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Congress is dedicated to the honorable Professor Jurgis Brédikis
13-15 September 2017
Kaunas, Lithuania

ONLINE ABSTRACT BOOK
The content of the abstracts presented is the responsibility of their authors and co-authors. The abstracts are arranged in sequence according to the congress program.
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**Chairs:** Jūratė Staikūnienė, Kecheng Xu

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Chair
Prof. Rimantas Benetis

Members
Prof. Jurgis Brédikis
Dr. Algimantas Budrikis
Prof. Mindaugas Jievaltas
Prof. Algimantas Tamelis
Prof. Skaidra Valiukevičienė

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ORAL PRESENTATIONS

SESSION I  |  GENERAL

I-I

Historical Note: Kaunas Experience on CryoCardiac Surgery treating Cardiac Arrhythmias
Jurgis Brēdikis¹, Rimantas Benetis
1. Lithuanian University of Health Sciences, Lithuania
2. Department of Cardiac, Thoracic and Vascular Surgery, Lithuanian University of Health Sciences Medical Academy, Lithuania

In 1971 in Kaunas University Hospital we started surgical treatment of tachyarrhythmia, successfully performed ablation of His bundle in closed beating heart by using electrocoagulation technique. Cryoablation technique seemed to us more preferable – precisely determined site of destruction, no tissue defects. We used liquid nitrogen for cryoablation.

Experimental studies demonstrated a possibility of cryoablation inside the beating heart. We found that parameters for cryoablation are the same as for external cryoablation: temperature – 60-70, exposure – 90-180 s. Historically, in 1977 we firstly introduced our original technique for “off-pump” closed heart cryodestruction of the His bundle, for both right and left parietal accessory pathways (Kent bundle) and for other tachyarrhythmias: atrial ectopic tachycardia, ventricular tachycardia, cryofragmentation of low right atrium in cases of atrial flutter, also for electric isolation of certain cardiac zones.

Our closed heart intracardial cryoablation technique may be considered as a prototype of contemporary catheter cryoablation.

I-II

History of Cryosurgery
Sajio Sumida¹, Masashi Watanabe²
1. Sajio Sumida Clinic, Japan
2. Omori Medical Center, Toho University, Japan

Human life is repetition of failure and success. But, failure is never tolerated for physicians in the clinical medicine, because the failure often makes them lead to the medical lawsuit. Therefore, we must brush up our surgical technique to evade such troublesome problems. Once more, we should learn the history above all. History gives us a comprehensive knowledge of human nature. George Santayana (1863-1952) reminded us that “those who cannot remember the past are condemned to repeat it.” Without knowing the history of medicine, including cryosurgery, of course, our progress will be too hard. We know very well that anyone fails, but when we are afraid of failure, we can’t do any surgical operation.

The answer, which appeared in “The Physician Himself“ in 1931 by Daniel W. Cathell (1839-1925), noted that “the more one believes in the possibility of error, the surer will he be to avoid mistakes”. Before him, Rudolf Billroth, who succeeded the first gastrectomy in 1881, said “Only the man who is familiar with the art and science of the past is competent to aid in its progress in the future.”

LTM has been originally recognized as a regenerative medicine, and played an important role in the clinical medicine. LTM was often compared to a double-edged sword, the one for cryopreservation and the other for cryodestruction that is cryosurgery. For the treatment of cancer, the sward has been used for many years. In those process, a mythical phenomenon of cryo-immune response, as a cancer vaccination. The best skill has been unattained that is to freeze and to thaw a target at optimal temperatures precisely.

Recently in China, cryosurgery was adopted into the traditional Chinese medicine as for a promising strategy, “the green treatment of tumor” not only in Guangzhou Fuda Cancer Hospital but in Beijing Dongfang Hospital, resulting in the prolongation of the survival period and the improvement of quality of life of the elder or advanced primary lung cancer patients.

I should explain some historical events of cryosurgery since James Arnott in 1845, who treated cancer applying a special container with an ice and salt solution to achieve local cooling to -24°C. Subsequently, we came to be able to generate subzero temperature anywhere by the discovery of a physical principle by J.P. Joule (1818-1889) and
W. Thomson (1824-1907). From the end of 19th century to the mid-20th century, many skin diseases were treated with dry ice and other refrigerants. Terminologies of cryo-congelation (Arnott 1851) or cryotherapy appeared at that time (The first renaissance).

Cryosurgery in the late 20th century was targeted to treat not only body surface diseases but also internal organ diseases by the liquid nitrogen probe developed in 1961 by I.S. Cooper and A.S. Lee. This innovative instrument produced a limited controlled region of freezing of internal organ. However, monitoring of the freezing range and adjustment of the cooling temperature of target organs had been still the difficult problems. Furthermore, the perioperative prediction of limited destruction of the disease tissue was very hard. For those purpose, trocar cryoscopy (Reuter 1871), guide button or palpable nob behind the freezing part of the probe (Gage 1965, Garamy 1968, Reuter 1971), thermocouple, cryoimpedancemetry (Le-Pivert 1975), direct cryoablation of AV node-His bundle (Bredikis J, 1977), ultrasound image (Onik 1984), X-ray fluoroscopy, and MRI (Isoda 1989, Rubinsky 1993) have been used as the navigator, and made cryosurgery much more popular (second renaissance).

Now, we have a needle sized (1 ~1.5mm in diameter) cryoprobes, which reappeared in the 21st century, using 2D or 3D array with perfection of the peripheral equipment, such as guiding cannula and wound dilator to insert cryoprobes, and thus enabled the percutaneous insertion of a thin probe into the target tumor of internal organs under MRI systems more safely and correctly ( The third renaissance). However, the inevitable accident occurred that the hairy sized tube built-in the thin cryoprobes, which produces the J-T effect to be required in cooling, resulted in clogging (Barron 1971). History repeated itself in cryosurgery too.

In 1971, U.S. President R. Nixon declared cancer destruction and signed the National Cancer Act, but the result was not successful. The cancer patients in the world are still complaining the pain. In other words, that campaign brought a good grade in the preventive medicine of cancer, for example, the reduction of smokers and the improvement of eating habits.

Nobody knows whether many results that we obtained by the clinical treatment and by the animal experiment of cancer lead the establishment of the ultimate therapy. "Science and Hypothesis" by H. Poincare (1854 -1912) about a study philosophy of scientist suggests that science is built up of facts, as a house is built of stones; but an accumulation of facts is no more a science than a heap of stones is a house.

We hope the immense development of cryomedicine in the near future to give a comfortable quality of life, and to give a hope to live longer for the patients of cancer and the other incurable diseases.

I-III

Translational Research on Ultra-Low Temperatures in Biomedical Science: From Fundamental Research to Clinical Implications

Nikolai N. Korpan
International Institute of Cryosurgery, Austria

Background: As any physical process, the effect of the factor of cold on biological living substance and the use of low temperatures in biology and medicine can be both positive and negative. The process of biological living structure damage consists of a series of reactions in response to freeze-thawing. However, all pathogenetic mechanisms were not known over the decades.

Objectives were aimed at profoundly understand the pathogenetic mechanisms referring to response of living biological substances and tissues to ultra-low temperatures in biomedical science based on fundamental research and clinical implications and change paradigms in medicine against malignant diseases.

Material and methods: Numerous of fundamental studies, i.e. theoretical models, experimental research on living matter in vitro and in vivo and different clinical investigations in patients with malignancies of liver, pancreas, breast, skin, stomach, colon, rectum, soft tissue were developed and carried out over last three decades.

Results: The new terminologies, definitions and innovative concept of thermal cascade phases were described and introduced into the modern biomedical science using ultra-low temperatures. Biocryoimmunology in living matter was defined and clinically introduced with formation of new allogenic substances originating and resulting from physiological and pathological living substances followed initiation of multiplied primarily local and systemic immunological response. The observed ultrastructural cellular changes in parenchymal organs - liver and pancreas tissue - given rise to a new concept concerning the technical requirements of cryogenic equipment using in modern biomedical science, especially in surgical oncology and biogenic immunology. that early and late cryosurgical vascular changes and circulatory stagnation together with the following cryoaponecrosis and cryoapoptosis as well as cryosurgical anti-angiogenesis and blocked the tumor vascularisation with immediately stopping oxygen supply to the tumor cells are the main important mechanisms of living liver tissue damage as a response to ultra-low
temperatures via freezing-thawing process namely double-deep-freeze. This initiated an irreversible damage tissue process and finally leads to complete destroying of malignant tumor mass. These are the main mechanisms of biological living tissue injury following the low temperature exposure which were elucidated and illustrated at ultra-low temperatures of (-170 °C) – (-180 °C). The unique phenomena have been observed and discovered in living matter which has become fundamental discoveries of importance to all of medicine. The nano-structures of the living substance were fragmented and denaturated into smaller nano-particles following ultra-low temperature exposure, leading to the deep low temperature interitus of the living matter on a nanometric scale and, finally, to local irreversible and avital lethal cell cryogenic explosion, namely cell cryo-non-vita. The findings of the investigations allow clearly defining the clinical implication for the main technical requirements to cryosurgical devices. The main technical parameter stipulating for the effective tissue cryo-devitalization is the provision of a high freezing rate of the biological tissue followed by further deliberate thawing. The summarized a long-term follow-up clinical experience based on the data of the 10-year prospective, randomized clinical trial suggested that hepatic cryosurgery is effective in the treatment of resectable and non-resectable malignant liver tumor.

**Conclusions:** The scientific implication of longstanding research is clearly to conclude that surgery using ultra-low temperatures lead to cryo-anti-angiogenesis, cryo-necrosis and cryo-apoptosis of malignant tumor with prevention of local recurrence, regional and distant dissemination of malignant cells with benefit for human civilization in science and medicine globally.

**SESSION II | ORTHOPEDIC**

**II-I**

**Histological Analysis of Liquid Nitrogen Treated Tumor-Bearing Bones that were Retrieved After Clinical Implantation**

Norio Yamamoto, Katsuhiro Hayashi, Akihiko Takeuchi, Yu Aoki, Takashi Higuchi, Kensaku Abe, Yuta Taniguchi, Hisaki Aiba, Yoshihiro Araki, Hiroyuki Tsuchiya

Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kanazawa University, Japan

**Background and objectives:** Currently, in many countries, allograft combined with recycled bone is often used as graft material for biological reconstruction due to cultural, religious, and/or cost concerns. Only a small number of studies, however, have been reported on the histological outcome of the recycled bone when retrieved at the time of revision surgery. We have used liquid nitrogen processing to allow for recycling of the resected tumor-bearing bone. In this study, we examined the histology of retrieved frozen autograft after having been implanted for limb reconstruction.

**Material (patients) and research method used:** Frozen tumor-bearing autograft specimens treated with liquid nitrogen were obtained from seven patients with a mean age of 34.4 years (8-68 years). These specimens were retrieved at the time of revision surgery for local tumor recurrence or complications at a mean of 31.7 months (2-96 months) postoperatively and were studied histologically.

**Findings/results in sufficient details to support conclusions:** Tumor cells were completely eradicated from the frozen bone in all cases. In a specimen retrieved 5 months after implantation, a small area of the bone showed active osteoblasts and osteocytes. In four cases retrieved greater than 1 year after implantation, active osteoblasts and osteocytes were observed widely throughout the frozen bone implant indicating the onset of osteogenesis commencing soon after implantation. Osseous incorporation was noted along the cortical host-graft junctio as indicated by the continuity of bone trabeculae. Notably, in the specimen which was retrieved 8 years after implantation, excellent bone revitalization and incorporation was observed.

**Discussion:** Joint preservation during limb reconstruction is a key element for retaining good limb function postoperatively. The use of recycled bone as a reconstruction material is more suitable to other alternatives as it allows for perfect anatomical joint reconstitution and the bio-integration of stabilizing ligaments. In this study, even at less than 6 months after implantation, bone revitalization was initializing and noted histologically; this progressed to continued graft-host osteointegration over time. It has been reported that MMP-7, which is important in bone metabolism, was better preserved in liquid nitrogen-treated bone compared with heat-treated bone. This supports the possibility that liquid nitrogen treatment might preserve other proteins important in bone metabolism. Overall, liquid nitrogen-treated bone appears to be a good biological material for use as a reconstruction material in limb salvage surgery.
Conclusions and recommendations: Our results suggest that liquid nitrogen-treated tumor-bearing bone might have superior biologic characteristics making it a better implant for bone replacement in limb reconstruction surgery and should be considered one of the most useful recycled materials for biological reconstruction.

