TUMOUR DEVASCULARISATION

A COMPASSIONATE USE PROTOCOL

PILOT STUDY OF AN IMMUNOSURGICAL INTERVENTION OF METASTATIC SOLID TUMOURS

Main researcher:
David Kopsky, MD, secretary of the foundation IOCOB, www.iocob.nl
e-mail: djkopsky@iocob.nl telephone: 00-31-628671847, address: Vespuccistraat 64-III, 1056 SN, Amsterdam, the Netherlands

Co-researchers:
Prof. dr. Jan M. Keppel Hesselink, MD, PhD, chairman of the foundation IOCOB
Remco Liebregts MD, anaesthesiologist

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SUMMARY

Rationale: At the moment, no validated curative therapy is currently available for metastatic solid tumours. Adjuvant therapy can prolong life, though side effects influence strongly the quality of life. Chemotherapy and radiotherapy can prolong life expectancy, though side effects influence strongly the quality of life.
In the Czech Republic a surgical technique is developed to treat certain metastatic solid tumours. This technique is called tumour devascularisation and can be seen as a variation of autoimmunisation techniques such as cancer vaccination using autologous tumour cell-BCG vaccine (OncoVAX) or autologous heat shock protein gp96-tumour peptide complexes (Oncophage). Non-resorbable sutures are used during the procedure to isolate the primary tumour from its vascularisation, and the devascularised tumour is left in situ. Animal models and human case reports show that the devascularised tumour necrotises following the intervention and the metastases can go in regression. Based on these preclinical experiments and the collected case reports this tumour devascularisation procedure seems safe, it does not induce sepsis and the intervention might result in reducing cancer pain. This technique could be of a great value in developing counties, because it is cheap, simple, easy to learn, quick and no chemo- or radiotherapy is involved.
Objective: The objective of this pilot study is to investigate and confirm the safety and feasibility of the devascularisation technique for metastatic solid tumours.
Study design: Pilot study using an open uncontrolled design; observational study
Study population: Twelve patients with metastatic colon cancer, renal cell carcinoma or melanoma, in which chemotherapy or radiotherapy is no clinical relevant option and the patients are not immune compromised.
Intervention (if applicable): The tumour will be devascularised by sutures. This can be the primary tumour or metastases in case of absence of the primary tumour.
Renal cell carcinoma: the hilar structures (arteries, veins and ureter) will be closed.
Colon carcinoma: the mesenteric blood vessels will be closed, the lumen of the intestine with the tumour will be closed, and a new anastomosis of the healthy intestine will be created.
Melanoma: sutures will be placed at the base of skin metastases; metastases of the lymph nodes will be devascularised and party debulked if the lymph nodes are superficial.
Main study parameters/endpoints: The purpose of the pilot study is to confirm the feasibility and safety of the technique, documented on a 5 point scale. The tumour load before and after the operation will be recorded.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: When a patient has metastatic melanomas superficially on the skin and lymph nodes, the procedure can be done under local anaesthesia and is not a great burden. In case of a metastatic renal cell carcinoma or colon carcinoma the burden is the same as a standard operation for removing the primary tumour. After the tumour devascularisation, patients can have 4 days of sub febrile elevation of temperature. Other well known complications due to a surgical procedure may also occur. No significant risks are involved in the follow up, which will consist of a standard follow-up in cancer patients (see table).

The risk-benefit ratio is relatively low. Stage IV carcinoma indicates that the patients will die in the near future. Several case reports, different animal studies and the latest understanding of tumour immunisation have shown that this technique has a chance to reduce metastases in other places of the body or prolong the progression free survival.

	Screening	1-7 days	2 weeks	4 weeks	3 months	6 months
Informed consent	X					
Medical History	X					
Demographic details	X					
In- exclusion criteria	X					
EORTC-30 questionnaire	X				X	X
Lab[1]	X	X	X	X	X	X
Appropriate monitoring techniques will be used according to the type of tumour:						
CT-abdomen	X			X	X	X
PET/ MRI	X				X	
Colonscopy	X				X	
X-thorax	X			X	X	X
Skin photo's	X			X	X	X

1) CRP, LDH, AF, leucocytes with differentiation and subpopulations, CD3+, CD4+, CD8+, CD16+/CD56+, CD28+, Th/Tc ratio, IgG total, IgM en IgA, liver and kidney parameters, Tumour markers, Full standard lab analysis.

INTRODUCTION AND RATIONALE

At the moment, no validated curative therapy is currently available for metastatic solid tumours. In western countries between 20 to 30% of the patients with colorectal cancer present themselves in stage IV.[1] [2] After resection of the primary tumour, often recurrence of cancer arises on the basis of micro metastases. Chemotherapy and radiotherapy can increase life expectancy, though side effects influence strongly the quality of life. Also medical cost increases with increasing tumour stage, mainly due to adjuvant treatments and palliative care.[3]
In developing countries delayed presentation of solid tumours in an advanced stage is another concern. More than two thirds of the patients with colorectal cancer have stage III or IV at presentation.[4,5] The late presentation of advanced cancer in developing countries counts also for other types of tumours like prostate cancer,[6] Wilms tumour,[7] nephroblastoma,[8] breast cancer,[9,10] melanocarcinoma,[11,12] orofacial tumours.[13] A higher incidence of advanced cancer is seen in patients with a poor socio-economic status.[14] Chemotherapy is not given regularly, because most of the patients are poor and a free health care facility is not available in most developing countries, these drugs are not affordable to many of the patients.[5] Many countries of low or middle income have limited access to radiotherapy, and 22 African and Asian countries have this service not at all.[15,16]

The last decade activation of the immune system is the main focus of the development of new cancer therapies, because some tumours are immunogenic. Phase I and II studies of melanoma-specific vaccines, like whole tumour cells, antigen peptides, antigen-pulsed dendritic cells, recombinant viruses, plasmids or naked DNA, and heat-shock proteins, were promising, though in the few large phase III randomized clinical trials the vaccines have altogether failed to prove their efficacy.[17] These individual techniques only use one part of the mechanism of the immune system to attack tumours.
In the Czech Republic a novel surgical technique is being developed to treat metastatic solid tumours. This technique is named devitalisation, though in this protocol it will be called tumour devascularisation.[18] Tumour devascularisation reduces the tumour suppressive properties by reducing the tumour load. This technique also activates anti-tumour immune reaction, due to the release of a huge amount of tumour antigens, the release of Heat Shock Proteins and stimulation of the dendritic cells. Furthermore a high infiltration of malignant cell clusters with the helper and cytotoxic T-lymphocytes might lead to further reduction of tumour load.

