Autologous tumor immunizing devascularization of an invasive colorectal cancer: A case report and literature review

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Abstract. Colorectal cancer is the third most common cancer. Approximately 20% of patients have at the time of presentation metastasized colorectal cancer, which is incurable in ~80% of cases. The present case report describes a typical case diagnosed with an advanced invasive colorectal adenocarcinoma, with two suspect hypodense lesions in the liver, as revealed by sonography. Judged inoperable for a curative outcome by radical resection, the patient was treated with a novel surgical technique based on stimulating the immune system, termed 'autologous tumor immunizing devascularization' (ATID). The tumor was isolated from its surroundings by ligature of arteries and veins, and subsequently the completely devascularized tumor was left *in situ*. The distal part of the rectum was closed, and a stoma was made from the proximal part of the colon. Following ATID, the stressing pathophysiological condition of the completely isolated tumor provoked a generalized cellular immune response, which led to the elimination of the devascularized tumor and distant lesions without causing sepsis. The patient did not experience any serious side-effects following the operation, and refused any adjuvant chemo- and/or radiotherapy. To date, the patient has no complaints and remains in good health after the ATID intervention, already more than 14 years. The present case study provides a typical demonstration of the clinical safety of ATID, and also indicates both the immunizing and the curative potential of the method.

Introduction

Colorectal cancer is the third most common cancer (~1.2 million new cases are diagnosed each year), and the fourth

cancer-associated cause of mortality in the world's population (1). Approximately 20% of patients have at the time of presentation metastasized colorectal cancer, which is incurable in ~80% of cases (1). Although, during the last two decades, there has been an increase in the survival rate of end-stage patients of 8-14 months, and even 2 years or more in certain cases, predominantly due to novel chemotherapeutic agents, the quality of life may be severely affected (1). International guidelines recommend that an incurable metastasized colorectal cancer should not be resected although, in practice, the therapeutic approach varies widely: Two-thirds of cases are operated on in the United States whereas, in The Netherlands, the majority of patients do not receive surgery (1). Considerable perioperative mortality and morbidity following incurable colorectal cancer resection should be weighed against a supposed longer survival rate (1).

An innovative approach to metastasized colorectal cancer is provided by surgically induced auto-immunotherapy. In this therapy, the tumor is completely devascularized by the ligature of all arteries and veins, and left *in situ* in the body. This provokes a generalized immune response to tumor antigens, and leads not only to the elimination of the devascularized tumor, but possibly also to the elimination of distant lesions without causing sepsis (2). This technique, formerly termed 'devitalization', has been renamed as 'autologous tumor immunizing devascularization' (ATID) (2). The Czech surgeon, Karel Fortýn, coincidentally discovered this technique when he operated a patient wounded by gunfire. Besides the injury, the patient was also suffering from a metastasized inoperable stomach tumor. To prolong the life of the patient, the surgeon arrested all hemorrhage and performed a jejunal stomach bypass. Due to its invasive nature, the tumor could not be completely resected, and was therefore left completely devascularized in the body. Unexpectedly, the patient fully recovered in the following months, with no relapse of the cancer years later (2). Following this emergency operation, the surgeon realized that the remaining completely devascularized tumor apparently provoked an immune response against tumor cells.

After this case, following the identical procedure, Dr Fortýn operated several patients presenting predominantly colorectal cancer, though also patients with stomach and kidney cancer (3-5). Animal models and other studies elucidated the