II-II
Cryosurgery For Low Grade Intramedullary Chondrosarcoma
Yehuda Kollender, Solomon Dadia, Ortal Segal, Alexander Nirkin, Amir Sternheim, Yair Gortzak
The National Unit for Orthopedic Oncology, Tel-Aviv Medical Center, Israel
Tel-Aviv University, "Sacler" School of Medicine, Israel

Symptomatic intramedullary condroid lesions may be treated in various ways, the decision to treat is guided by combination of radiological and clinical features of the tumor, pathology reports are not always helpful and it is difficult even for a very skilled pathologist to make the decision whether a lesion is benign or considered a low-grade malignancy in this kind of intramedullary chondral lesions.

For that reason, the decision for surgery should be upon clinical or radiological changes. Curettage, cryosurgery and internal fixation is a well-established method for treating benign and low-grade bone tumors.

From 1988 to 2015 more than 800 cryosurgical procedures were performed in our unit. The aim of this study was to evaluate the oncological and functional outcome associated with intralesional curettage, burr drilling and cryosurgery for chondral intramedullary tumors.

From 1988 to 2015 we treated 132 patients with intramedullary chondral lesions; the decision for surgery was made after clinical and or radiological dynamics.

Patient's age ranged from 8-77 years (mean-52 years). Male female ratio was 67/65.

The anatomical location was mostly around the distal femur and proximal humerus and the rest in the proximal tibia, proximal femur, iliac bone, distal tibia, carpal and metacarpal bones and distal tibia.

The decision to treat was made after consultation with at least two senior surgeons from our staff. Surgical procedure included curettage, burr drilling, cryosurgery and internal fixation as needed.

Two patients were excluded from the study due to a change of the pathology to high grade.

NO local recurrence was noted in any patient.
We had very few complications that included fractures, infections, wound healing problems and degenerative changes at the adjacent joint.

Functional outcome was excellent or good in the majority of the patients.

Fenestration, curettage, cryosurgery and internal fixation is the method of choice for treating grade 1-2 intramedullary chondrosarcomas with a very low complications rate and no local recurrences.

II-III
Long-term Results of Epiphyseal-Preservation and Biological Reconstruction Using Tumor-Tone Sterilized with Liquid Nitrogen for Childhood Osteosarcoma Around the Knee
Hiroyuki Tsuchiya, Norio Yamamoto, Akihiko Takeuchi, Katsuhiro Hayashi, Yu Aoki
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kanazawa University, Japan

Background and objectives: Various methods exist to reconstruct large bone defects after tumor excision such as tumor prostheses or biological reconstructions using allograft, autograft, or recycled tumor-bearing bone. In 1999, we developed a reconstruction method using tumor-bearing autograft treated by liquid nitrogen. The purpose of this study was to evaluate the clinical outcome of this technique for treating osteosarcoma around the knee in children after epiphyseal sparing tumor resections.

Material (patients) and research method used: We retrospectively reviewed 17 children (7 boys and 10 girls) with osteosarcoma around the knee with an average age of 12 years (range 6-16). The mean follow-up period was 49 months (range 19-87). In 11 cases, the lesion was in the distal femur, while in 6 patients it was in the proximal tibia. Two distinct freezing techniques -- resection and freezing (11 cases) and pedicle freezing (6 cases) -- were used based on the location of the tumor. Complications and oncological outcomes were evaluated and graded using the Musculoskeletal Tumour Society (MSTS) scoring system.
Findings/results in sufficient details to support conclusions: The mean MSTS score was 95% (range 63-100). Complications that required additional surgery were observed in 7 (41.2%) patients. The 7 complications consisted of 4 fractures, 2 local recurrences arising from the surrounding soft tissue, and one superficial infection managed by irradiation and IV antibiotics. Limb shortening (>3 cm) was observed in 6 patients; 2 of these patients underwent subsequent limb lengthening. One frozen-autograft (5.9%) was surgically retrieved to manage the soft tissue recurrence. Clinically, 14 patients were able to run normally. Final oncological status was CDF in 13, NED in 3, and DOD in 1.

Conclusions and recommendations: The long-term outcome of epiphyseal-preservation surgery using tumor-bone sterilized with liquid nitrogen for childhood osteosarcoma around the knee was satisfactory and promising. This procedure proved to be very beneficial for children with osteosarcoma.

II-IV

Revitalization of Frozen Autologous Bone Graft by Adipose Derived Stem Cells

Yu Aoki, Norio Yamamoto, Katsuhiro Hayashi, Hiroaki Kimura, Akihiko Takeuchi, Shinji Miwa, Hiroyuki Inatan, Takashi Higuchi, Kensaku Abe, Yuta Taniguchi, Hisaki Aiba, Yoshihiro Araki, Hiroyuki Tsuchiya
Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kanazawa University, Japan

Background and objectives: Adipose-derived mesenchymal stem cells (ADSCs) are highly safe and pluripotent stem cells. We are conducting a research on tissue regeneration using adipose-derived regenerative cells and ADSCs. In this study, we report the ability of ADSCs to regenerate frozen bone in vitro and in vivo for clinical application.

Material (patients) and research method used: Culture supernatant of ADSCs served as the conditioned culture group, whereas Dulbecco's Modified Eagle Medium (DMEM) was used as the control group. Rat osteoblasts were cultured using a medium consisting of conditioned or control medium as an in vitro experimental model, and the proliferative capacity of osteoblasts was examined using the WST-8 assay. In addition, RNA was extracted from rat osteoblasts co-cultured with rat ADSCs in non-contact co-culture (co-culture group) and non-co-cultured rat osteoblasts (control group), and the gene expression level was examined using various types of real-time PCR. Next, as an in vivo experimental model, the femoral shaft of the rat was removed and was frozen with liquid nitrogen, which was then reimplanted with an autologous bone transplantation. Using type I collagen gel plus 5.0 × 10^6 ADSCs (ADSC group) and without ADSCs (control group), histopathological examination of bone regeneration ability was conducted.

Findings/results in sufficient details to support conclusions: In vitro, osteoblasts in the conditioned culture group significantly proliferated compared with those in the control group (0.24 vs. 0.45, p < 0.01), suggesting that ADSCs induce proliferative humoral factors for osteoblasts. BMP2 expression of osteoblasts in the co-culture group was significantly lower than that in the control group. In vivo, bone regeneration was observed in two of five cases in the control group at week 8, whereas bone regeneration was observed in all five cases in the ADSC group at week 8. Moreover, the osteogenesis-promoting action of ADSCs for frozen bone was suggested.

Conclusions and recommendations: In vitro, the promotion of osteoblast proliferation and osteogenesis-promoting action of ADSCs was confirmed. In vivo, the possibility of promoting frozen bone regeneration was suggested, and its clinical application is expected in the future.

II-V

Cryosurgery in the Setting of Ewing’s Sarcoma of the Extremities in the Pediatric Population

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The National Unit for Orthopedic Oncology, Tel-Aviv Medical Center, Israel
Tel-Aviv University, the "Sacler" School of Medicine, Israel

Background: Adjuvant cryosurgery for bone tumors is very well established in the literature. The orthopedic-oncology department in Tel Aviv is using cryosurgery since 1994 mostly for benign aggressive and low grade bone tumors. Since 1994 more than 700 cryosurgery procedures were performed. The use of cryosurgery for highly malignant bone tumors is controversial.
Here we summarize our experience with seventeen patients treated for Ewing’s Sarcoma (high grade bone sarcoma) of the extremity that have undergone intercalary resection and re-implantation or curettage with adjuvant cryoablation of the involved bone.

**Patients and methods:** Seventeen patients with non-metastatic Ewing sarcoma in long bone of the extremity, age 12.6 (range 2-17), underwent wide resection or curettage of the involved bone, depending on the amount of extramedullary involvement. The resected bone was stripped of soft tissue and the medulla was curetted out, followed by immersion in liquid nitrogen for 20 minutes, followed by 15 minutes in sterile water. The bone segment was filled with cement and fixation was achieved by plates, intramedullary nailed or a combination of these and local bone graft. Curettage only was performed in cases where there was no or minimal soft tissue extension, cryoablation was performed with a closed Argon-Helium gas based system. Weight bearing was initiated 3-4 months post-surgery and patients progressed to FWB according to clinical inspection and repeat imaging showing consolidation of the osteotomy/curettage sites.

**Results:** 14 patients are alive and three have died at 57 months of follow up (range 10-192). One patient had positive margins, one patient had a local recurrence and three developed distant metastases. Of the 12 patients treated with resection and re-implantation, six achieved union at the time of writing. Five needed additional surgery. One patient treated at the age of two with curettage and cryo-ablation needed several surgical interventions to achieve proper leg length and function at maturity. The functional outcome as measured by the MSTS score is 25 on average. No deep infections occurred and two fractures healed. All patients ambulate without support and have satisfactory function.

**Conclusions:** Cryo-ablation in the setting of Ewing’s Sarcoma of long bones of the extremities is an attractive treatment option, enabling conservative surgery coupled with good oncological and functional outcomes. The advantage of re-implantation of weight bearing long bone segments after cryoablation with liquid nitrogen ensures a perfect fit and is readily available. Union of the osteotomy sites will be achieved as long as proper osteo-synthesis rules are followed.

Table 1, demographic and treatment parameters of the study group.

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SESSION III | CRYOIMMUNOLOGY

III-I
Past, Present and Future of Cryoimmunotherapy
Israel Barken
Prostate Cancer Research and Education Foundation, USA
Anti – Cancer Lab, USA
University of California, San Diego, USA

Background and objectives: Review of cryoimmunology since Dr. Ablin initial cryoablation work.
Review Animal Work demonstrating the effect of GMCSF and IL-2 on metastatic disease in the experimental model. Clinical experience from patients treated with Cryoablation and CheckPoint inhibitors.

Material (patients) and research method used: Experimental Prostate Cancer Model in Dunning Rats. Use of GM CSF G and IL-2 and Cryoablation in different combination with control group.

Findings/ results in sufficient details to support conclusions: Implanted tumor that were not treated directly by Cryoablation responded. Including affecting improved survival of the animal.

Conclusions and recommendations: Using immune modulators that historically demonstrated an activity synergistic to Cryoablation. Opening the possibility of using less expensive and less toxic immunomoulators.

Future application and combination of immune modulators for metastatic: Disease in the bone, is an ongoing work done in our Anti Cancer Laboratory in Beijing.

III-II
Clinical Efficacy of Percutaneous Cryoablation Combined with Allogenic NK Cell Immunotherapy for Advanced Non-Small Cell Lung Cancer
Lizhi Niu, Kecheng Xu
Department of Oncology, Fuda Cancer Hospital of Jinan University, China

Background and objectives: In this study, the safety and clinical efficacy of cryosurgery combined with allogenic NK cell immunotherapy for the treatment of advanced non-small cell lung cancer (NSCLC) were evaluated.

Material (patients) and research method used: From July 2016 to March 2017, we enrolled 60 patients who met the enrollment criteria and divided them into two groups: (1) the simple cryoablation group (n = 30) and (2) the cryoablation combined with allogenic NK cell group (n = 30). The changes in immune function, quality of life, and clinical response were evaluated.

Findings/ results in sufficient details to support conclusions: We found that allogenic NK cells combined with cryosurgical treatment for advanced NSCLC have a synergistic effect, which not only enhancing the immune function of patients, improving the quality of life, and significantly increasing the response rate (RR) and disease control rate (DCR) compared to cryoablation group.

Conclusions and recommendations: This study is the first clinical trial of allogenic NK cells combined with cryosurgery for the treatment of advanced NSCLC and preliminarily its safety and efficacy.

III-III
Cryoablation Combined with Allogenic Natural Killer Cell Immunotherapy Improves the Curative Effect in Patients with Advanced Hepatocellular Cancer
Kecheng Xu
Department of Oncology, Fuda Cancer Hospital of Jinan University, China

Background and objectives: In this study, the clinical efficacy of cryosurgery combined with allogenic natural killer cell immunotherapy for advanced hepatocellular cancer was evaluated.

Material (patients) and research method used: From October 2015 to March 2017, we enrolled 61 patients who met the enrollment criteria and divided them into two groups: 1) the simple cryoablation group (Cryo group, n = 26); and 2) the cryoablation combined with allogenic natural killer cells group (Cryo-NK group, n = 35), the safety
and short-term effects were evaluated firstly, then the median progression-free survival, response rate and disease control rate were assessed.