1.1. The reason of this pilot study

This pilot study on tumour devascularisation could be of great value, because there has not been done yet an observational study on this interesting immunosurgical intervention. This pilot study will investigate the safety and feasibility of the devascularisation technique for metastatic solid tumours. The clinical, laboratory and imaging technique investigations before and after the procedure can perhaps help to indicate how much tumour load has to be devascularised to activate efficiently the immune system for reducing the non-devascularised

metastases. The laboratory measures and imaging techniques also monitor the patient after the procedure. This study has to be done to confirm the safety of the technique: the only described side-effect of the technique is 3 to 4 days sub febrile elevation of body temperature. The possible advantages of this technique are reduction or stabilisation of metastases that are not devascularised due to activation of the cellular immune system and the possible reduction of chemo- and/or radiotherapy with its' burden and diminishing pain due to tumour in growth.

1.2. Scientific and social relevance

The last decade a lot of research has been done on tumour immunology. Immunologic cancer therapies have been developed to treat cancer. Mainly only a part of the whole immunologic mechanism is focussed on in the novel cancer therapies, like tumour antigen antibody therapy, pulsed dendritic cell therapy, or the use of TNF-α or interleukin 2. Tumour devascularisation attacks the tumour by stimulating the immune system in multiple ways, which combines the immunological targets of novel cancer therapies.
On the social level this technique could also be preformed easily in third world countries, because of its' low costs and the more frequent presentation of metastatic cancer. Because patients in third world countries usually have to pay their own doctors visit, they present themselves usually with an advanced disease like metastatic cancer.[19] This simple surgical technique could have great implications on the future treatments of solid cancer, if proven to be effective and safe. The clinical and preclinical studies are in alignment with the latest understanding of tumour immunisation therapies.[20]

1.3. Rationale behind the study population

The study population is selected because of the following reasons.

- It has been demonstrated that melanoma,[21] colon carcinoma[22] and renal cell carcinoma[23] are immunogenic and the tumour devascularisation technique has already been developed for these tumours in patients and animals.
- There is no effective cure for stage IV melanoma, colon carcinoma stage IV and renal cell carcinoma stage IV.
- Chemotherapy and radiotherapy are often burdened with many side-effects, limiting its use and diminishing quality of life. This technique could be an effective alternative.
- The population should not be immune compromised due to other diseases, chemo- or radiotherapy, because the effectiveness of the tumour devascularisation depends on a healthy and functional immune system.

1.4. Human studies

The surgeon Karel Fortýn who developed the tumour devascularisation technique, operated 20 patients, and 7 of them are described as case reports in journals. His patients didn't die due to the operation and probably also not due to the metastatic cancer. Four other surgeons have gained experience with this technique and operated each around 30 patients.
Most experience was gained with metastatic intestinal tumours and melanomas.

Five patients with metastatic colon cancer, treated with surgical tumour devascularisation, all recovered from their metastatic disease.[24,25,10] Two patients with a schirrus carcinoma without metastases recovered fully after the devascularisation.[8] One patient with a gastric carcinoma underwent successfully devascularisation, and survived 3 years without any difficulties. His death was due to a hemorrhagic stroke.[26] Furthermore tumour devascularisation was performed on a patient suffering from a metastatic renal cell carcinoma instead of the routine of extirpation of the kidney, due to peroperative complications. Three years after the operation the patient enjoyed a good state of health.[27]
Three patients with metastatic melanomas where treated using the devascularisation technique. One patient was treated successfully for palliation. In another patient the devascularisation of a metastasis in the neck reduced the trigeminus neuralgia completely, and stabilised the metastases in the lungs. The third patient underwent twice partially debulking and devascularising of lymph nodes in the groin and was still after 2 years in complete remission.[28,29]
In all cases during and after performing the surgical tumour devascularisation, no serious side effects, like sepsis were observed. For more detailed description of the case reports see chapter 7.

1.5. Animal safety studies: devascularisation of healthy organs

To explore the safety of the devascularisation technique various animal models were used like the rat, rabbit and minipigs. The effect of the devascularisation technique had been thoroughly investigated for different healthy organs. The stomach, kidney, lung, uterus, rectum, sigmoideum, colon, ileum and jejunum in minipigs could be devascularised without any serious side effects due to the devascularisation technique.[8,9,30,31,32,33,34,35] No sepsis was observed after using this method. All the experiments have been done in the Institute of Animal Physiology and Genetics, Laboratory of Tumour Biology, Academy of Sciences of the Czech Republic, in Libechov.
In the minipigs the intestine was devascularised till the length of 180 cm. The first week every day and then every week biopsies were taken to follow the process of degradation and absorption of the tissue.
12 hours after devascularisation of the intestine the subserosa showed a mild autolysis and after 48 hours mono- and polymorphonucleair leucocytes start to infiltrate the intestine. In the mucosa and lumen a dense accumulation of eosinophile leucocytes was visible. Also macrophages, histiocytes and fibrin fibres were present. Between the 3rd and the 5th day the serosa was covered with fibrin fibres. After 1 week also muscularis externa was prone to autolysis. During 2 weeks, the original cell components of the intestinal wall were replaced by fibrinous connective tissue. After four weeks conditions in the peritoneal cavity was completely normal; there were no adhesions and the peristalsis was regular. In the 8th week the devascularised intestine was completely replaced by connective tissue and it was hard to find the site of the operation.
In ten minipigs an injection of a large dose of pathogenic micro organisms (Escherichia coli type 01, 04, Citrobacter and Enterococci, 10^5-10^9/1ml and 4-10ml injected) in the lumen of

the devascularised intestine did not provoke any sepsis; the process of degradation was exactly the same as in the other animals.[15]
Between 1971 and 2001 the devascularisation technique was preformed on 160 minipigs, 163 rats and 4 rabbits on various organs.[10]