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immunological working priniciple of this technique (2,6). It has thus been demonstrated that total devascularization of the primary tumor, with the interruption of all blood vessels (arteries and veins), promotes apoptosis of tumor cells, while circulating leukocytes are massively mobilized and accumulate at the periphery of the devascularized tumor under the influence of liberated stress-signaling factors, predominantly heat-shock proteins (6). This allows phagocytosis of cellular debris to occur by antigen-presenting cells, and the normal processing of non-denatured tumor antigens for presentation to cytotoxic T-lymphocytes. Finally, this leads to the specific activation of cytotoxic T-lymphocytes by all tumor-specific antigens and tumor-associated antigens through co-stimulation, also involving tumor-infiltrating lymphocytes and memory T-lymphocytes, which conjointly effect the elimination of lesions (2). This process, elicited via ATID, is termed 'immunolysis' (2). The method resembles other immune therapies, such as specific tumor antigen vaccines and tumor cell lysate-pulsed, dendritic cell-based cancer vaccines, with the main difference being that, for the ATID technique, the specific activation of cytotoxic T-lymphocytes against all the potential tumor antigens proceeds entirely *in vivo*, with the involvement of all co-stimulating mediators of the immune system.

In the present study, a case is described of a patient who presented with an invasive colorectal carcinoma, after having undergone ATID with 14 years of clinical follow-up.

Case study

A 48-year-old man with a history of rectal bleeding and increased frequency of loose stools for 6 months underwent colonoscopy in the polyclinic Železniční, Olomouc Czech Republic, on January 25, 2001. A polyp (measuring 3x5 cm) located 17 cm from the anus was detected as the source of hemorrhage. The distal part of the polyp had color changes. Five biopsies were taken, and directly fixated in 10% formalin and paraffin, whereafter slices of 3-4 μm were stained with hematoxylin and eosin. Evaluation with a light microscope revealed a tubulovillous adenoma with high-grade dysplasia. CT scans with contrast per os and per rectum of the pelvis and rectum did not reveal any other suspect lesions or enlarged lymph nodes. Due to the histological outcome, with no suspicion of malignant transformation, a second colonoscopy was performed only to resect the whole suspect adenoma polyp. Due to its size, the polyp was resected in parts on February 7, 2001. Histology of the whole polyp, performed as described above, revealed adenocarcinoma invading the muscularis propria, free of tumor cells at the cutting edges, staged as pT2, NX, MX. Follow-up with rectoscopies in March, June and September 2001 and biopsy of the scar from the previous tumor resection taken in March 2001 did not reveal any malignancy. In September 2001, ultrasound of the liver did not reveal any lesions. Also, the level of carcinoembryonic antigen (CEA) was revealed to be negative.

All further examinations were negative up to January 14, 2002, at which time ultrasound of the abdomen revealed two suspect lesions in the right liver lobe. The suspect lesions in the liver necessitated staging examinations. With a colonoscopy also performed on January 14, 2002, a small polyp of 2 mm in diameter was resected near the distal part of the scar from the previous polypectomy performed in 2001. Biopsy samples from the scar and its surroundings were subsequently taken. Further histological analysis revealed adenocarcinoma *in situ* originating from the cylindrical colon epithelium, although invasion was not detected. The patient was informed of the histological outcome and the suspect lesions in the liver, which urged the necessity to undergo further examinations to reveal the extent of the cancer. This was refused, and the patient sought directly the possibility of an ATID intervention at the Podripska Hospital.

In Podripska Hospital, ultrasound examination of the liver confirmed the presence of two suspect hypodense lesions in the right liver lobe measuring 14 mm (1.5 cc) and 9 mm (0.5 cc) in diameter. This confirmation made the requirement for surgery even more urgent. Therefore, the decision was taken not to wait for CT scans of the rectum and abdomen, which at that time could not have been performed in the same hospital and would lead to a delay of several weeks, but to perform ATID directly.

The body weight and height of the patient were 72 kg and 175 cm, respectively (body mass index: 23.5). Initial biochemical analyses revealed only a slight increase in the levels of non-conjugated and overall bilirubin. C-reactive protein (CRP), CEA and cancer antigen 19-9 (CA19-9) values were revealed to be within normal limits, or negative. Furthermore, blood counts were within the normal range.