**Findings/ results in sufficient details to support conclusions:** All adverse events experienced by the patients were recorded, and included local (e.g., pain, pleural effusion, and ascites) and systemic (e.g., chills, fatigue, and fever) reactions, fever was more frequent. Other possible seriously side effects (e.g., blood or bone marrow changes) were not detected. Combining allogeneic natural killer cells with cryoablation had a synergistic effect, not only enhancing the immune function, improving the quality of life of the patients, but also reducing the expression of AFP and significantly exhibiting good clinical efficacy of the patients. After a median follow-up of 8.7 months (3.9 –15.1 months), median progression-free survival was higher in Cryo-NK (9.1 months) than in Cryo (7.6 months, P = 0.0107), median progression-free survival who received multiple natural killer was higher than who just received single natural killer (9.7 months vs. 8.4 months, P = 0.0011, respectively), the response rate in Cryo-NK (60.0%) was higher than in Cryo (46.1%, P < 0.05), the disease control rate in Cryo-NK (85.7%) was higher than in Cryo group (69.2%, P < 0.01).

**Conclusions and recommendations:** Percutaneous cryoablation combined with allogeneic natural killer cell immunotherapy significantly increased median progression-free survival of advanced hepatocellular cancer patients. Multiple allogeneic natural killer cells infusion was associated with better prognosis to advanced hepatocellular cancer.

**III-IV**

**Prospective Study of Percutaneous Cryoablation Combined with Allogenic NK Cell Immunotherapy for Advanced Renal Cell Cancer**

Lizhi Niu, Kecheng Xu
Department of Oncology, Fuda Cancer Hospital of Jinan University, China

**Background and objectives:** In this study, the clinical efficacy of cryosurgery combined with allogenic NK cell immunotherapy for advanced renal cell cancer was evaluated.

**Material (patients) and research method used:** From July to December 2016, we enrolled 60 patients who met the enrollment criteria and divided them into two groups: (1) the simple cryoablation group (n = 30); and (2) the cryoablation combined with allogenic NK cells group (n = 30). The clinical efficacy, quality of life, immune function, and other related indicators were evaluated.

**Findings/ results in sufficient details to support conclusions:** Combining allogeneic NK cells with cryoablation had a synergistic effect, not only enhancing the immune function and improving the quality of life of the patients, but also significantly exhibiting good clinical efficacy of the patients.

**Conclusions and recommendations:** This study is the first clinical trial that has evaluated the safety and efficacy of allogenic NK cells combined with cryosurgery for the treatment of renal cell cancer.
Suppurative wounds were detected in 3 patients (12.0±2.3%) in the main group and in 5 (20.0±3.9%) patients in the control group (p>0.05). Suppurated trophic ulcers were revealed in 4 patients (16.0 ±3.1%) in the main group and in 3 (12.0±2.3%) patients in the control group (p> 0.05). Purulent complications of diabetic angiopathy were detected in 3 patients (12.0±2.3%) in the main group and in 2 (8.0±1.5%) patients in the control group (p>0.05). Cryogenic exposure to the purulent focus was carried out by the steam-drop method.

**Results:** Hyperthermia in the main group was noted for 2.5±0.4 days from the beginning of the study, while in the control group it was observed for 3.3±0.5 days (t = 1.6; p>0.05); local hyperemia and edema in the main group lasted 3.2±0.3 days, in the control group – 4.1±0.5 days (t = 2.5; p<0.05); purulent exudation in the main group was pointed out for 3.1±0.5 days, and in the control group it was for 4.7±0.4 days (t = 2.6; p<0.05).

There was no change in the composition of pathogens in both groups until the end of the observation period. The decrease in CFU (less than 10^4 in 1 ml) was observed regardless of the microorganism species in 20 (80%) patients in the main group and 18 (72%) in the control group on the fifth day of the study (p<0.05).

Calculation of cytograms from the smears of the wounded was performed. On day 3 of the beginning in the control group, a degenerative-inflammatory type was noted in 5 (20.0±3.9%) patients, inflammatory type - in 14 (56.0±11.1%) patients, inflammatory-regenerative type - in 6 (24.0±4.7%) patients; in the main group, a degenerative-inflammatory type was detected in 15 (60.0±11.9%) patients, inflammatory type - in 10 (40.0±7.9%) patients. On day 5 from the beginning in the control group, the inflammatory type was noted in 4 (16.0±3.1%) patients, inflammatory-regenerative in 4 (16.0±3.1%) patients, regenerative - in 17 (68.0±13.5%) patients; in the main group, a degenerative-inflammatory type in 1 (4.0±0.7%) of the patient, inflammatory in 5(20.0±3.9%) patients, inflammatory-regenerative - in 9 (36.0±7.1%) patients, regenerative - in 8 (32.0±6.3%) patients. All differences in the groups are statistically significant (p<0.001).

**Conclusions:** The obtained data showed the expressed multidirectional curative effect of local cryoexposure in cases of various pyoinflammatory diseases of the skin and subcutaneous tissue and prove the necessity of applying cryosurgical technology with the aim of reducing related with PIDSST complications and mortality. To use it more fully, it is necessary to continue the new engineering, medical development and further research of biological, biochemical and biophysical changes in the purulent focus when using LC.

**IV-II**

**Intralosional Excision Combined with Intralosional Cryosurgery for the Treatment of Oversized and Therapy-Resistant Keloids of the Neck and Ears**

Lior Har-Shai3, Norbert Pallua1, Justinas Grasys1, Issa Metanes2,3, Yaron Har-Shai2,3

1. Department of Plastic Surgery, Hand Surgery, Burn Center, RWTH University Hospital, Germany
2. Plastic Surgery Unit, Carmel Medical Center, Israel
3. The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Israel

**Background and objectives:** The aim of this prospective study was to evaluate the efficacy of a new method for the treatment of oversized and therapy-resistant keloids of the neck and ears employing intralosional excision combined with intralosional cryosurgery.

**Material (patients) and research method used:** Three patients suffered from therapy-resistant keloids of the auricles, and one patient of a gigantic keloid of the anterior neck which followed skin scarification. The keloids were excised in an intralosional approach and the remaining base and lateral margins of the scar tissue were frozen using intralosional cryoneedles. After complete thawing of the frozen tissues, the margins of the scar were approximated and sutured.

**Findings/ results in sufficient details to support conclusions:** The follow-up period extended over 18-24 months. Over a period of 12 months the scars gradually flattened with no hypopigmentation. No signs of recurrence of the keloids were seen. Marked improvement in the patient general appearance and overall satisfaction was evident.

**Conclusions and recommendations:** Intralosional excision of oversized and therapy-resistant keloids combined with intralosional cryosurgery could well be an additional tool to the plastic surgical armamentarium, for treating this group of patients.
IV-III

Cell Population in the Blister Fluid Following Intralesional Cryosurgery for the Treatment of Keloids and Their Possible Role in Scar Rejuvenation

Yaron Har-Shai,1,3 Zuchman Rina2,3 MSc., Nir Gal-Or1,3, Nir Eyal1,3, Tamar Ziv2,3, Arie Admon2,3
1. Plastic Surgery Unit, Carmel Medical Center, Israel
2. The Ruth and Bruce Rappaport Faculty of Medicine and the Proteomics Center, Israel
3. Department of Biology, Technion-Israel Institute of Technology, Israel

Background and objectives: Following intralesional cryosurgery, histomorphometric studies have demonstrated rejuvenation of the treated keloid scars, i.e., parallelization, and a more organized architecture of the collagen fibers when compared to the pre-treated scars. The aim of this study was to classify the type of the cells in the blister fluid, formed after intralesional cryosurgery of keloid scars, which may explain the above findings.

Material (patients) and research method used: This study was approved by the institutional ethical committee. 10 healthy patients suffering from keloids were treated at the Unit of Plastic Surgery, Carmel Medical Center, by intralesional cryosurgery. Following the cryo-treatment a blister has developed. A fluid sample of the 10 keloid blisters was aspirated up to 24 hours post-treatment using a 19G sterile needle. The volume of the sample ranged from 0.4cc to 4 cc. The samples were stored at 4°C. Cells from the samples were washed fixed and stained using fluorescent antibodies against specific epitopes CD34, CD184 (positive markers of stem cells and multipotential progenitor cells), E-Cadhrin, and CD45 (negative marker of stem cells). FACS Arie Ilu Cell sorter was used to analyze the cell populations of the samples.

Findings/ results in sufficient details to support conclusions: Several populations of cells were identified in the blister fluids. The results showed high levels of Neutrophils (50-80%) and Erythrocytes. In order to detect stem cells that may exist in low level, gradual centrifugations were used. CD34 positive cells were detected in low amounts (about 5%) in all the samples that were tested. CD184 was detected in 1-4% and were enriched after 10 days in culture of the blister fluids.

Conclusions and recommendations: Cryosurgery may promote the delivery of multipotential progenitor cells into the treated scar tissue. The noteworthy finding of multipotential progenitor cells in the blister fluid of keloids treated by intralesional cryosurgery may explain the rejuvenation of the treated scars and the successful clinical results obtained by the intralesional cryosurgical method and may elucidate the low recurrence rate which has been observed clinically. In addition, the results of this study might ignite further research for possible treatment of keloid scars by multipotential progenitor cells.

IV-IV

Cryotherapy for Primary Localized Cutaneous Nodular Amyloidosis

Gintarė Linkevičiūtė¹, Vaiva Jievaltaitė¹, Jaunė Stumbrienė¹, Vesta Kučinskienė¹, Jurgita Makštienė², Skaidra Valiukevičienė¹
1. Department of Skin and Venereal Diseases, Lithuanian University of Health Sciences, Hospital of Lithuanian University of Health Sciences, Kauno klinikos, Lithuania
2. Department of Pathology, Lithuanian University of Health Sciences, Hospital of Lithuanian University of Health Sciences, Kauno klinikos, Lithuania

Background and objectives: We present a case report of Primary Localized Cutaneous Nodular Amyloidosis (PLCNA) which was treated with cryotherapy (CT). This method showed partial clinical response and patient was satisfied with performed treatment result.

Case report: 52-year-old woman had 10 years history of reddish-yellow infiltrated plaques on both cheeks. Examination of skin lesions with dermoscope showed peripheral telangiectasia and homogeneous yellow diffuse infiltrate in the centre. Histopathological examination of plaques in the skin revealed eosinophilic deposits in the dermis which were positive for Syria and Congo red staining. The material stained by Congo red produced green birefringence under polarized light. Immunohistochemical analysis disclosed that the amyloid deposits are positive for lambda light chains and negative for kappa light chains. Serum protein concentrations of α1 globulin were 3.1%, correspondingly Kappa and Lambda light chains - 21.7 mg / l and 73.6 mg / l, ratio of them- 0.29. Separation of blood monoclonal protein fractions was negative. Trephine biopsy did not show any specific abnormality. Serological blood tests and radiological investigation of internal organs were without significant changes, except
mitral regurgitation and nodules in the thyroid occurred. The course of open spray technique CT procedures (4 times every 4 weeks) of skin lesions showed partial clinical response with patient positive satisfaction.

Conclusions and recommendations: Treatment of this rare disease is based on published several case reports and remains controversial. Different approaches like CT, CO2 laser, local corticosteroids injections or excision can stabilize this condition. Different from other published case report in our case the course of CT procedures had partial clinical response and the patient was satisfied with treatment results. Also, there is a risk of PLCNA progression to systemic amyloidosis from 7% to 50% of cases. Therefore a regular follow-up of patient every 6 or 12 months is important.

SESSION V  |  RENAL-BLADDER AND PROSTATE

V-I  

Cryoablation of Inoperable and Multiple Tumours in Solitary Kidney  
Mantas Trakymas, Albertas Ulys, Rūta Grigienė  
National Cancer Institute of Lithuania, Lithuania

Background and objectives: Cryoablation and thermal ablation are well established methods for treating T1a kidney tumours for elderly patients or patients with comorbidities who are not suitable for surgery. Ablation is a nephron sparing method as it only slightly reduces the number of nephrons and does not require intraoperative ischemia as kidney resection does. Cryoablation does not induce scarring what is important issue while treating central tumours and it has this advantage to thermal ablation. Cryoablation of malignant tumours in Lithuania is performed at National Cancer Institute (NCI) from 2012 for inoperable tumours. We would like to present the results of cryoablation of inoperable and multiple tumours in patients with a solitary kidney. This indication is approved by authorities in Lithuania.