1.6. Explorative animal studies: tumour devascularisation

Tumour devascularisation was tested in various species, like minipigs, dogs and in a cat. In 86 dogs with mammary gland carcinoma (some with lung metastases), 3 dogs with metastatic melanoma, one dog with gastric cancer and one dog with a lymphoma tumour devascularisation had been preformed upon. Between 1975 and 2001 92 minipigs with metastatic melanomas were devascularised.[10] One cat with fibrosacroma was successfully operated with the tumour devascularisation technique. All these animal experimental studies have been preformed in the Institute of Animal Physiology and Genetics, Laboratory of Tumour Biology, Academy of Sciences of the Czech Republic, in Libechov. From the 86 dogs with mammary gland carcinoma, one year survival was better in a group of younger dogs (age 4-8 years: 92%) than in a group of older animals (age 9-11 years: 70%). In two veterinary practices some dogs with cancer disease underwent tumour devascularisation.[10]

Because of the tumour devascularisation technique, it has been possible to breed a strain of minipigs with metastatic melanomas, the MeLiM minipigs. In this animal model the immunological mechanism of this surgical technique could be well analysed. This animal model is relevant for human situation, because the metastatic melanoma in the minipigs resembles the human metastatic melanoma[36] in the following aspects.

- Macroscopically, [37] the melanomas are usually multiple, nodular and exophytic with vertical growth phase, and multiple metastases in the inner organs such as lungs, spleen, lymph nodes. Necrotic destruction was sometimes observed in central parts of larger skin melanomas. Also in some animals, multiple metastases in the organs were observed, without cutaneous melanomas, which appear in 5% of the human melanoma cases.
- The detection of metastatic activity of the melanomas based on finding of circulating melanoma cells in the peripheral blood[38]
- Histopathologically, [19,39] the melanoma cells showed a polygonal, round or spindle shape with a very high concentration of melanin. The nodular cutaneous melanoma cells were usually concentrated into trabecular or alveolar clusters running from the basal layer of the epidermis to the corium.
- Biochemically, [40] the presence of typical melanoma enzymes: tyrosinase, α-mannosidase and γ-glutamyltransferase. A very high concentration of eumelanin and low concentration of phaeomelanin was found in melanoma cells, which makes the probability of photochemical regression of MeLiM melanomas negligible. The melanomas have an extremely high level of melanosomes, which suggests a high differentiation of the MeLiM melanoma and is consistent with its mechanical rigidity.
- Immunohistochemically, [19,22] most melanomas were positive for HMB-45 and S-100.

- RT-PCR techniques showed that in the MeLiM melanomas Tenascin C was 3.6 times elevated, compared to normal skin, whereas Tenscin X decreased 30 fold in the melanomas. This alteration of these extracellular glycoproteins shows a strong tumourigenesis and metastatic activity in the MeLiM.[41]
- Genetically, it shows that Sus Scrofa chromosomes 1, 6 and 7, respectively, have counterparts on human chromosomes (HSA) 9p, 16q and 6p, harboring melanoma candidate loci.[42] Another study showed that loci on the minipig chromosome, associated with melanoma, corresponded with the DNA counterparts of the human melanoma (1p36, 3p25, 9p21, 9q21 and 16q24).[43]
- FDG PET-scanning data showed that the metabolic ratio was correlated with the evolution of the melanoma, and is effective in staging and follow-up as in human melanoma. [44]

Ten years of selective breeding established the MeLiM strain (Melanoblastoma-bearing Liběchov Minipigs). 34% of these pigs die in 2 months due to metastatic melanomas. More than forty minipigs of the MeLiM strain with a bad prognosis where chosen for tumour devascularisation. These animals showed an undernourished state, progressively growing multiple cutaneous nodular melanomas (5 -120 mm) with vertical growth and metastases in the inner organs such as lymph nodes, spleen and liver. After 2 weeks of tumour devascularisation disintegration of the tumour cells was documented in the devascularised cutaneous tumour as well as all non-treated tumours in the skin and organs. 2 months after the procedure intact tumour cells were found very rarely in the cutaneous tumours. At 4 to 6 months neither tumour cells, nor their destroyed particles were detected in the cutaneous tumour sections. All tumour areas were totally replaced with fibrotic connective tissue. Macroscopically, this process was documented by change of tumour colour from black to grey-white and by flattening of the tumours. The cachectic minipigs started to increase their weight rapidly after devascularisation, reaching nearly the normal weight during several months. All minipigs recovered fully.[19]

1.7. Immunohistochemical, immunological and biochemical effects induced by tumour devascularisation

In the melanoma minipig model the effects of tumour devascularisation on the devascularised tumour and the other non-ligated tumours and organ metastases have been studied. The results show that tumour devascularisation induces the T-cell mediated immune reaction.

After tumour devascularisation a high concentration of tumour infiltrating leucocytes (T helper and cytotoxic T lymphocytes) has been seen in the non-ligated and devascularised tumours, characterised by flow cytometry as $CD^{8+}CD^{3+}CD25^{-}CD45RC^{-}$ and $CD^{4+}CD^{3+}CD25^{-}CD45RC$. In the melanoma coupes very little B lymphocytes, natural killer cells and phagocytes were present. The melanomas in the minipig model suppress the production of immunoglobulines (IgG_1, IgG_2, IgM, IgA) and red blood cells (hematocrite below 10%). Four months after the tumour devascularisation these parameters were normalised, as in the control group.[45,28,29]

The expression of heat shock proteins (HSP: HSP70 and gp96) in the devascularised tumour tissue, demonstrated immunohistochemically, was strongly increased. The high concentration of the HSP was maintained during 2 weeks after the treatment. [27,46]
Biochemical analyses revealed a remarkably high concentration of melanin in the melanomas (>15% by weight), whereas after tumour devascularisation the melanin level decreased to very low values.[22]
The typical melanoma enzymes, tyrosinase and α-mannosidase, were present in the growing tumours of the minipigs. Six months after melanoma devascularisation, the not-ligated tumours went in regression and showed a reduction in both the α–mannosidase and tyrosinase activities that approached almost zero or very low levels, respectively.[22]
The extra cellular proteins, Tenascin C and fibronectin, play a role in tumour invasion and are present in high concentrations in the minipig melanomas. Two months after tumour devascularisation, these proteins are nearly not present in the devascularised tumours and the metastases that went into regression.[27,47]