On February 15, 2002, the patient underwent ATID through a lower middle laparotomy. A tumor (measuring 8 cm by 5 cm; 220 cc) was identified, located 10 cm proximally from the anus. The tumor, which had invaded perirectal fat tissue, the prostate, periosteum of the os sacrum and os coccygis, was first enucleated from the invaded tissues. Liver metastases were not palpable. Due to its invasive nature in surrounding tissues, without considering lesions in the liver, the tumor was determined to be at stage T4, NX, MX. The mesorectum was subsequently released and ligated. Then, the rectum and its lumen were closed by ligatures applied 1 cm proximally, and 3 cm distally, from the tumor. The proximal part of the colon was used to make a terminal colostomy. Finally, the tumor was completely devascularized and left *in situ*. Drains were placed inside the pelvis, and the laparotomy was closed according to the normal procedure with sutures: Peritoneum and aponeurosis together with a continuous resorbable suture, subcutaneous tissue with single resorbable sutures, and skin with single non-resorbable sutures. Antibiotic regimen was applied with amoxicillin 1,000 mg and clavulanic acid 200 mg every 8 h and gentamicin 240 mg every 24 h intravenously starting 30 min prior to operation during 7 days. Hereafter amoxicillin 875 mg and clavulanic acid 125 mg per os twice daily was given 3 days.

The first day after the intervention, the temperature of the patient rose to ~38˚C. The patient reported only mild abdominal pain. Due to discharges from the rectum 13 days following the operation, a rectoscopy was performed, and biopsy samples of possibly necrotic tissue were taken at a distance of 8 cm from the rectum. No perforation of the intestinal wall was identified. Histological examination did not reveal the presence of malignant cells. The patient's condition returned to normal within 2 days. The patient was released from hospital 2 weeks after

Table I. Retrospective simulation of immunolysis.

		Primary tumor				Metastases						
Sim. no.	Case	TDT days	DV cc	TI days	CL %	VM$_1$ cc	TI days	CL %	VM$_2$ cc	TI days	CL %	Rd
(1)	CS	30	220	80	50	1.5	40	80	0.5	30	90	110
(2)	CS	14	220	80	50	1.5	50	75	0.5	30	83	110
(3)	CS	10	220	80	50	1.5	60	75	0.5	50	81	110
(4)	CS	7	220	80	50	1.5	75/R	56	0.5	70	64	110
(5)	EC	7	220	80	50	1.35	80/R	52	0.65	90	45	110
(6)	EC	7	220	80	50	1.25	90/R	45	0.75	100	40	110
(7)	EC	10	20	55	68	1.5	65	60	0.5	45	78	10
(8)	EC	10	10	45	78	1.5	70	60	0.5	50	74	5
(9)	EC	10	6	45	78	1.5	65	60	0.5	45	78	3
(10)	EC	7	20	55	68	1.5	75/R	56	0.5	75	60	10

Sim. no., simulation number; TDT, tumor volume-doubling time; DV, devascularized volume of tumor; TI, time of complete immunolysis or beginning of regrowth; CL, percentage of the maximum systemic concentration of cytotoxic lymphocytes following time TI; VM$_{(1/2)}$, volume of individual metastases 1 or 2; CS, case study; EC, exploratory calculation; Rd, ratio of devascularization [DV/(VM$_1$ + VM$_2$ + ...)]; R, regrowth.

the operation. No inflammatory complications were present during hospitalization. Increased postoperative levels of CRP and the white blood count returned to within the normal range in 2 months. The patient refused any adjuvant therapy, including chemotherapy, radiotherapy or immunotherapy. Regular check-ups were not performed due to the patient's non-compliance.

Eleven years later, as determined on May 23, 2013, subsequent CT scans did not reveal any malignancy, and all tumor and inflammation markers were within the normal range. In august 2016 the patient reported to be in good health. He gained weight (now 80 kg) and works as a farmer.

Discussion

The presented case of an invasive colorectal tumor reveals the clinical safety of ATID, and also indicates both the immunizing and the curative potential of this method.