Material (patients) and research method used: Seven patients (median age 63, range 28-75) with inoperable or multiple tumours in solitary kidney were treated using argon helium cryoablation system at NCI from 2012 to 2016. All ablations were performed under CT guidance. Intentional ablative margin was 5-10 mm where feasible. Contrast enhanced CT was used for follow up.

Findings/ results in sufficient details to support conclusions: There were 14 tumours (median size 18mm, range 13-42 mm) treated in nine cryoablation sessions. All tumours were biopsy proofed to be clear cell renocellular carcinomas Fuhrman grade II. One patient with von Hippel Lindau syndrome had six tumours that were treated in two sessions with 27 months interval. There were no major complications observed after all ablations. The detected median minimal ablative margin was 2 mm (range 0-9mm). Despite the fact that only two tumours had an ablative margin of 5 mm or more, on median follow up of 27 months (range 3 - 54 months) no local tumour progression was observed. The mean increase in creatinine concentration next day after the ablation was 24 µmol/l 95% CI 19-29.

Conclusions and recommendations: Cryoablation is a safe and effective treatment modality for T1a single kidney tumours even in inoperable circumstances that provides possibility to preserve solitary kidney and its function. Effectiveness for T1b tumours should be further investigated.

V-II  
Percutaneous Cryoablation of Renal Cell Carcinoma in Patients with Von Hippel-Lindau Disease: 2 Clinical Cases  
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Background and objectives: Von Hippel-Lindau (VHL) syndrome is a rare genetic disorder characterized by the formation of fluid-filled cysts and benign tumors in many different organ systems. Renal cell carcinoma (RCC) is the main cause of death in patients with VHL disease. Because of VHL renal tumors have a high incidence of regrowth and finally bilateral nephrectomy may be needed. Therefore, nephron-sparing treatment such as
cryoablation is recommended to preserve renal function as long as possible. We overview 2 clinical cases of WHL disease treated with renal cryoablation.

**Material (patients) and research method used:** Both patients received few cryoablation procedures with a real-time ultrasound and/or computed tomography guidance and temperature change tracking. We used 17G Ice Sphere cryoablation probes during the procedure. **First case:** A 33-year-old women with diagnosed both kidneys RCC (right kidney - cT1aN0M0; left kidney - cT2N0M0). The patient had a single kidney, because of the left nephrectomy in 2008. From 2012, the patient was treated at our institute. In 2012, after computed tomography, multiple renal cysts similar to RCC were found and the renal radiofrequency ablation procedure was performed to treat a 14x17 mm tumor. Three years later RCC cryoablation was indicated because of 5 new primary RCC tumors. All of them were completely treated. After a year the renal cryoablation procedure was performed on the right kidney. Another primary 14x19mm tumor was frozen. There are no new renal tumors until now. Also the patient suffers from other complications of VHL: eye removal, multiple cysts in pancreas and liver.

**Second case:** A 24-years old women with both kidneys RCC (cT1aN0M0), which was diagnosed at the beginning of 2013. Left kidney cryoablation was performed to treat a 17x23 mm RCC tumor with a left kidney stenting due to pyelonephritis. Because of multiple tumors in both kidneys, bilateral renal cryoablation was performed after 5 months. A 17x23mm left kidney tumor and a 11x17mm right kidney tumor were frozen. Also left ureter stenting was done due to the prevention of stricture. After 2 years another cryoablation procedure was performed to treat two right kidney tumors (27x31mm and 21x22mm). Now patient has one more RCC tumor on the left kidney which is planned to remove.

**Findings/ results in sufficient details to support conclusions:** After overall 6 RCC tumors treatment and 28 months follow-up in the first case, patient’s renal function is quite good with serum creatinine 108 µmol/l and glomerular filtration rate 68 ml/min/1.73 m². In the second case, after the treatment of 5 RCC tumors and 48 months follow-up, patient’s renal function is excellent with serum creatinine - 67 µmol/l and glomerular filtration rate 123 ml/min/1.73 m². Only one complication after procedure was pyelonephritis.

**Conclusions and recommendations:** Our cases shows that percutaneous renal cryoablation of renal tumors in patients with von Hippel- Lindau disease is effective, safe and kidney function saving minimal invasive treatment.

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**V-III**

The 1st Laparoscopy-Assisted Cryoablation of Kidney Cancer in Lithuania (Case report)

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**Background and objectives:** Kidney cancer is the seventh most common cancer in Europe. Lithuania has high and still increasing kidney cancer incidence rate. Approximately 90% of all kidney cancers are renal cell carcinomas (RCC). Partial nephrectomy is considered as a first-line therapy for the patient with tumour up to 7cm. But some patients are not suitable for surgery even with smaller tumours: patients older than 70 years with tumours <3cm characterized with high surgical risk, solitary kidney, compromised renal function, hereditary RCC or multiple bilateral tumours). A less invasive treatment e.g. cryoablation, microwave or radiofrequency ablation are currently being widely used for such patients. The aim of this presentation is to show the benefits (effectiveness and safety) and wider possibilities of cryoablation with aim of laparoscopy to eliminate injury of critical organs for a patient with a solitary kidney from our experience.

**Material (patients) and research method used:** 71 yo woman after right radical nephrectomy for RCC (1991) and with diagnosed solid renal masses 3cm in upper pole of a solitary left kidney. She underwent Laparoscopy-assisted cryoablation in OCT 2014. Histological diagnosis was clear cell renal cell carcinoma pT1a, G2. Tumour was imaged on contrast enhanced CT before cryoablation. Under general anaesthesia laparoscopically separated upper pole of kidney from neighbor organs and ultrasound guidance was used to place the cryoprobes into renal tumour. An argon helium based cryoablation system was used with two freeze-thaw cycles, at a probe tip temperature of -40°C. Follow-up imaging with CT and physical examinations were done at the first day after cryoablation, 3 months after ablation and every 6 months afterward. Renal function was analyzed before ablation and on first day after ablation, 3 months and every 6 months afterward. She is currently well and being followed up as an outpatient for 34 months.

**Findings/ results in sufficient details to support conclusions:** CT scans before cryoablation (will be added). CT scans after cryoablation OCT2014, FEB2017 (will be added).
Results: hospital’s stay – 4 days. Postoperative complications – 0. Residual tumour – no evidence, cancer free survival >34 months. Treatment success – 100%. Postoperative creatinine - 82mcml/l - 86mcmol/l.

Conclusions and recommendations: Laparoscopically assisted cryoablation enlarges possibilities of use of cryoablation and is an effective and safe method in the management of patients with tumours in a solitary kidney who are poor candidates for surgery. To conclude about the safety and efficacy more cases and a long-term follow-up are required to confirm these results.

V-IV

Percutaneous Cryoablation of Renal Tumours: Single Institution Experience
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Background and objectives: Cryoablation is a minimal invasive procedure used to treat kidney cancer. We want to report our experience in treating renal tumours with computed tomography guided cryoablation.

Material (patients) and research method used: Retrospectively we analysed the data of 14 patients who underwent cryoablation at our institute from January 2010 until April 2017. Patient demographics and basic clinical characteristics, number of kidneys, number of procedures, size of tumours, GFR before and after operation, stage of cancer, patients follow-up and Charlson comorbidity index (CCI) was recorded. Patients were divided in two groups by CCI: A group <7, B group ≥7.

Findings/ results in sufficient details to support conclusions: Overall 22 cryoablation procedures were performed on 26 renal tumours. The mean of age was 62.9 ± 18.2 years (range, 24-83). 57% (N=8) of all patients were women and 57 % (N=8) had single kidney. The mean of tumour size was 23.2mm ± 7.4. We had 13 cases with T1a renal cancer and 1 case with metastases in kidney. Overall 50% of all patients creatinine raised after operation. The mean of GFR before the procedure was 70.50 ± 23.2 ml/min/1.73 m², but at last follow-up it reduced until 62.2 ± 21.8 ml/min/1.73 m². The recurrence of RCC was noticed in 1 patient with single kidney, when ablative margin was not possible to calculate. The median of CCI was 4.6 ± 3.6. A group consisted of 10 patients (66.7%) and B group of 4 patients (26.7%). The median of follow-up was 30.5 months (range, 3-89).

Conclusions and recommendations: Percutaneous cryoablation of renal tumours is a safe and effective minimal invasive technique for RCC patients with comorbidities and/or solitary kidney, with good functional outcomes.

V-V

First Experience of Focal Cryotherapy for Local Prostate Cancer at NCI
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Background and objectives: Prostate cancer is the most frequent cancer among males in Europe. On these days we have large selection of prostate cancer treatment options and which we choose it is depends on prostate cancer risk level. One of them is focal primary cryotherapy of prostate controlled by 3D MRI and ultrasound mapping system. A focal treatment might reduce the risk of adverse events while maintaining cancer control in carefully selected patients. For this day focal primary cryotherapy stands between active surveillance and radical prostatectomy and should be used for low and moderate risk group.

Material (patients) and research method used: Cryotherapy is a method of tissue ablation through local induction of extremely cold temperatures. In 2015, this procedure was approved by the Lithuanian accreditation service and confirmed by EAU. The procedure is performed with real-time transrectal ultrasound and MRI guidance that allows monitoring the insertion of probes and freezing. Freezing and thawing cycles are performed with argon and helium gases respectively. CT, MRI, ultrasound scanning, bone scintigraphy was performed for patient before treatment. No pathological bone changes were detected. Prostate cancer was diagnosed by multiparametric MRI and transperineal template biopsy. Findings were validated with Magnetic Resonance Prostate Imaging Reporting and Data System (PI-RADS).
For 68 years old patient, prostate cancer cT1cN0M0 Gleason 6, was diagnosed by saturation transperineal template biopsy in 2014. MRI showed masses on the left side of central zone (PI-RADS 3/3/3. At that time PSA level was 5,37 ng/ml. For patient was offered active surveillance, but he choosed primary focal prostate cryotherapy because of low complication risk. In 2015 focus cryotherapy was performed. After treatment in 2015.07 PSA level was 4,36ng/ml. In 2015.09 PSA still decreasing. At 2015.12 prostate volume 86 cm³. At 2016 MRI any masses on prostate was not detected. From 2017-05 patient is taking Dutasteridum and tamsulosinum hidrochloridum because of benign prostatic hypertrophy. The last PSA level at 2017-04 was 7.2 ng/ml. At 2017.05 MRI was performed and any masses of prostate was not detected. Currently is continued active surveillance.

Findings/ results in sufficient details to support conclusions: We described the first experience of primary focal prostate cryotherapy in Lithuania. Saturation transperineal biopsy was performed because of disease verification and target location selection. After cryotherapy any signs of disease progression was not detected. Any complications were not detected. Patient has benign prostatic hypertrophy and for this reason is taking Dutasteridum and tamsulosinum hidrochloridum.

Conclusions and recommendations: Our initial experience shows that focal cryotherapy is effective alternative minimally invasive option for the treatment of localized prostate cancer. Focal therapy has the potential to offer an array of treatments that stand midway between active surveillance and radical therapy for patients with low-to intermediate-risk disease. Transperineal (TP) biopsy with three-dimensional (3D) mapping system helps to detect the cancer and it is suitable for focal cryotherapy.

V-VI
Salvage Cryotherapy for Recurrent Prostate Cancer with Follow Up
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Background and objectives: Prostate cancer is the most frequent cancer among males in Europe. Biochemical failure after primary external beam radiotherapy for prostate cancer isn’t rare, and a significant proportion of these failures are due to local residual or recurrent disease. These patients with locally recurrent prostate cancer after primary radiotherapy can be eligible for salvage techniques treatment. We review the 6 patients treated with cryotherapy on salvage of locally recurrent prostate cancer after primary radiotherapy for all of them, PSA was increasing after radiotherapy and local recurrence was found on MRI and biopsies.

Material (patients) and research method used: Patient 1: 76 years old, in 2004 was diagnosed prostate cancer cT2aN0M0, Gleason 3+3=6, PSA 8,9 ng/ml. Patient received 70 Gy dose radiotherapy. In 2012, PSA 6,69 ng/ml. Then was started neoadjuvant hormone therapy and salvage cryotherapy. Because of urinary retention suprapubic cystostomy was performed, followed by TURP. After cryotherapy PSA was 0,02 ng/ml. In 2014 PSA, 0,66 ng/ml. In 2016 patient was studied with MRI and any masses on of prostate was not detected.