1.8. Side effects of tumour devascularisation in animals

In the MeLiM minipigs which underwent tumour devascularisation changes in colour coat was regularly seen and being most conspicuous in black minipigs. Firstly, a narrow circle of white bristles appeared around all tumours, including the devascularised melanoma, approximately one month after the intervention. This depigmentation continued in some animals to get almost totally white about 6 to 10 months after the tumour devascularisation. No other side effects were observed.[19]

1.9. Immunological response on tumour antigens

It is known that melanomas,[48] renal cell carcinomas[49] and colon carcinomas[50] can activate the cellular anti-tumour immune reaction. When tumours go in regression, often cytotoxic T cells are seen in the tumour tissue, which is associated with a favourable prognosis. Though for a sustained immunogenic reaction, besides cytotoxic T cells, co-stimulation is needed with heat shock proteins (HSP), antigen presenting cells (APC) and T-helper cells.

1.10. Stopping the tumour immune suppression

Active suppression of tumour-specific T lymphocytes can limit the efficacy of immune surveillance. Through tumour devascularisation the tumour disintegrates and stops producing tumour-derived factors that inhibit the ripening of dendritic cells.[51] Thus, the suppression of the cellular immune system will stop around the devascularised tumour.

1.11. Overcoming the weak antigenecity of tumour cells

Tumour associated antigens are usually weak antigenic and are mostly not recognised by the immune system. Also the tumour associated antigens and the MCH I are diminished in expression on the cell surface of tumour cells.
After pig melanoma devascularisation a huge amount of HSP with bound tumour peptides were released into extra cellular milieu due to necrosis of tumour cells. These complexes can

activate the APC which present the tumour peptides to the effector cells of cellular immune system. The release of HSP stimulates this antigen presentation.
Due to continuous release of HSP and tumour antigens during several weeks after tumour devascularisation, the cellular immune system stays continuously activated.

1.12. The possible advantages of compared to existing therapies

The aim is to strive for cure in otherwise incurable patients. The procedure triggers a cellular immune reaction against the cancer cells. The advantage of tumour devascularisation over conventional treatments is that the cellular immune reaction is activated in more than one way:

- Reduction of tumour load, thus diminishing tumour-derived factors that inhibit the ripening of dendritic cells,
- Continuously elevated production of HSP by necrotizing the treated tumour
- Releasing all the tumour antigens in the devascularised tumour, instead of targeting only one or a few

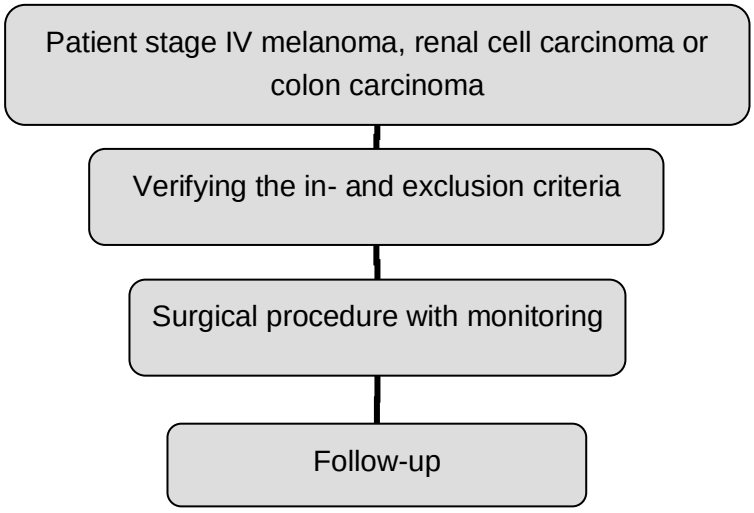
These effects of tumour devascularisation could create a long active immunisation against the autologous tumour cells.

OBJECTIVES

Primary Objective: The objective of this pilot study is to evaluate the safety and feasibility of the devascularisation technique for the metastatic melanoma, colon carcinoma and renal cell carcinoma.

STUDY DESIGN

The pilot study will be an observational study using an open uncontrolled design. The study will be preformed in a medical centre. The duration of the study will be 6 months.



STUDY POPULATION

Twelve patients with advanced cancer will be recruited.

1.1 Inclusion criteria

- Patients suffering from melanoma, colon carcinoma, and renal cell carcinoma stage IV
- ECOG-PS (Eastern Cooperative Oncology Group-performance status) ≤ 2 [52]
- Karnofsky Performance Scale > 50 [53]
- Clinical Prediction of Survival > 4 months
- Informed consent
- Written permission to post mortem examination
- Accepted for total anaesthesia and operation
- TNM classification cTxNxMx – pTxNxMx
- Peroperative showing invading tumours in the surrounding
- Leucocytes total: >2,0 10^9/l, CD4: >0,500x10^6/l, CD8: >500x10^6/l, CD3: >800x10^6/l

1.2 Exclusion criteria

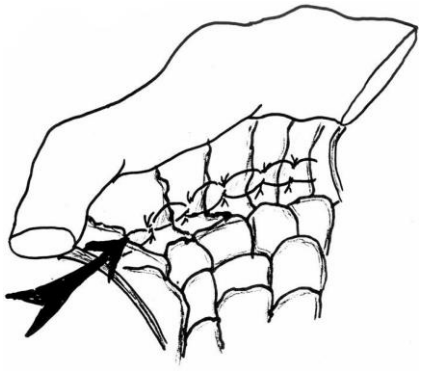
- Immune compromised situation, due to the use of corticosteroids (more than 20 mg prednison per day) or methotrexate (more than 15 mg per week).
- Other primary tumours
- Leukemia
- Less than six months before the tumour devascularisation the use of chemo- or radio therapy.
- Less than half year before the tumour devascularisation the use of perfusion therapy in case of a melanoma metastasis.

TREATMENT OF SUBJECTS

1.3 Investigational treatment

1.3.1 General description

The aim of tumour devascularisation is to isolate the tumour from the vascular and lymphatic system, with surgical not resorbable sutures.
The devascularised tumour will be left in situ, which will activate the cellular immune system. The proportion devascularised tumour will be around one third of the total amount of tumour load.



