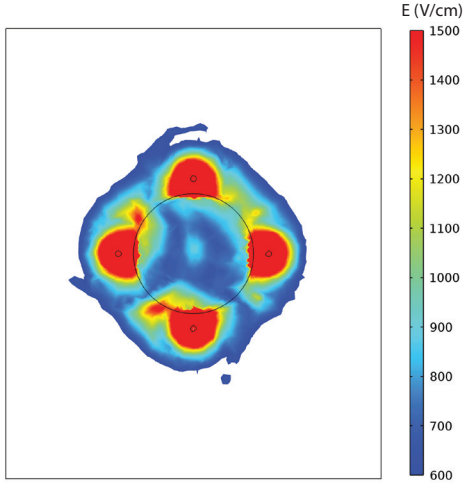
A)



B)

Electrode pair	Distance between electrodes (cm)	Voltage (V)	Pulse duration (µs)	Pulse number	Calculated current (A)
1 - 2	1,41	2100	90	100	22,92
2 - 3	1,41	2100	90	100	24,26
3 - 4	1,41	2100	90	100	24,82
4 - 1	1,41	2100	90	100	25,23
1 - 3	2,00	3000	90	100	37,64
2 - 4	2,00	3000	90	100	38,50

C)



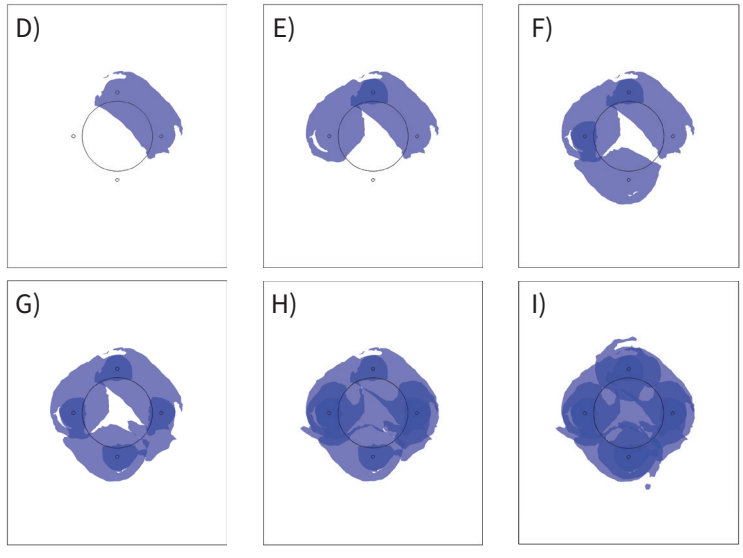


Figure 5: A) A simplified numerical model of a liver tumor, surrounded by four needle electrodes. B) Chosen parameters of electric pulses for the six active electrode pairs in the model. C) Final distribution of the electric field in the tissue. The round contour of the tumor can be seen. The colour scale is adjusted to the range from 600 to 1500 V/cm for better visibility. D/I) At the selected model parameters, the threshold for irreversible electroporation of the tumor is approximately 600 V/cm. The D/I images show the contributions of individual electrode pairs to the total coverage of the target tissue with an electric field greater than 600 V/cm. We see that the entire volume of the tumor is already covered by the contributions of the first five pairs.

6 / A shows the calculated temperature in the tissue after delivery of 100 pulses to the first pair of electrodes. We see that the temperature rises sharply already in the first pair, which is then followed by the delivery of pulses to a another 5 pairs. Figure 6 / B shows the highest calculated tissue temperature for all 6 electrode pairs. With rising tissue temperatures the electrical conductivity of the tissue also increases, and with it the electric current (Figure 6 / C). From the final distribution of the electric field on Figure 5 / C it is evident that the entire tumor is covered with electric fields above the default threshold for IRE (600 V/cm). We could further optimize the parameters and thus limit tissue heating and the risk for unwanted thermal damage. When preparing an actual patient-specific plan for the procedure, we use the patient's pre-intervention images, on the basis of which we create a simplified, but anatomically correct, numerical model of the target organ / tissue. We then determine the intervention plan in the model by calculating the electric field distribution and by optimizing the placement and voltage on the electrodes (66). The