Patient 2: 62 years old, in 2006 was diagnosed prostate cancer cT2bN0M0, Gleason 3+4=7, PSA 4,36 ng/ml. Patient received 74 Gy dose radiotherapy, complicated with radiation proctitis. In 2013 PSA increased to 7,84 ng/ml. MRI and transperineal biopsy verified prostate cancer (Gleason 6). After salvage cryotherapy PSA was 0,75 ng/ml. In 2014 PSA was 1,47 ng/ml. In 2015 PSA increased to 6,1 ng/ml. Then patient was studied with MRI and any masses on of prostate was not detected. In 2015.11 Choline PET was detected masses in pubic lymph nodes. After that MDT was decided treat with external radiotherapy.

Patient 3: 69 years old, in 2004 was diagnosed prostate cancer cT3bN0M0, Gleason 3+3=6, PSA 44 ng/ml. Patient received 70 Gy dose radiotherapy and hormone therapy was started. In 2011 PSA was 4,4 ng/ml. Transperineal biopsy verified prostate cancer (Gleason 6). In 2012 PSA was 5,2 ng/ml and cryotherapy was performed. After salvage treatment in 2015 PSA - 0,02 ng/ml. In 2016.09 PSA was 0,33 ng/ml.

Patient 4: 76 years old, in 2001 was diagnosed prostate cancer cT2bN0M0, Gleason 3+3=6, PSA 44 ng/ml. Patient received 70 Gy dose radiotherapy and hormone therapy was started. In 2011 PSA was 4,4 ng/ml. Transperineal biopsy verified prostate cancer (Gleason 6). In 2012 PSA was 5,2 ng/ml and cryotherapy was performed. After salvage treatment in 2015 PSA - 0,02 ng/ml. In 2016.09 PSA was 0,33 ng/ml.

Patient 5: 68 years old, in 2007 was diagnosed prostate cancer cT2bN0M0, Gleason 3+3=6, PSA 13 ng/ml. In 2008 patient received 70 Gy dose radiotherapy and hormone therapy was started. In 2012 PSA 0,288 ng/ml. In 2013 MRI and transperineal prostate biopsy verified local recurrence on left prostate side. PSA was 6,08 ng/ml. In 2014 cryotherapy was performed. After salvage treatment in 2014 PSA - 3,83 ng/ml. 2014.08 MRI any masses of prostate was not detected. In 2015 PSA 7,58 ng/ml, MRT – without local recurrence. Bone scintigraphy any
metastasis was not detected. 2016.08 PSA 10.13 ng/ml. Any masses by MRI and bone scintigraphy was not detected. We plan to perform pelvic MRI, bone scintigraphy, lymph nodes biopsies and after that MDT discussion. Patient 6: 79 years old, in 2007 was diagnosed prostate cancer cT2bN0M0, PSA 17.6 ng/ml. Patient received 70 Gy dose radiotherapy and hormone therapy was started. In 2012 PSA 5.74 ng/ml. MRI and biopsy verified local recurrence Gleason 6. In 2014 cryotherapy was performed. After salvage treatment in 2014.05 PSA - 0.91 ng/ml. In 2015 PSA was 4.38 ng/ml. 2015 patient was studied with MRI and metastasis in internal pubic lymph node was detected. Patient received 60 Gy dose radiotherapy to internal pubic lymph node zone. After radiotherapy PSA was 0.91 ng/ml. In 2016.05 PSA was 2.76 ng/ml. MRI and bone scintigraphy any masses was not detected. In 2016.11 PSA 6.09 ng/ml. Abdomen and pelvis MRI are planned.

Findings/ results in sufficient details to support conclusions: All patients had MRI performed after cryotherapy and none of them local recurrence was not detected. Urinary retention was observed for 2 of 6 patients. Temporary suprapubic cystostomy was performed followed up TURP for one patient. For two patients cancer progressed to lymph node and these patients were treated with radiotherapy.

Conclusions and recommendations: For all patients after salvage cryotherapy PSA was decreased and local recurrence disease was not found on MRI and biopsies. Prostate cryotherapy is a safe and effective treatment option for local prostate cancer recurrence after radiotherapy for selected patient without cancer spread to lymph nodes and metastases. In 2015, this procedure was approved by the Lithuanian accreditation service and confirmed by EAU.

SESSION VI | EXPERIMENT TECHNOLOGY

VI-I
Difference of the Ice Ball Size Between on MR Image and on Ultrasonogram
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Background and objectives: During image guided cryoablation, we employ ultrasonography (US), Computed Tomography (CT)) and/or Magnetic Resonance Imaging (MRI) for targeting and monitoring. Each imaging modality represents different physical characters of frozen tissue. The aim of this study was to evaluate the accuracy of the measured diameter of the frozen area on the US and MR image.

Material (patients) and research method used: We froze a phantom (swine muscle) using an Argon-based cryomachine. A 17G MR-compatible cryo-needle was placed in the phantom, and the phantom was frozen on the table of the MR imager (0.3T, open type). A ultrasound equipment was set beside the MR table outside the 5 gauss zone. We measured the diameter of the frozen area on both US and MR images every minute.

Findings/ results in sufficient details to support conclusions: Variances of the measured ice ball sizes on US image and on MR image were homoscedasticity by F-test. And the diameter on the US image was significantly larger than on the MR image by t-test (p<0.0001). The mean difference of the size between the two modalities is 1.02±1.04 mm.

Conclusions and recommendations: Measured diameter of the frozen area is larger on US images than on MR images during cryoablation.

VI-II
Study of Cryopreserved Adipocyte Derived Mesenchymal Stem Cells (MSCS) Using Cinemicroscopy
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Cryopreservation and culture of human MSCs are indispensable techniques for the study of their use in regenerative medicine. Currently, we used NIKON D800 camera and a specially designed mini-incubator sized of 25×85×125 mm (BLAST Inc. Japan), which was incorporated on the stage of inverted microscope to make the
time-lapse cinemicroscopy of proliferation and mitosis of MSCs, which derived from frozen-thawed human adipose tissue.

The explants (about 100–300 mg) of subcutaneous and peri venous adipose tissue of 108 patients were harvested from July 1972 to April 2017 and cryopreserved in 15% glycerol in TC199 or in 10% Me2SO in IMDM solution at −196 °C. After thawing at +40°C, the explants were cultured first in the conventional incubator followed in the mini-incubator. The length of cell cycles of MSCs correctly measured by the time lapse microphotography was about 107 minutes (n=12) as for the time from condensation of intracellular structure to cleavage furrow, and about 5 hours as for the time from one MSC to two daughter MSCs. Morphologically, those MSCs were mostly fibroblasts of fusiform or stellate formed MSCs sizing 100~400 µm. It is not rare that MSCs have two nuclei. MSCs fibroblasts responded to temperature change or trypsin, and quickly transformed from fusiform to globe form. There are still several problems of MSCs that should be resolved before clinical use, one of which will be whether the MSCs cultured in the Petri dish out of the human body can work jointly with the resident MSCs in the body of the patients which have no exposure to or contact with a plastic surface. The cryopreservation delayed budding and growth of MSCs after the thawing, but there seems to be room for the improvement. Additional basic study will be necessary the further elucidation of the MSCs action in vivo.

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VI-III
Newly Developed Cryosurgical System Using Thermosiphon: a Fundamental Study and an Animal Experiment

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6. Zeon Medical Inc., Japan
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11. Strategic Innovation Division, Hamamatsu Agency for Innovation, Japan

Background and objectives: Argon-based cryosurgical devices need a large compressed gas cylinder with a pressure of 300 atmospheres. To overcome these drawbacks, we developed a new cryosurgical probe with thermosiphon effect and liquid nitrogen-cooled aluminum thermal storage blocks. The purpose of our study was to investigate the freezing capabilities of our new cryosurgical system with the aid of a poly-acrylamide gel phantom and to assess the feasibility of cryosurgical therapy with use of this system for tumor bearing rabbit model.

Material and research method used: We made a cryoprobe about 27 cm in length with separate lumens inside for liquid and gaseous ethylene to be used as a thermosiphon. The probe needle was 8 cm in length and 3 mm in outer diameter. In the fundamental study, we inserted the needle 5 cm into the poly-acrylamide gel phantom warmed to 36.5 degrees centigrade. Aluminum blocks cooled in liquid nitrogen were attached to the condenser of the probe and replaced with cooled blocks every 5 minutes to compensate for warming. We took continuous digital camera images of the ice ball and measured the temperature in certain locations of the cryoprobe and gel phantom. In the animal experiment, we used eight Japanese white rabbits inoculated with VX2 tumor cells into the muscle of the right thigh. Four days after the inoculation, a tumor approximately 2 cm in diameter was created in the thigh muscle. With the rabbits under general anesthesia we inserted the needle under ultrasound guidance. Aluminum blocks, cooled at -196 degrees centigrade in liquid nitrogen, were attached to the condenser of the probe. Thirty minutes of cooling was repeated with ten minutes of thawing period in between. During the therapy, the temperature in certain locations on the surface of the cryoprobe, the tumor and in the surrounding muscle was continuously recorded. After the cryotherapy, non-contrast MRI was performed to identify the area of ice ball formation. Two days after the therapy, precontrast and contrast enhanced MRI was again repeated to assess the
effect of cryosurgery. The animals were then euthanized and the right thigh was removed for histopathological assessments.

Findings/ results in sufficient details to support conclusions: The fundamental study showed that ice ball formation started at 30 seconds after cooling. The size at 30 minutes after the start of the procedure was 4.6×3.7 cm and the temperature of the needle reached -70 degrees centigrade. In the animal study an ice ball started forming around the needle one minute after starting cryosurgery and the needle reached -80 degrees centigrade. The average size of the ice ball measured by MRI at 16 to 20 minutes after the procedure and thawing started was 5.2×3.8 cm. The average size of the non-enhanced area on MRI was 5.6×4.3 cm and completely included the tumor geometry in six cases. In the other two cases, there were tumor cells at the peripheral margin of the treated area, which we thought was caused by needle misplacement. There were no viable tumors in the treated area of the histopathological specimen.

Conclusions and recommendations: This newly developed, compact and easy-to-handle cryoprobe using thermosiphon may be a promising device for cryosurgery of localized tumors.

VI- IV
Dependency of Cancer Stem Cell Resistance to Cryoexposure on Tumour Growth Stage
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Background and objectives: Nowadays the cryosurgery is widely applied in clinical practice when treating malignant tumors. The main task of cryosurgery, being the basis for successful tumour cryoablation, is the maximum damage of not only the common pool of tumour cells, but primarily cancer stem cells (CSCs), which are the key structure of pathogenetic tumor growth. To date there are no convincing data on the dependency of CSCs cryoresistance on tumour growth stage. Of interest was also to find out how many freeze-thawing cycles would result in a complete inhibition of tumour cell growth depending on the stage of tumour growth. Despite the fact that the cell suspensions and tissues respond differently to cryoexposure factors, in both cases the common regularities of CSCs response to freezing will be observed. Proceeding from the mentioned above, this research purpose was to study the effect of multiplicity of freezing cycles on structural and functional features of CSCs in the Ehrlich carcinoma (EC) model system, being the analogue of human breast cancer.

Material and research method used: The EC cells were obtained on the days 7 (EC-7) and 14 (EC-14) of their culturing in peritoneal cavity of BALB/c mice. The EC-7 and EC-14 cells underwent a single and multiple (2-4 times) freeze-thawing cycles in ascitic fluid with no cryoprotective agents. Proliferative potential of post-thaw EC cells was assessed on their doubling. Cell concentration with CSCs markers: CD44+CD24- and CD44high in total EC pool was evaluated with FACS Calibur flow cytometer using monoclonal antibodies to CD44+CD24- and CD44high phenotype contained in them, depending on the tumor development stage.

Findings/results in sufficient details to support conclusions: We revealed the fact that a single cryoeffect demonstrated the inhibition of functional potential for EC-7 culture and the stimulation for EC-14. After two and three-fold freeze-thawing cycles the proliferative activity of cells was inhibited for both growth terms. The inhibition rate of proliferative activity after these cryoexposures for EC-14 was more pronounced than for EC-7. There were no CD44hi cells after four-fold freeze-thawing in EC-7 and EC-14 suspensions and they lost growth potential.