1.3.2 Intestinal tumours

A tumour in the intestine will be isolated by closing the intestine on both sides in where the tumour is located. The mesenteric veins, arteries lymph vessels are closed 2 cm from the intestine with sutures, returning to the same location. The intestinal continuity is restored by a side-to-side or end-to-end anastomosis. The mesenteric ligatures are tied together, leaving the devascularised intestinal segment in its original localization, see figure. For more detailed description of the tumour devascularisation for specific parts of the colon and small intestine, see the appendix with the case reports.

1.3.3 Renal cell carcinoma

Access to the kidney with the renal cell carcinoma is gained transperitoneally, through a paraduodenal incision. The hilar structures (arteries, veins and ureter) will be closed with non resorbable sutures.

1.3.4 Skin melanoma

Melanoma on the skin can be devascularised by mattress sutures inserted specifically to the exact place obstructing reliably the nutritive vessels at the tumour base.

1.3.5 Lymph node melanoma

The ligature is inserted at one edge of the node. Then the node is circled by one thread, which is then knotted after its firm tightening.

1.3.6 Material

For performance of the tumour devascularisation technique, only the non-resorbable suture material (silon, oxsilon, or silk) should be used. Usual anaesthesia and post operative care is given.

1.4 Escape medication

All escape medication is accepted.

2. METHODS

2.1 Study parameters/endpoints

2.1.1 Primary endpoints

The primary endpoints are descriptive. The safety and feasibility of the surgical technique will be evaluated.

- Feasibility of the devascularisation technique, measured on a 5 point VAS score. (1= very feasible, 2= enough feasible 3= neutral, 4= rather difficult, 5= not feasible)
- Feasibility of the recruitment of the patients
- Safety of the procedure, monitored on a 5 point VAS score (1= very safe, 2= slightly safe 3= neutral, 4= rather unsafe, 5= not safe at all)

2.1.2 Secondary endpoints

The EORTC-C30 quality of life questionnaire.[54,55,56]

- Progression free survival
- The overall survival

2.1.3 Descriptive parameters

- Adverse events due to tumour devascularisation during and after the operation, and how the complications are solved
- The percentage of devascularised tumour in relation to the total metastatic tumour
- Possible difficulties while using the technique

2.2 Study procedures

The tumour of the patient will be staged according to the standard procedure. The patient will be evaluated on the in- and exclusion criteria. Standard laboratory tests will be done to decide if the patient can undergo total anaesthesia and the operating procedure. The patient has to complete the informed consent form. The surgeon will fill in the case record form to register the feasibility and the safety of the technique, the total amount of devascularised tumour, the possible difficulties of the technique and the possible complications during and after the operation. The patient will be monitored according to the standard procedures after an operation. The follow-up of the patient will consist of standard monitoring techniques. For more detailed information see the scheme in the summary.

2.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

2.3.1 Specific criteria for withdrawal

The withdrawal of the study will be effectuated when the patient or surgeon decides.

2.4 Replacement of individual subjects after withdrawal

The study will be conducted during a time period of 6 months, the study will be stopped once 12 patients have been treated and follow up data of those patients has been gathered.

2.5 Follow-up of subjects withdrawn from treatment

Follow-up of patients withdrawn from treatment will be monitored till the end of the study period.

2.6 Premature termination of the study

The study will be prematurely terminated when the surgeons will not find any patient meeting the in- and exclusion criteria in the first 4 months. The premature termination of the study is also justified if in the first 5 patients the safety is 4 or more on the primary end point and the feasibility of the technique is 4 or more.

3. SAFETY REPORTING

3.1 Section 10 Medical Research Involving Human Subjects Act event

In accordance to section 10, subsection 1, of the Medical Research Involving Human Subjects Act, the investigator will inform the subjects and the reviewing accredited MREC (Medical research ethics committee), if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited MREC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

3.2 Adverse and serious adverse events

Any serious adverse event will be treated directly.
Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that at operation results in death;

- is life threatening (at the time of the event);
- requires prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, major safety finding from a newly completed animal study, etc.

All serious adverse events will be reported to the accredited MREC that approved the protocol, according to the requirements of that MREC.

3.3 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

4. STATISTICAL ANALYSIS

4.1 Descriptive statistics

Only descriptive data will be gathered.

5. ETHICAL CONSIDERATIONS

5.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version, 09-10-2004, www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act and other guidelines, regulations and Acts.

5.2 Recruitment and consent

Recruitment of patients will be done by a medical centre. When patients meet the inclusion and exclusion criteria, their surgeon will inform them about this pilot study. The patient will be given written information about tumour devascularisation and the study procedures. When a patient is willing to participate in this pilot study, he will be asked to fill in the informed consent. The patients will have 3 weeks to consider this technique.

6. ADMINISTRATIVE ASPECTS AND PUBLICATION

6.1 Handling and storage of data and documents

The data of the patient will be stored and written in the patient's dossier. Only doctors who will treat the patient and the investigator will have access to the data.

6.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited MREC has been given. All amendments will be notified to the MREC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited MREC, but will be recorded and filed by the institute.

6.3 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited MREC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the

trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

Because of the many studies on this subject done in the Institute of Animal Physiology and Genetics, Laboratory of Tumour Biology, Academy of Sciences of the Czech Republic, an annual progress report will also be sended to the head of the laboratory, Dr. Vratislav Horák.

6.4 End of study report

The investigator will notify the accredited MREC of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit, 3 months after the operation.

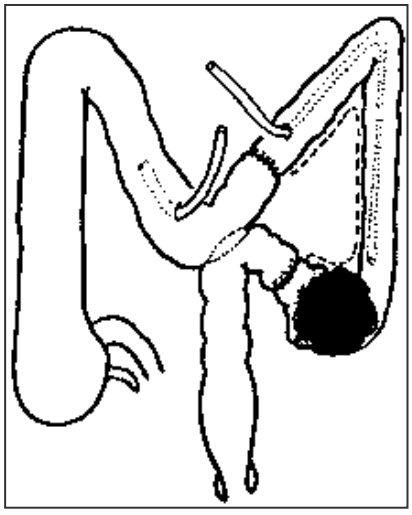
In case the study is ended prematurely, the investigator will notify the accredited MREC including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited MREC.