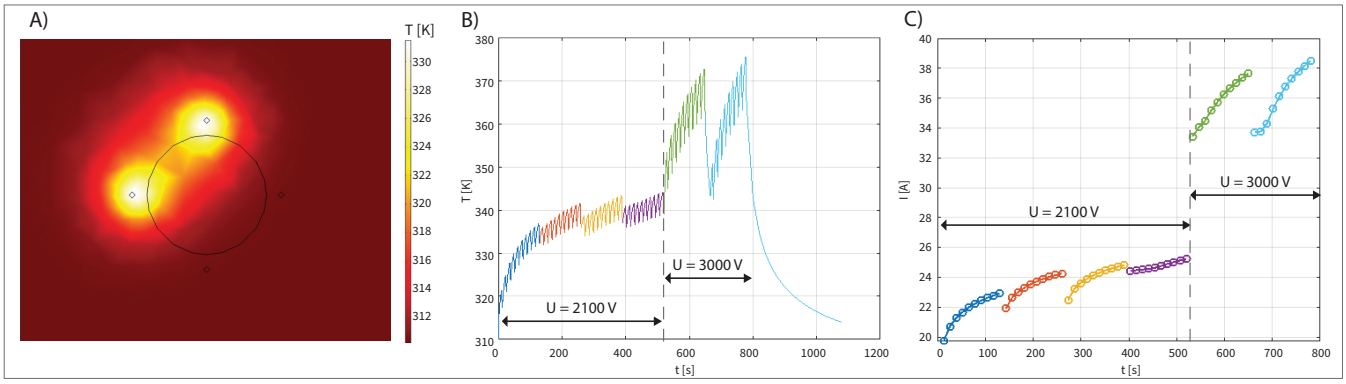


Figure 6: A) Tissue temperatre after the first 100 pulses; starting temperature is around 310 K - 37 °C. B) Maximal tissue temperature calculated after each individual train of 10 pulses. C) The temperature rise causes an increase in tissue conductivity and therefore an increase in the electric current.

pre-operative treatment plan then has to be imported into the intervention imaging domain on the day of the procedure, which requires the registration of three-dimensional pre-intervention and intervention images in the same coordinate system. Image registration is an extremely difficult procedure, especially with soft tissues, where deformations are often present. In addition to the limited precision of image registration, the time complexity is also problematic, as the registration process is still too time-consuming for routine clinical use (60). The pre-operative plans are thus currently used as a support when performing the actual procedure.

2.4 Thermal aspects of irreversible electroporation

Ablation effectiveness with irreversible electroporation is not dependent on temperature, so the method is often characterized as a non-thermal ablation metod, which can lead to erroneous thinking that IRE does not cause any tissue heating. Soft tissues are good conductors and their electric conductivity increases during electroporation, which can cause high electric currents and consequent Joule heating of surrounding tissue. The temperature rise is most evident in the immediate proximity to electrodes, where the temperature can exceed 60 °C, which can cause coagulation of cell proteins and immediate cell death even with brief exposures. Longer durations of exposure can lead to cell death even at 43 °C. The increase in temperature is proportional to the number of electric pulses and the frequency of pulse delivery. In larger tumors, where many electrodes are used and whose contributions overlap, hundreds of pulses can be cumulatively delivered to the tissue locally, which can lead to high temperatures throughout the target tissue, thus losing the non-thermal nature of IRE. One of the reasons for choosing IRE ablation over thermal methods is its use in anatomical locations and organs where thermal damage is not acceptable. In such cases the tissue temperature needs to be controlled and considered during planning for the procedure. Heating and cooling of tissues and pulse delivery dynamic need to be considered in calculations as well.

In the numerical model, we take into account the change in the conductivity of the medium both due to electroporation and due to heating. Figure 6 / C shows the increase in electric current as a result of heating for each active pair of electrodes separately. Although the V/d ratio is the same in all electrode pairs, we can see that

in pairs with lower voltage the current increases by approximately 3 A after the application of all 100 pulses, while in pairs with a higher voltage the current rises by 5-6 A. The increase in electric current is often considered in literature as one of the indicators of successful electroporation, although there are multiple factors for this increase; among others, as we can see in Figure 6, there is also an increase in tissue temperature, which is by no means negligible during IRE.

3 Challenges in introducing irreversible electroporation into the clinical practice

Although irreversible electroporation has proven to be a promising alternative to existing methods of ablation of various soft tissues, there are still many problems to be solved when introducing it into the clinical practice.