Conclusions and recommendations: Thus, we may conclude about the individual features of CSCs response to single and multiple freezing cycles. Each CSCs population responds in a different way to cryopreservation with tumor process development. Our findings emphasize the need of a correct choice of terms and number of cryoeffect cycles in cryoablation performance.
VII-I

Application of Cryodestruction in Locally Spreaded Pancreatic Cancer

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**Background and objectives:** To improve the quality of life, and with the use of chemotherapy, an increase in life expectancy in patients with pancreatic cancer.

**Material (patients) and research method used:** Since 2012, we have performed cryodestruction of pancreatic tumors in 65 patients (men - 35 (53.8%), women - 30 (46.2%). The average age was 52 ± 6 years. Adenocarcinoma was detected in 59 patients (90.7%), cystadenocarcinoma - in 6 (9.3%). The size of the pancreas is 2.5 to 10 cm. In 7 pts, simultaneous cryoexposure to the pancreatic tumor and liver metastases was applied. The local destruction was supplemented by the formation of bypass Anastomoses in 42 cases (64.6%).

We used Russian devices "KRIO-MT" and "KRIO-01", as well as porous-sponge applicators made of titanium nickelide. Cryo-applicators with a diameter of 2 to 5 cm were used. The target temperature was -186 °C. Exposure time - from 3 to 5 min. The number of sessions depended on the size of the tumor and ranged from 1 to 5 (2.4 on average).

All patients subsequently underwent adjuvant chemotherapy, in 10 cases, supplemented with regional chemoembolization.

**Findings/results in sufficient details to support conclusions:** There were no lethal outcomes during the intervention and in the nearest postoperative period. Intraoperative bleeding Intraoperative bleeding was noted in 3 (4.6%) patients (due to cracks appearing on the border of the pancreatic tissue and "iceball" -1, the artery was damaged in the process of biopsy after cryodestruction - 1, bleeding from the mesenteric artery (1) stopped by X-ray endovascular intervention). Acute pancreatitis was noted in 5 (7.7%) cases; Suppuration in the manipulation zone - in 2 (3.1%), eventification - in 1 (1.5%). Ascites were detected in 12 (18.5%) patients (they were stopped conservatively for 5-8 days). After cryodestruction, the complete disappearance of the pain syndrome was observed in 39.2%, a significant decrease in its intensity - in 41.6%. Survival in Kaplan-Meier in patients with pancreatic cancer: 6 months. - 56%, 12 months. - 28%, 24 months. - 15%, 28 months. - 4.3%.

**Conclusion:** The proximity of the main vessels is not a contraindication to the conduct of cryodestruction of malignant pancreas. Cryodestruction in pancreatic cancer in inoperable patients is an intervention that significantly improves the quality of life of cancer patients, primarily by reducing the pain syndrome. With the use of chemotherapeutic treatment, there is a slight increase in survival rates.

VII-II

Cryofixation of Pancreas Resectable Malignant Solid Tumors

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**Background and objectives:** The level of radically operated patients with pancreatic cancer 5-year survival rarely exceeds 6% even in specialized centers. At the time, the pancreatic cancer is diagnosed the radical resection is possible in only 15-20% cases. In 80% of radically operated patients during first 2 years after the operation localized relapses of the disease, peritoneal dissemination, distant metastases are developing. It is connected to the intraoperative dissemination of tumor cells (TC) during the stage of tumor mobilization. The cryofixation of pancreas resectable malignant solid tumor (PRMST) before its radical removal is a promising method of intraoperative TC dissemination prevention, for which ablasic and antiblastic effects are inherent. However, the method remains completely unformed and needs to be optimized.

**Objectives:** Determine the gradient of temperature changes in the tumor tissue during cryofixation at discrete depths of 3 mm, 8 mm, 13 mm and 18 mm from the working surface of cryoapplicator and quantitatively assess the nature of the morphological changes at the points of temperature measurement.
Material (patients) and research method used: During 2015 - 2016 period the cryofixation of PRMST was performed on 21 patients (n=21) with subsequent implementation of the classic version of the resection gland depending on localization of the tumor. Ductal adenocarcinoma of pancreas was diagnosed in all cases. Cryofixation was performed using a universal cryosurgical device «Cryo-puls»: the 30 mm diameter cryoapplicator was used with temperature of - 180°C on its working surface; the period of freezing was 10 minutes; cryosurgery was performed in 2 cycles followed by spontaneous thawing after each cycle. During cryofixation, the temperature at discrete depths in the tumor at a distance of 3 mm, 8 mm, 13 mm and 18 mm from cryoapplicator working surface were registered by the developed intraoperative thermocouple four-channel complex of measuring (CMIT - 4). The average temperature at discrete depths in the tumor was evaluated, which was attained at the end of the 10th minute of the second cryofixation cycle, presented in (Table 1). After tumor removal, cuts were performed at depths, with recorded temperature (3 mm, 8mm, 13 mm and 18 mm), histological micropreparations were prepared. By using a microscope OLYMPUS CX 41 with a built-in camera we conducted a count of TC with irreversible destructive changes percentage (Table 2) in cutting manually and automatically using software Quick PHOTO MICRO 2.3 under standard conditions. Statistical data processing was carried out in the program IBM SPSS Statistics 22 and the author's package of MedStat.

Findings/ results in sufficient details to support conclusions: Number of patients with localization of the tumor in the head of pancreas (n = 14), with localization in body – (n = 6), in pancreas tail – (n = 1). The average age of patients was 57,9 (±7,8) years old. The average volume of the tumor with localization in pancreas head (n = 14) was 17,9 (±3,1) cm³, which is statistically significantly less than the volume of tumor with localization in pancreas body and tail (n = 7) – 31,6 (±5,2) cm³ at the level of significance (p<0,001).

<table>
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<tr>
<th>Depth of the temperature mesurment in the tumor (mm)</th>
<th>Temperature in Pancreas tumor (n=21), M±σ</th>
<th>The level of differences significance (p)</th>
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<tbody>
<tr>
<td>Head (n=14)</td>
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<tr>
<td>T1 (3 mm)</td>
<td>- 54,7 (±3,0)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>T2 (8 mm)</td>
<td>- 30,2 (±2,2)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>T3 (13 mm)</td>
<td>- 12,9 (±1,7)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>T4 (18 mm)</td>
<td>2,3 (±2,1)</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Body, tail (n=7)</td>
<td>- 70,1 (±1,3)</td>
<td></td>
</tr>
<tr>
<td>T1 (3 mm)</td>
<td>- 60,38 (±10)</td>
<td></td>
</tr>
<tr>
<td>T2 (8 mm)</td>
<td>- 36,3 (±5,8)</td>
<td></td>
</tr>
<tr>
<td>T3 (13 mm)</td>
<td>- 16,5 (±4,7)</td>
<td></td>
</tr>
<tr>
<td>T4 (18 mm)</td>
<td>-1,4 (±5)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Depth of the temperature mesurment in the tumor (mm)</th>
<th>Average temperature (t°C) in Pancreas tumor, M±σ</th>
<th>The percentage (%) of with irreversible destructive changes, M±σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (3 mm)</td>
<td>- 60,38 (±10)</td>
<td>99,6 (±0,7)</td>
</tr>
<tr>
<td>T2 (8 mm)</td>
<td>- 36,3 (±5,8)</td>
<td>78,5 (±7,6)</td>
</tr>
<tr>
<td>T3 (13 mm)</td>
<td>- 16,5 (±4,7)</td>
<td>28,5 (±7,4)</td>
</tr>
<tr>
<td>T4 (18 mm)</td>
<td>-1,4 (±5)</td>
<td>2,7 (±2,9)</td>
</tr>
</tbody>
</table>

Table 2

More than 70% of TC had signs of irreversible destructive changes because of primary cryofixation at a depth of 3 mm and 8 mm from the cryoapplicator working surface, where high freezing rates were reached in the temperature range from - 180°C to - 30°C. At the depths of 13 mm and 18 mm from the cryoapplicator working surface the percentage of TC, with irreversible destructive changes significantly decreased and correlated with the values of achieved temperatures at discrete depths. Investigation of the Pearson correlation coefficient showed that there is a linear correlation relationship, R<0 (R= -0,980), at the level of significance p=0,020 between the level of reached temperature and the percentage of died TC.

Conclusions and recommendations:
1. In patients with localization of a PRMST in body and tail during the cryofixation reliable lower mean temperatures at the depth of 3 mm, 8 mm, 13 mm, and 18 mm are achieved compared to patients with tumor...
localized in the pancreas head, which is due to the massive heat transfer from the main vessels in the projection of the pancreas head.

2. Cryofixation of PRMST with double cycle of 30 mm diameter cryoapplicator and the time of the freezing period exposition of 10 minutes with subsequent spontaneous thawing does not guarantee 100% irreversible destruction of TC due to primary cryodamaging at depth of 3 mm, 8mm, 13mm and 18 mm from cryoapplicator working surface.

3. We are looking forward to the development of effective ways to enhance cryofixation.

VII-III

Local Cryoapplication in the Treatment of Infected Pancreatic Necrosis

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3. IC Biomedstandart, Russia

Background: Local cryoapplication (LC) promotes primary breakage of the pathological biochemical and morphological changes cascade in the pancreas and retroperitoneal tissue, leads to changes in the pathological process.

Objective: To estimate the results of LC application to pancreas in different forms of infected pancreatic necrosis (IPN).

Materials and methods: 208 patients with IPN and failure of medical therapy underwent surgery in the hospital surgery clinic of Volgograd State Medical University. The patients were divided into two groups with comparable severity of condition. The main group included 95 patients, who were treated with LC. The control group consisted of 113 patients. 63 (66,5±6,5%) in the main group and 80 (70,8±6,6%) in the control group had initial complication of IPN with puruloncrotic parapancreatic necrosis (PNPPN) (p>0,05). Extensive purulent peritonitis (EPP) was initially detected in 27 (28,4±3,0%) cases in the main group and 34 (30,1±2,9%) in the control group (p>0,05). Cryotherapy on pancreas was conducted by application method.

Results: After the laparotomy progression of PNPPN was observed in 12 (19,0±2,3%) cases in the main group and 29 (36,3±4,0%) in the control group (p<0,001). There were no cases of progression of EPP in the main group and 4 (11,8 %) in the control group. 2 patients (2,1%) in the main group and 5 (4,4%) in the control group had complication with bleeding. Septic course of disease was identified in 11 (11,6%) cases in the main group and 26 (23,0%) in the control group. 1 patient (1,1%) in the main group and 3 (2,7 %) in the control group had pancreatic fistulas and also 2 (1,8%) patients in the control group had intestinal fistulas. All differences are statistically significant (p<0,001).

The patients in the main group showed considerable improvement of hemodynamics and reduction of gastrointestinal tract paresis on 6-7 day of postoperative period. The control group demonstrated positive changes in organ functional characteristics only on 10-14 day.

Postoperative lethality with IPN in the main group accounted for 14,7±1,5% (14 out of 95 patients) in the main group and 27,4±2,5% (31 out of 113 patients) in the control group (p<0,001).

Conclusion: Our data give evidence of significant multidirectional medicinal effect of LC. This effect is recorded in all the forms of pancreatic necrosis and proves the necessity of LC to reduce the complications and lethality of IPN. For its full-scale use new engineering and technical, and medical developments and further research of biological, biochemical and biophysical alterations in P with LC should be continued.

VII-IV

Experience of Cryochemical Ablation in Complex Treatment of Patients with Focal Liver Lesions

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1. Bogomolets National Medical University, Department of General Surgery, Ukraine
2. Kyiv Center of Hepatopancreatobilary Surgery named after Zemskov, Ukraine

Background and objectives: There was a need to develop new and improve existing methods of local cytoreductive surgery, due to unsatisfactory long-term results of focal liver lesions treatment. Among the large
arsenal of local destruction methods of focal liver lesions, in our opinion, the most promising is the cryosurgical method. Despite its wide application in modern surgical hepatology, some issues related to potentiation of its cytodestructive effect remain unresolved.

Aim was to evaluate the results of cryochemical ablation in the complex treatment of patients with focal liver lesions.