7. APENDIX

7.1 Intestinal tumours

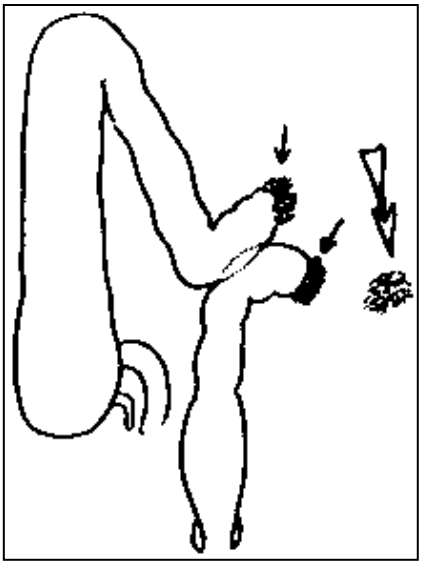
7.1.1 Coloncarcinoma in colon descendens and sigmoideum



A 57-year old woman had a subtotal obstruction of the intestine due to a double fist size tumour in the colon descendens and sigmoideum, infiltrating the left vasa ilicaca, the left urether and the abdominal wall. Lymph glands were turgid in the course of vasa ilica sinistra. On the liver there were several cancer metastases.

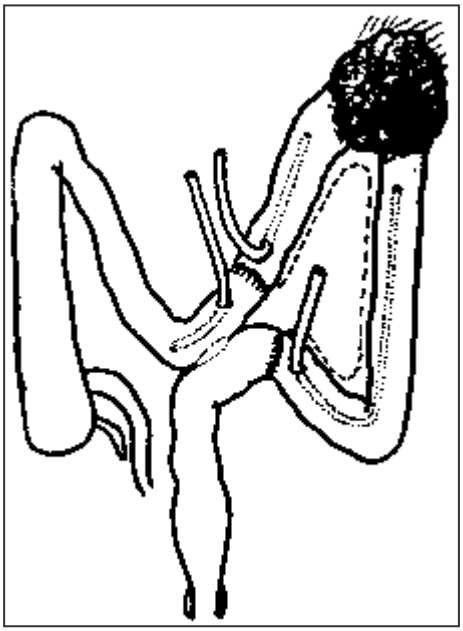
The tumour was devascularised with closing the vasa colica media and vasa colica sinistra. An anastomosis was made between the colon transversum and colon sigmoideum. In area of anastomosis the arteries were left (arteria rectalis cranialis and arteria sigmoidea ima). For decompression of the intestine anastomosis and for bringing the securing drainage out from the left part of colon transversum, a parietal colostomy was performed. The first faecal discharge came on the third day after the operation. The drainages were removed from the peritoneal cavity on the fifth day. The drain from the devascularised part of the intestine was gradually shortened, and removed definitively on the fourteenth day. The wound after the colostomy closed quickly. After the serous secretion from the operation

wound an excellent healing of the wound followed. The patient was released from the hospital 4 weeks after the operation without complications.



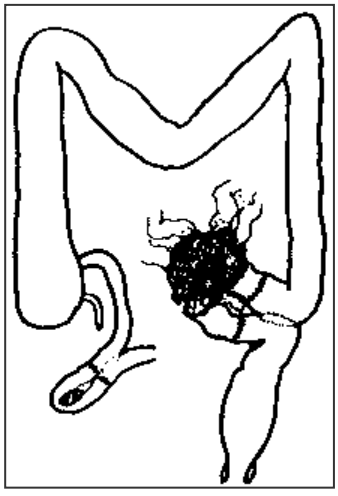
After the rectoscopic and irrigoscopic checks also a pararectal laparotomy was performed. A good functioning anastomosis was seen and only one and a half centimetre of fibrotic tissue was seen in the place of the devascularised part. At the endings of the colon, close by the side-to-side anastomosis, only hardly recognizable small flat fibrous parts remained (the 2 little black arrows on the picture). No liver metastases could be found. The patient was released on the 10th day after the relaparotomy. The patient is in the time of publication (7 years later) in a state of very good health.[24]

7.1.2 Coloncarcinoma in flexura lienalis



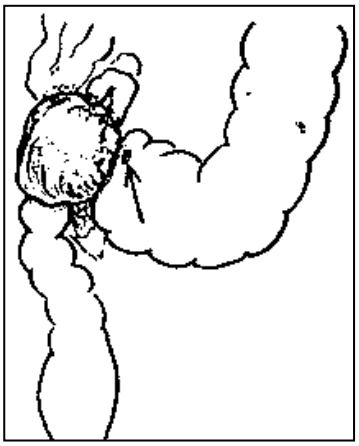
A 63-year old woman underwent a laparotomy due to suspicion of an ileus. The flexura lienalis of the colon contained a tumour of 12 by 15 centimetres, infiltrating in the spleen, pancreas, colon transversum, the left kidney accompanied with liver metastases and lymphadenompathy. Due to the inoperability of the tumour with regular techniques, tumour devascularisation was preformed. A side to side anastomosis of the colon transversum and sigmoideum was made, where after the left part of the vasa colica media, vasa colica sinistra and vasa sigmoidea where closed. The lumen of the intestine with the tumour was closed on both sides. Decompressing drains were established in the part of intestine before the anastomosis and into the two devascularised parts of intestine. All drains were brought out through the parietal colostomy. Other metastases on the liver were discovered, and numerous turgid lymphatic glands in mesocolon transversum and along the vasa colica sinistra. Faecal discharge began from the 4th day. The drains were removed between the 5th and 7th day.
One month after her operation, she could leave the hospital without complications.
6 month later a revision laparotomy showed a good functioning anastomosis and only one small hard fibrotic tissue was seen in the place of the devascularised part. No metastases were seen in the abdomen. The patient is in the time of publication already 7 years in a state of good health.[24]