One of the key challenges is the lack of an indicator of the technical success of IRE ablation during or immediately after the procedure. IRE causes immediate tissue edema, so imaging immediately after the procedure does not provide reliable information on the area of ablation. Due to tissue regeneration following IRE (and associated shrinkage) it is difficult to reliably demarcate the area of ablation on post-operative imaging, which makes it difficult to develop and validate numerical models for intervention planning (68,69).

Studies in the last few years have often used an increase in electric current as a measure of successful irreversible electroporation. Martin and colleagues in a study of pancreatic IRE ablation state that with successful tissue IRE, the electric current increases by at least 12 A (45). If an adequate increase in current is not achieved for an individual pair of electrodes after the introduction of all pulses, an additional 70–100 pulses are suggested (45,70,71). This way of »controlling« the ablation size leads to a large number of pulses and thus to significant heating, and it has not been proved that additional pulse delivery increases the size of ablation (72). In addition, the conclusions of the study (45) refer to the results of ablation in the pancreas; soft tissues have a variety of electrical properties, so the direct transfer of conclusions to other tissues is not sufficiently warranted without further research. In the study (73) O'Brien et al. studied the influence of internally cooled electrodes on effectiveness and safety of IRE ablation in the liver. Results of the study have shown that lower temperatures and lower electric currents are achieved with the use of cooled electrodes compared to traditional electrodes without a change in the size of the ablation area. Ruarus et al. have shown in their study (74) that although an increase in current correlates with the success of ablation of colorectal metastases, an increase of current for 12 A was observed in only 10% of patients. The size and increase of the electric current depend on tissue composition and also on heating – either as a consequence of an increase in conductivity or due to impaired tissue cooling due to local vascular occlusion caused by electroporation. In the numerical model (Figures 5 and 6), we considered the dynamic properties of the tissues and calculated the increase in electric current during IRE ablation of the tumor in the liver. We have shown that despite a low increase in electric current we can achieve complete coverage of the target tissue with a safety margin, as shown by Ruarus et al. in their study (74). Increase in current during IRE ablation can serve as a potential indicator, but it does not represent a reliable method for predicting treatment success (or failure); its misuse can even lead to increased risk of damage to nearby

delicate anatomical structures. Despite an increasing number of clinical trials in various organs, there are no studies in which IRE ablation has been chosen as the primary treatment option. In most medical centers, IRE ablation is suggested for patients in whom current established treatment options have failed or are contraindicated for other reasons. Patients in which success of IRE ablation has been tested so far have often been previously treated with systemic chemotherapy, have recurrent disease or tumors in hard-to-reach places. When comparing it to other established ablation techniques, we must therefore be aware of the bias to the detriment of IRE ablation. In addition, there are currently no standardized protocols for IRE ablation, which leads to high variability between studies in terms of the number of electrodes used, the number and duration of pulses, the size of the target area, and so on. Inconsistencies in protocols may adversely affect treatment outcome, increase patient risk, and therefore impede further development. The publication of the first standardized electrochemotherapy treatment protocols in 2006 (75) has, for example, greatly accelerated the development and introduction of electrochemotherapy as a new method of treatment of primary and secondary tumors into clinical practice. The unification of treatment protocols in different clinical centers has expanded the use of electrochemotherapy and contributed to new knowledge, so in 2018 a new, updated and expanded version of standardized protocols was published (76). Introducing similar standardized protocols for ablation with irreversible electroporation would increase the replicability of studies and enable easier evaluation of the clinical value of this new ablation method.

4 Conclusion

Irreversible electroporation is an alternative method to thermal ablation techniques, such as radiofrequency ablation, microwave ablation and cryoablation, since its mechanism of cell destruction does not depend on tissue temperature. Because of the mostly non-thermal mechanism of action it is especially interesting for use in organs and in anatomical locations where thermal damage to surrounding tissue is not acceptable. Numerous clinical studies have demonstrated the effectiveness and safety of irreversible electroporation in various tissues/organs – for ablation of deep-seated tumors in the liver, kidneys, pancreas and prostate, and more recent studies for pulmonary vein isolation in AF treatment. The main guidelines for the future dictate the development of standardized protocols for the ablation of deep-seated tumors and the development and validation of tools for pre-operative treatment planning. Standardization of a direct indicator for technical success of IRE ablation is one of the key challenges with introducing this new and promising ablation method into the clinical environment. Despite the challenges that need to be overcome, irreversible electroporation is a promising new method of soft tissue ablation. We can certainly expect further development and use of irreversible electroporation in various branches od medicine.

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