**Material (patients) and research method used:** In the period from 2012 to 2017 cryochemical ablation was performed in 112 patients (mean age 58.4 years): primary liver cancer (n = 6), metachronous metastases (n = 81), synchronous metastases (n = 25). The cryochemical ablation technique included intraoperative chemical ablation of the tumor focus with a 10% solution of calcium chloride + focus cryodestruction. A total number of 397 (min-1, max-5) tumor lesions were combined. The diameter of the foci was from 1.0 to 8.6 cm (average - 3.7 cm): up to 3.0 cm - 214 (53.9%), 3.1-5.0 cm - 152 (38.3%), more 5.0 cm - 31 (7.8%). All patients in the postoperative period received chemotherapy according to accepted standards in accordance to tumor pathology. Postoperative morbidity and mortality, tumor response (RECIST1.1), conversion to resectability, frequency of local recurrence were assessed. The median follow-up period was 22.4 months

**Findings/ results in sufficient details to support conclusions:** Indications to cryochemical ablation were: bilobar multiple lesions, central localization of foci, insufficiency of functional reserves of the liver parenchyma, the presence of general contraindications to resection, the patient's refusal from resection. The postoperative morbidity associated with cryochemical ablation was 6.3% (n = 7). The severity of postoperative complications associated with cryochemical ablation, according to Clavien-Dindo classification, corresponded to the I-II grade. Postoperative mortality associated with cryochemical ablation was not noted. According to RECIST1.1 by MRI data: a complete response was obtained in 22.8% of tumor sites, partial response - in 46.7%, stable disease - in 25.1% and progressive disease - in 5.4%. In 36.4% of patients it was possible to perform a liver resection later.

**Conclusions and recommendations:** Cryochemical ablation is a safe method of local cytoreduction in the treatment of patients with liver lesions, which promotes conversion to resectability, and can be used for local tumor control. However, further study of the effect of cryochemical ablation on long-term oncological outcomes are required.

**VII-V**

**Possibility of Cryosurgery Application in Primary and Metastatic Liver Cancer**

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**Background and objectives:** To improve the quality of life, and with the use of chemotherapeutic treatment, an increase in life expectancy in patients with primary and metastatic liver cancer.

**Material (patients) and research method used:** Since 2012 we have performed cryodestruction (CD) in 10 patients with primary and 40 metastatic liver cancer. Among patients (5 men and 5 women) with primary cancer of HCC was observed in 6 and HCCC – 4. The average age was 49+6 years. In patients with secondary liver lesions (25 men, 15 women) interventions were performed for metastases of colorectal cancer (CRC) (17), pancreatic cancer (13), gastric cancer (3), neuroendocrine tumors (2), cancer of the bile ducts (2), MDP-cancer (1), breast cancer (1), and kidneys (1). The mean age was 57+6 years.

CD was used by the Russian devices "CRYO-MT" and "KRIIO-01", as well as porous, spongy applicators Nickel-titanium. Used cryoapplicator diameter 1 to 5 cm of the Target temperature to -186 °C. Exposure time - from 1 to 5 minutes. The number of sessions depended on the size of the tumor and ranged from 1 to 8 (average is 3.6). All patients were subsequently conducted adjuvant chemotherapy in 16 cases, supplemented by regional chemoembolization.

In the group of patients with primary liver cancer CD 3 observations were made in combination with liver resection and RFA. Such combined surgical treatment in patients with metastatic defeat of a liver was used in 6 cases. In 5 patients with HCC and 1 with metastatic CRC CD was supplemented by percutaneous alcoholization in the postoperative period.

**Findings/ results in sufficient details to support conclusions:** In the postoperative period in the group of patients with primary liver cancer was noted intra-abdominal bleeding in 1 (12.5%) case; in one case (12.5%) renal and hepatic failure led to the development of the syndrome of multiple organ failure and then to death on the 47th day after the intervention (liver resection+ CD+RFA).
In the group of mts of the liver in the postoperative period was marked by the following complications: intra-abdominal bleeding -2 (5%) monitoring, gastric bleeding in 1 (2.5%), biliary fistula – 1 (2.5%), ascites in 6 (15%), hydrothorax – 4 (10%), right lower lobe pneumonia - 2 (5%). The survival rate according to Kaplan-Meier in patients with primary liver cancer: 6 months. – 87.5%, 12 mo. – 75%, 24 months. – 62.5%, 28 months. – 50%. The survival rate according to Kaplan-Meier in patients with liver MTS 6 months. – 95%, 12 months. – 72%, 24 months. 60% in 36 months -28%.

Conclusions and recommendations: Cryodestruction in primary and metastatic liver cancer have unresectable patients is the intervention greatly improves the quality of life of cancer patients, primarily by reducing pain. In connection with the improvement of the quality of life on a background of appropriate adjuvant chemotherapy and noted some improvement in survival rates. A multidisciplinary approach to treatment is key in the successful treatment of such patients.

VII-VI
Cryo-Assisted Resection en bloc, and Cryo-Ablation in situ, of Primary Breast Cancer Coupled with Intraoperative Ultrasound-Guided Tracer Injection

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2. International Cryosurgery Center for Cancer, China
3. Jinan University School of Medicine, Fuda Cancer Hospital, China
4. Toho University, Omori Medical Center, Japan
5. Interventional Drug Delivery Systems and Strategies, USA

Background: The therapy for breast cancer is multimodal. This is the first study to look at the transport and loco-regional distribution of a dye injected into a breast tumor, subjected to simultaneous cryo-assisted resection and cryoablation.

Objective: The aim of the study was to perform cryosurgery on a primary breast tumor, coupled with simultaneous peritumoral and intratumoral tracer injection of a blue dye, to evaluate lymphatic mapping. We explored the ability of our strategy to prevent tumor cells, but not that of injected tracers, to migrate to the lymphovascular drainage during conventional resection of frozen breast malignancies.

Material and methods: Seventeen patients aged 51 ± 14 years (mean ± SD), presenting primary breast cancer with stage I-IV, were operated on, and included in this preliminary clinical study. Under intraoperative ultrasound, fourteen patients underwent curative cryo-assisted tumor resection en bloc (CATR), coupled with peritumoral tracer injection (PTI), which consisted of complete tumor freezing and concomitant peritumor injection with a blue dye, before resection and sentinel lymph node dissection (group A). In group B, three patients previously refused any standard therapy and had palliative tumor cryoablation in situ (TCA) combined with intratumoral tracer injection (ITI). Double-deep-freeze starts with first freeze concomitantly with peritumoral dye injection, and ice block formation before resection. This freeze-inject-thaw-refreeze procedure takes about 16 minutes from beginning to completion. The blue dye injected at the tumor margin (peritumoral) at the end of the first freezing migrates diffusely into the tumor tissue (TT) and distributes widely into the breast parenchyma. After second freezing, the ice block containing the TT is completely resected on an unfrozen breast margin with the following sentinel lymph node dissection.

Results: The IOUS facilitated needle positioning and dye injection timing. In group A, the frozen site extruded the dye that was distributed through the unfrozen tumor, the breast tissue, and the resection cavity for 12 patients. One to four lymph nodes were stained for 10 out of 14 patients. The resection margin was evaluable. Cryo-assisted tumor resection en bloc and peritumoral tracer injection, in patients with primary breast carcinoma, i.e. intraoperative sequence showing cryo-assisted tumor resection en bloc, concomitant with peritumoral tracer injection of a breast tumor, located in the upper right outer quadrant (group A). The cryoprobe (CP) tip, 25 mm in diameter, freezes and sticks to the surface of a 19-mm-wide tumor and a margin of breast parenchyma. The injection needle (white arrow), inserted into a deeper, not yet frozen part of the tumor, at the tumor margin, is shown injecting 2 ml of patent blue vital dye under US imaging (white arrowhead). The longitudinal view sonogram shows the hyperechoic ice margin (blue arrow) in the anterior aspect (ant.) of the tumor. The flow (three yellow arrows) of the dye is detected at the posterior (post.) aspect of the tumor about to be frozen. A fluid dye channel, anechoic, is located between the ice margin and the breast tissue. The CP and the needle have been removed after the second freeze, but the tumor surface is still frozen and the blue staining is visible at the frozen
tumor margin (arrows) just before the tumor resection en bloc. Sentinel lymph node dissection reveals a stained node (arrow) in the axillary region. Some staining of the resection cavity is seen.

**Conclusions:** Our IOUS-guided performance revealed the injection and migration of a blue dye during the frozen resection en bloc and cryoablation in situ of primary breast tumors. Sentinel lymph node mapping, pathological determination of the tumor and resection margins were achievable. The study paves the way for intraoperative cryo-assisted therapeutic strategies for breast cancer. We have shown that our cryosurgical technique of repeatedly freezing deep tumors for en bloc resection or for in situ ablation of primary breast cancer, facilitated by IOUS monitoring, can be combined with the simultaneous injection of dye tracers during conventional surgery, which then allows for lymphatic mapping.

**VII-VII**

**Cryoballoon Ablation of Atrial Fibrillation: Advantages and Challenges**

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Catheter ablation of atrial fibrillation (AF) is a well-established method of interventional treatment of symptomatic patients suffering from this most common type of sustained tachyarrhythmia. Pulmonary vein isolation (PVI) is considered as a cornerstone of AF ablation procedure, and could be extended with additional ablation maneuvers in a case of persistent or long-standing persistent AF [1]. Two comparable energy sources could be used for PVI procedure to achieve tissue necrosis and electrical isolation of atrial tissue blocking the ectopic activation from the pulmonary veins (PV): radiofrequency (RF) energy (heat) and cryoballoon (CB) ablation (cold) [2]. The effectiveness and safety of two competing methods is comparable: acute electrical isolation of PV could be achieved in 98 - 100% of cases using both energy sources [3,4], freedom from AF relapse was 65.6% using CB and 60.1% using RF ablation, rate of procedure-related complications was 4.7% for CB and 4.3% for RF procedures [5,6]. As RF ablation procedure is performed as point-by-point energy applications and CB is a single-shot device isolating entire circumference of PV entrance area, PVI using CB is related with shorter procedure time and steeper learning curve [7]. The only more frequent complication with CB ablation is a phrenic nerve palsy [5], which usually resolves within 1 year from the ablation procedure.

**VII-VIII**

**Cryoanalgesia - part of interventional chronic pain management**

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Cryoanalgesia, also known as cryoneuroablation or cryoneurolysis, is a specialized technique for providing pain relief in chronic neuropathic pain conditions. It is a method of relieving pain, by freezing the affected nerve branches, involved in conduction of the pain. The procedure is used for the treatment of various intractable pain conditions and involves the precise location and freezing of the nerves associated with the neuropathic pain that originates from relatively small, well localized lesions such as neuromas, entrapment neuropathies, and postoperative pain. It has also been used to obtain pain relief in biomechanical pain syndromes including lumbar or cervical facet syndromes, and coccygodynia. The most common use for cryoanalgesia for lower back pain is the long-term treatment of lumbar facet pathology. Sustained blockade of afferent impulses with cryoanalgesia may reduce plasticity (windup) in the central nervous system and lead to long lasting analgesia. Permanent blockade does not usually occur, since the affected axons regenerate. Furthermore, cryoablation avoids the complication of causing or exacerbating pain. This can occur with a neurotomy or non-pulsed thermocoagulation with radiofrequency. With cryoablation, the axons and myelin sheaths are lysed (Wallerian degeneration), but the epineurium and perineurium remain intact, which facilitates successful nerve regeneration. The affected axons are unlikely to form neuromas, often associated with traumatic, surgical, and thermal lesions that interrupt perineurium and epineurium. The contact surface area of a cryoablation probe on larger nerves provides more complete neurolysis than pulsed radio frequency procedure. Furthermore, cryoablation provides immediate analgesia in the affected area while there is a delay of up to a week with pulsed radiofrequency.
Despite small risk of procedure and good results in case reports or case series there is not much randomized control studies we can find in literature.

In conclusion cryoneuromaoblation could be an effective interventional pain management technique that providing significant neuropathic pain relief, but still more randomized control trials are needed.

SESSION VIII | THORACIC-BRONCHIAL

VIII-I

4D Radiation Therapy for Lung Cancer

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2. National Cancer Institute, Lithuania
3. Vilnius University Hospital Santaros Klinikos, Lithuania

Background and objectives: The aim of the work was to determine the difference between 3D and 4D CT models for irradiated volumes in lung cancer radiation.

Material (patients) and research method used: Prospectively analyzed the anonymize data of 9 patients with non-small cell lung cancer in the National Cancer Institute 2016-2017. When planning radiotherapy, studies are carried out to localize the irradiation target and calculate its volume. For all patients was done computer tomography of the chest. Sections were made every 2.5 mm. The above six patients were additionally subjected to a four-dimensional (4D) protocol using the MIP (maximum intensity projection) protocol. During the study, breathing movements are recorded. The data were correlated with 3D CT imaging kits for the same patients, evaluating 5 respiratory phases. The precise dosage calculation uses the Radiation Therapy Planning System (TPS), the Dose Count Algorithm - Anisotropic Analytical Algorithm (Version 10.0.28). For statistical analysis was used SPSS version 23, the c2 test was used for statistical reliability and t-test for independent samples. The chosen statistical reliability level was p < 0.05.