7.1.3 Coloncarcinoma in colon sigmoideum and metastases in small intestine

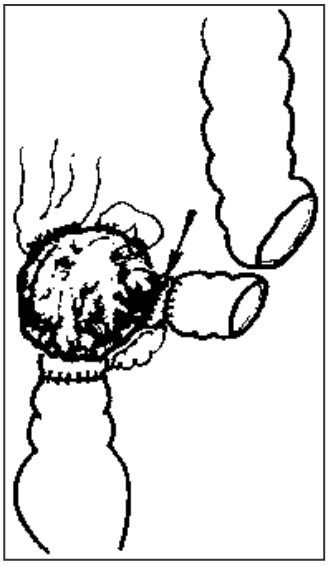


A 66-year old man, with suspicion of intestinal obstruction, underwent a medial laparotomy. At 60 cm from valvula ileocoecalis, the small intestine was curved by a metastasis and in the colon sigmoideum there was a fist-size tumour with infiltration into several neighbouring loops of the small intestine. Lymphatic glands in mesosigmoideum were enlarged, and several liver metastases were present. At first about 10 cm long piece of the small intestine with the metastasis, on both ends was closed by double ligature and a side-to-side anastomosis was made. Then tumour in the colon sigmoideum was devascularised with a side-to-side anastomosis on the rest of the S-loop. Patient left the hospital in good condition. He died 5 years after the operation for cardiac infarct. No post mortem examination is done.[24]

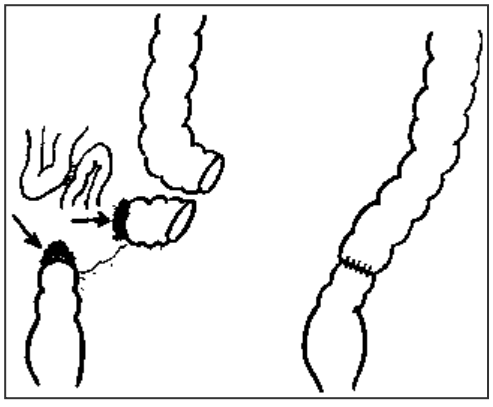
7.1.4 Coloncarcinoma in colon sigmoideum with infiltration in small intestine



A 75-year old patient with chronical obstipation and current pain in the left part of the hypogastrium, was suspected of obstruction in the large intestine, and the lower medial laparotomy was done. In the colon sigmoideum a fist-size tumour was found, with infiltration into the neighbouring loops of the small intestine. During the revision prevent perforation of the intestine in the close proximity of the tumour and rupture of mesosigmoideum happened. After stopping of bleeding, devascularisation was preformed of the tumour and its surrounding, because, there was no chance for radical primary removal of the tumour. Before and behind the tumour of the sigmoideum, closure of the intestine was made by double ligature, and stitched it down by the sero-muscular button-sutures. Both open ends of the sigmoideum were made as an axial colostomy. Into the Douglas space a drain was placed. Antibiotics were applied.



After the operation the first four days, his temperature was between 38ºC and 39ºC. Since the third day excretion went through the colostomy. During remaining two days a turbid fluid flew through the drain in the amount of up to 100 ml per day. Secretion was then decreasing and fully stopped on the 8th day. One day later the drain was removed. The wound was perfectly healed in spite of all unfavourable circumstances. No secretion was coming out from about 4 cm long lower part of colostomy. Condition of the patient was continually improving, so he could be released from the hospital after 4 weeks. Before this, a rectoscopy was performed, which detected presence of still about 7 cm long piece of the closed intestine.

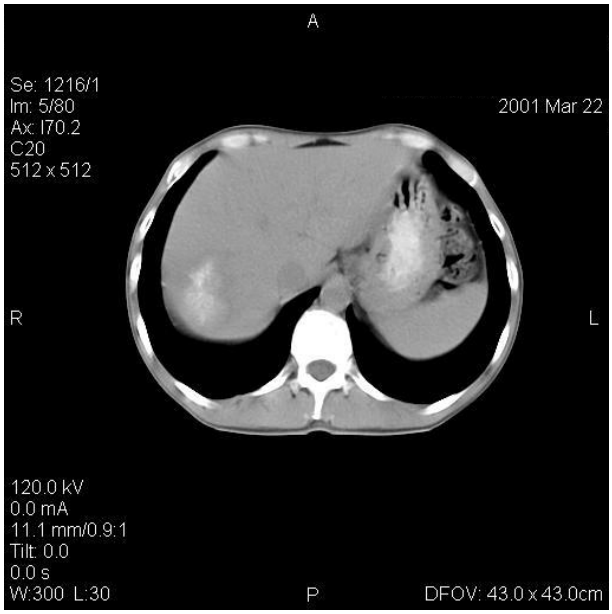


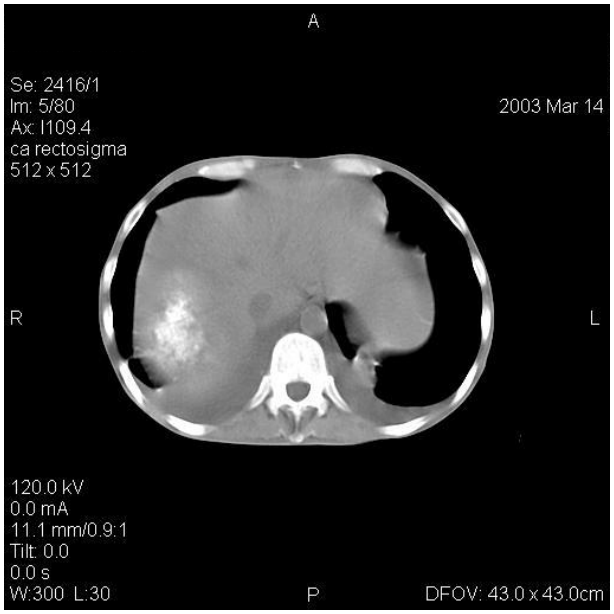
After 4 months the patient was in good condition and a revision was preformed. The aboral part of colostomy was around 3 cm long and ended in solid tissue, same as the aboral part of sigmoideum. The loops of the small intestine, joint originally with the tumour, were free except some small adhesions. The peritoneal cavity was free of metastases, no recurrence of cancer proliferation. An end-to-end anastomosis of the sigmoideum was preformed with resection of lower part of the colostomy. After smooth post-operative period the patients was released four weeks later.

Patient was then observed for 4 years - no signs of recurrence of the tumour. Patient died from myocardial infarction due to chronic heart ischemia. During post mortem examination no recurrence of cancer in the intestines or metastases was found. Also no adhesions good be detected and the passage of the anastomosis was good.[8]

7.1.5 Coloncarcinoma of the rectum

A 41-old man underwent in 2000 an explorative laparotomy and was diagnosed with an adenocarcinoma in the rectum and sigmoideum with liver metastases. He could not bare chemotherapy and stopped it in December after the first dose. The man was very weak, cachectic and could not walk anymore to the toilet. On 23 January 2001 the tumour was devascularised and a stoma terminalis of the sigmoideum is made. The patient did not receive antibiotics. After the operation, the patient had only the first 4 days an elevation of body temperature and had slightly pain when the belly was touched. On the 5th of February he was dismissed from the hospital. After the operation two CT-scans where made. On the 22th of March 2001 slight calcification of the liver metastases is seen. The CT-scan on 14th of March 2003 shows more calcification of the liver metastases (white coloured). No new development of cancer was detected.[10]





7.1.5 Coloncarcinoma (scirrhus) of the rectosigmoideum

A 79-year old man underwent a lower medial laparotomy due to symptoms of ileus. On the border between rectum and colon sigmoideum, the passage was obstructed by a 4 x 3 cm big tumour. No metastases were found. Tumour was identified as a scirrhus carcinoma. After emptying the intestine, tumour devascularisation was performed. Invagination into the rectum of the devascularised part performed with use of seromuscular button sutures. The intervention was finished by axial colostomy on the sigmoideum.

After the smooth post-operative period, the necrotized section of intestine with the tumour separated itself after 15 days, and left the body via the anus. Histologically the diagnosis was confirmed. Four weeks after the rectoscopic examination an anastomosis of the sigmoideum with the rectum was made. After two weeks the patient was released from the hospital in good state with perfectly healed wound. He was in good health till the age of 83, when he died at the internal medicine department of the same hospital for icterus. During the post

mortem examination, no signs of cancer proliferation were found, as well as no post-operative adhesions in area of the operation.[24]

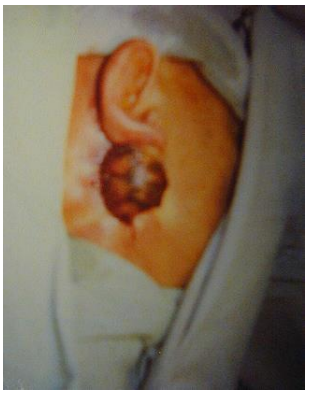
7.1.6 Coloncarcinoma (scirrhus) of the rectosigmoideum

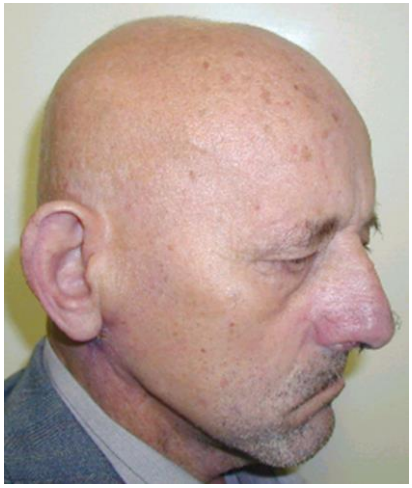
A 82-year old patient with colic pains and no flatus or faecal discharge underwent a laparotomy. In area of the rectosigmoideum there was a walnut size tumour of harder consistence. No metastases were detected. Tumour devascularisation was performed by isolating the tumour, closing the intestine, as well as inserting of tumour into the rectum secured by several button-sutures. Operation was finished by an axial colostomy of the sigmoideum. The post-operative period went smooth. On the 16th day, the tumour separated itself. Histological examination showed a scirrhus carcinoma. Four weeks later the colostomy was terminated and a new anastomosis was made. With perfectly healed operation wounds and in good state, the patient was released. He lived till his 87 years. During the post mortem examination, icterus was determined as the cause of death. No signs of cancer relapse were found in the peritoneal cavity.[24]

7.2 Melanoma

7.2.1 Melanoma in head area

In 1996 a 60-year old man underwent an excision of a melanoma in the head hair area and metastases in the right part of the neck followed by adjuvant chemo- and radiotherapy. In 1997 mediastinal and lung metastases were found. In the same year and 1999 excision of alocal recurrence of a melanoma the neck was preformed. In 2001 he underwent a treatment with interferon α 2a. In September 2002 the patient presented himself with a facialis paresis, facial pain and a 4 cm big and bleeding recurrent melanoma in the neck. The thoracic CT-scan showed several lung metastases. The tumour was devascularised. Ten days later the patient did not have facial pain anymore, the facial paresis persisted. In the follow-up thoracic CT-scan 10 months later a slight regression of the lung metastases was seen. The control CT-scan of March 2003 showed a status quo. De patient is immunologically under control and receives Interferon α-2a.[57]





7.2.2 Recurrent melanoma in groin

A 64-year old woman, with in her history an excision of a melanoma on her right foot, came in October 2000 with enlarged lymph nodes in her right groin. Echography and the clinic examination revealed melanoma metastases and was treated by immunotherapy. On the 5th

of January 2001 tumour devascularisation was performed on her request. A pigmented tumour of 7 cm was found and indicating stage III B. Partial tumour extirpation and partial tumour devascularisation was done. Histopathological examination showed an amelanotic melanoblastoma. In March 2001 a recurrent melanoma was found on the same place. Again partial extirpation and devascularisation was performed on the 13th of March 2001.[10] Controls in January 2003 did not show any sign of cancer recurrence. She receives immunotherapy.

7.3 Renal cell carcinoma

A 65-year old man had an adenocarcinoma of the right kidney. Access to the kidney was gained transperitoneally; through a paraduodenal incision the renal blood vessels were reached and denuded; an in growth of tumour tissue in the peritoneum was found. The closure of all hilar structures (blood vessels and ureter) was considered also as a preventive procedure cutting down the possibility of metastatic dissemination during handling the tumour. There was a sudden unfavourable change of the general condition of the patient (cardiac arrhythmia, decrease of blood pressure), therefore the surgical procedure had to be stopped speedily and the kidney left in place. The patient recovered very satisfactorily and was discharged after a few weeks. Three years after the operation the patient enjoyed a good state of health. He finally died of cerebral haemorrhage caused by high blood pressure. Unfortunately, no post mortem examination was carried out.[27]

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