Findings/ results in sufficient details to support conclusions: The mean age of patients was 65 ± 10.3 years. These patients have stage I-III non-small cell lung cancer (adenocarcinoma, platelet carcinoma of the lungs (G2-G3), 3D KT CTV median - 356.71 cm², median CTV equivalent spherical level - 7.27 cm, 4D KT CTV median - 165.70 cm², the median plane of the equivalent plane of the CTV is 5.75 cm. The median of the 3D CT PTV is 536.83 cm², the median of the PTV equivalent spherical level is 10.07 cm, the median of the 4D KT PTV is 369.21 cm², the PTV equivalent spherical level Median - 8.03.

Conclusions and recommendations: By applying 4D CT, a significantly lower volume of irradiated target is calculated. The planned lower volume of irradiated target can have a significant effect on the damage to the healthy lung tissue and critical organs.

VIII-II

Transbronchial Cryobiopsy in the Diagnostics of Interstitial Lung Diseases – First Experience in Hospital of Lithuanian University of Health Sciences Kauno Klinikos

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Background and objectives: Although in some cases clinical and radiographic features may be sufficient to establish a diagnosis of interstitial lung diseases (ILD), surgical lung biopsy is frequently required. Recently a new technique for bronchoscopic lung biopsy has been developed using flexible cryo-probes in Hospital of Lithuanian University of Health Sciences Kauno Klinikos. In this abstract, we will describe our first clinical experience using bronchoscopic cryobiopsy for diagnosis of diffuse lung disease.

Material (patients) and research method used: It was decided to perform bronchoscopic cryobiopsy by multidisciplinary team for patients who had clinical and radiographic features of ILD in high-resolution computed tomography (HRCT). Cryo-transbronchial lung biopsies (cryo-TBBs) were performed in the most affected lobes according to imaging studies of the chest. Due to the fact, that first cryobiopsy was performed on April 2017th, our study includes cases of just three patients.
All procedures were performed in bronchoscopy suite with supplement oxygen delivery and X-ray inspection. After deep sedation with Propofol and usage of muscle relaxants, a rigid bronchoscope was inserted in patients’ bronchial tree. High frequency jet ventilator (HFJV) was attached to rigid bronchoscope. Continuous records of oxyhaemoglobin saturation, heart rate and ECG were made. During flexible bronchoscopy, a flexible cryoprobe was introduced into the selected bronchus. The probe was placed at least 10mm away the rib cage, to avoid rupturing visceral pleura. Since bleeding can be, we applied an endobronchial balloon tamponade (Olympus balloon) proximal to the biopsied subsegment to immediately occlude the airway and provide better control of bleeding. Once brought into position, the probe was cooled and then retracted with the frozen lung tissue being attached on the probe’s tip. The tissue was processed for histology.

Findings/results in sufficient details to support conclusions: Our first patient, who underwent cryobiopsy, is 55 years male. This patient main complaints were dyspnea and sweating, lasting about six months. This patient is not smoking for 2 years, but earlier has 35 packs per year smoking history. Chest HRCT was performed and it showed interstitial changes in upper parts of lungs and ground glass opacity in the lower parts of lungs (on the left S8-10, on the right part – S9- S10). In dynamics, lung interstitial changes were progressive. For diagnosis specification, it was decided to perform cryo – TBB. Biopsy showed changes, typical to usual interstitial pneumonia (Fig. 1).

Second patient, who underwent cryo – TBB, is 64 years woman. Her complaint was like our first patient – dyspnea. She has anamnesis of chronic obstructive pulmonary disease (COPD), even though she hasn’t anamnesis of smoking. According HRCT, diagnosis of cryptogenic organizing pneumonia was formulated. There was no lymph node or pleural abnormalities. Since dyspnea was progressing in three months, it was decided to perform cryo – TBB. Histological specimen shows changes typical to MALT lymphoma (lymphocytic infiltration).

Our third patient is 65 years woman. Her main complaint is intensive dry cough, lasting for about one year, interrupting her normal day routine. Chest HRCT showed fibrotic changes in the lungs. Although, according cryo – TBB, where there not enough information for interstitial pneumonia.

There were no severe complications during or after cryo – TBB. All specimens were about 5 – 6 millimeters diameter. It was taken two specimens from most affected parts of the lungs from every patient (Fig.2).

Conclusions and recommendations: Cryo-TBB following our method is a minimally invasive, rapid, safe and economic technique that can be performed in a bronchoscopy suite under moderate sedation without the need for intubating the patient or using fluoroscopy, and without requiring general anesthesia. Transbronchial cryobiopsy is a novel technique which allows to obtain large biopsy samples of lung parenchyma that exceed the size and quality of forceps biopsy samples.
I


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2. Division of General Surgery, Taiwan
3. Comprehensive Breast Cancer Center, Department of Surgery, Taiwan
4. School of Medicine, National Yang Ming University, Taiwan
5. Division of Plastic and Reconstructive Surgery, Department of Surgery, Changhua Christian Hospital, Taiwan

Background and objectives: Robotic surgery, which incorporated 3-dimensional imaging system and flexibility of robotic arm, had been growingly used in different field of surgeries. Robotic nipple sparing mastectomy (R-NSM), which introduce Da Vinci surgical platform through a small axillary wound to perform NSM and immediate breast reconstruction, was reported to have potential to overcome the technique difficulty of endoscopic NSM and showed promising cosmetic outcome. In this study, we report the preliminary experience and clinical outcome of R-NSM.

Material (patients) and research method used: The medical records of patients who underwent R-NSM for breast cancer during the period March 2017 to August 2017 were collected from endoscopic breast surgery database at Changhua Christian Hospital (CCH), a tertiary medical center in central of Taiwan. Data on clinicopathologic characteristics, type of surgery, method of breast reconstruction, complications and recurrence were analysed to determine the effectiveness and oncologic safety of R-NSM.

Findings/ results in sufficient details to support conclusions: A total of 15 patients with breast cancer received R-NSM during the study period. The mean age of them was 45.1 ± 10.5 years (29-62), and the mean tumor size was 2.19 ± 0.85cm (1.20-3.43). The pathologic stage was 26.6% DCIS, 26.6% stage I, 33.3% stage II, and 13.3% stage III. All of them received immediate breast reconstruction (IBR) with Gel implant, and the mean operation time for R-NSM with IBR was 324.5 ± 93.2 mins (225-505). The mean hospital stay was 6.7 ± 1.3 days (5-9). The positive surgical margin rate was 0%. One patient suffered from delayed axillary wound healing, and one patient suffered from small area of skin flap blister formation. No major perioperative complication was found. No (0%) total nipple areolar complex necrosis case was observed. No local recurrence, distant metastasis or case mortality during follow-up.

Conclusions and recommendations: From our preliminary experience, R-NSM combined IBR with Gel implant is a safe procedure, with good cosmetic results, and cloud be a promising new technique for breast cancer patients indicated for mastectomy.

II

How PI-RADS determine different multiparametric magnetic resonance results

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Background and objectives: Standardized Prostate Imaging Reporting and Data System (PI-RADS) guidelines for the assessment of prostate alterations were de- signed for the assessment of prostate pathology. Published by the ESUR in 2012, PI-RADS v1 was based on the total score of different MRI sequences with subsequent calculation. PI-RADS v2 was published by the American College of Radiology in 2015 and featured different assessment criteria for prostate peripheral and transitory zones. The aim of our study was to assess the correlations of PI-RADS v1 and PI-RADS v2 with Gleason score values and to define their predictive values of the diagnosis of prostate cancer.

Material (patients) and research method used: A retrospective analysis of 66 patients. Prostate specific antigen (PSA) value and the Gleason score (GS) were assessed. One the most malignant focal lesion was selected in the peripheral zone of each lobe of the prostate (91 in total). Statistical analysis was carried out applying SPSS so ware, v.23, p < 0.05.
Findings/results in sufficient details to support conclusions: Focal lesions assessed by PI-RADS v1 score: 10% – 1, 12% – 2, 41% – 3, 23% – 4, 14% – 5. Assessment applying PI-RADS v.2: 20% – 1, 7.5% – 2, 26%, 29.5%, and 17% were assessed by 3, 4, and 5 scores. Statistically relevant correlation was found only between GS and PI-RADS ($p = 0.033$).
The positive predictive value of both versions of PI-RADS – 75%, negative predictive value of PI-RADS v1 – 46%, PI-RADS v.2 – 43%.
Conclusions and recommendations: PI-RADS v1 was more statistically relevant in assessing the grade of tumor. Prediction values were similar in both versions.

III

Sex, Age and Clinical Agents Influence on Melanoma Survival Prognosis

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2. National Cancer Institute, Lithuania

Background and objectives: Melanoma is the most dangerous form of skin cancer. Morbidity of this disease is increasing every year. Previous studies have revealed that there are some demographic and clinical factors having effect on melanoma survival prognosis. Purpose of our study to assess melanoma survival by age, sex, stage, depth, histology, and anatomical site.

Material (patients) and research method used: We investigated melanoma specific survival up to 10 years from diagnosis for 85 first primary cases of melanoma diagnosed at National Cancer Institute in 2006. Cox multivariate analysis was performed for 1-year, 5-year and 10-year survival. Data were processed using Microsoft Excel, data analysis was conducted using SPSS® software.

Findings/results in sufficient details to support conclusions: The study involved 51 women (60%) and 34 men (40%). The average age was 58 years. Females had better one-year (88.24%), five-year (69%) and ten-year (61%) survival than men (85%, 38%, and 32%, respectively). However, melanoma-specific survival dependent on sex did not have a statistically significant difference ($p = 0.121$). Patients given the diagnosis at the age of 65 and older had lower 10-year survival than younger people (44.44% vs. 46% and 75%). Melanoma-specific survival in different age groups did not have a statistically significant difference ($p = 0.455$). Melanomas that had been diagnosed at IV stage or were thicker than 4.00 mm had lower survival (accordingly 5-year survival: 12.5% and 26.66%). The stage ($p = 0.003$) and depth ($p = 0.049$) of melanoma had a statistically significant impact on melanoma survival. Nodular melanoma had the poorest 5-year and 10-year melanoma specific survival among histological subtypes (51.67% and 38.75%). Melanoma survival in different histological subtype groups did not have a statistically significant difference ($p = 0.364$). Melanoma diagnosed on the skin of the back/breast had the worst survival (one-year – 92.59%, five-year – 44.44%, and ten-year – 37.03%). However, melanoma-specific survival dependent on the anatomic site did not have a statistically significant difference ($p = 0.457$).

Conclusions and recommendations: Tendency of melanoma morbidity – two-thirds of patients are female and one-third male. The average age of patients with melanoma is 58–59 years. Melanoma thicker than 4.00 mm is statistically significant 1, 5 and 10 years melanoma survival prognostic factor. The stage IV melanoma is statistically significant 1, 5 and 10 years melanoma survival factor. There were no statistically significant results for 1, 5 and 10 years melanoma survival depending on sex, age, histology and anatomic sites.

IV

Combined Cryotherapy, Topical Imiquimod and Surgical Resection for Treatment of Locally Advanced Basal Cell Carcinomas

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Objective: The aim of the study is to demonstrate the efficacy of combined cryotherapy, topical imiquimod and surgical resection for treatment of giant basal cell carcinomas (BCC)
**Background:** Locally advanced BCC can sometimes pose significant treatment challenges. BCC is the commonest skin cancer in Caucasians, usually easily diagnosed and surgically resected, but can sometimes pose significant treatment challenges due to locally advanced presentation.

**Methods and patients:** We identified a number of mostly elderly patients with locally advanced huge BCC in craniofacial region where primary surgical resection was impossible. We used tailored approach where treatment was started with topical imiquimod followed by cryotherapy and finally surgical resection of residual tumor.

**Results:** All patients achieved complete response with minimal side effects. The duration of treatment varied due to cooperability of patients or their relatives but relatively good cosmetic outcome was finally achieved in all cases.

**Conclusions and recommendations:** Combined treatment of cryo+imiquimode+surgery offers a good effective modality for treatment of locally advanced BCC in cases where simple treatment options need to be substituted by tailored approach.
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