Total devascularization of the tumor mass (~220 cc) was well tolerated by the patient, without any serious side-effects and resulted in a curative outcome, with a symptom-free survival rate exceeding 14 years. Furthermore, following the operation, the patient reported only mild pain, comparable with that of a standard laparotomy. This case of colorectal adenocarcinoma could not be resolved by conventional oncological surgery, and corresponded with stage IV in view of its invasiveness and size alone.

ATID model calculations. Model calculations in order to predict the possibility of the elimination of metastases, and thus the curative effect of ATID, were performed retrospectively (Table I) (2).

In February 2001, histological examinations of a resected polyp revealed adenocarcinoma. On the ground of the presumption that the tumor was not completely resected, and therefore could grow back to the same volume it occupied at the time of the operation from only a micrometastase, a retrospective calculation was performed. The retrospective calculation was based on the estimated volume of the primary tumor (220 cc) and of two presumed metastases (1.5 and 0.5 cc) when ATID was performed a year later. Assuming that the primary tumor, with a hypothetical doubling time of ~14 days, developed over a period of 1 year (corresponding to an aggressive tumor), calculations revealed that the tumor (220 cc) would have originated from an undetectable locus or micrometastase of only 5.4x10^{-6} cc, i.e. ~5,400 tumor cells. Even in assuming the presence of a tumor that was much more aggressive, with a doubling time of only ~10 days, model calculations indicated possible elimination of the presumed metastases within ~60 days (Table I) (2). Therefore, assuming a doubling time of 7 days, according to the calculations larger metastases would regrow (Table I).

The rate of immunolysis of the tumor should be higher compared with the tumor growth rate. In the early development of a tumor, the growth rate may be much higher in comparison with the later stages, when the tumor is larger and detectable (7). This explains why Choi *et al* (8) were able to identify high variability in the tumor-volume doubling times (from 0.05-7.1 years).

Thus, the more aggressive the tumor, the greater the percentage of the total tumor mass that should be devascularized. For the purpose of clinical orientation, the amount of tumor mass devascularization is expressed as the ratio of devascularization (Rd): (Volume of devascularized tumor)/(total volume of remaining metastases). Calculating the Rd provides the clinician with an indication as to whether ATID could be curative. Besides Rd, the tumor volume-doubling time and the volume of the largest metastase are important factors in the calculation.

In more common cases with slowly growing tumors, such as in the case of colorectal cancer, Rd values of ~10 may be

adequate (2,8). The results of comparative simulations of immunolysis, calculated with various tumor-doubling times, the volume of the devascularized primary tumor and volumes of metastases, are shown in Table I.

Unfortunately, due to the non-compliance of the patient to undergo regular check-ups, the regression of metastases was not monitored. However, on CT scans 1 year following ATID, no hypodense lesions were observed. In future studies, the immunolysis of metastases should therefore be monitored with short intervals to allow further development of the ATID calculation model. To calculate the possible curative effect of ATID, magnetic resonance imaging scans and positron emission tomography scans, which are even more precise, are necessary for the estimation of tumor-volume doubling times (2).

Specific features of ATID. The curative outcome in the application of ATID, and absence of undesirable side-effects described in the present case study fully reflect the results obtained in numerous animal model experiments and clinical studies involving the devascularization of tissues and organs.

When performing ATID, it is very important to ensure perfect total devascularization. Double ligatures have to be tight and performed exclusively with non-resorbable stitching material. In anatomically indicated situations, in order to prevent collateral blood supply by microvessels along the periphery of the tumor, it is necessary to apply tight mattress stitches to isolate the tumor completely for optimal immunolysis. This is to ensure the longest possible duration of a high systemic concentration of activated cytotoxic T-lymphocytes (see the CL values, i.e. the percentage of the maximum systemic concentration of cytotoxic lymphocytes following the time of complete immunolysis or beginning of regrowth, in Table I).

On the macroscopic level, the devascularized tissue first increases in size; subsequently, shrinking occurs, and the devascularized tissue is eventually replaced by fibrous tissue (4). On the microscopic level, during the first 2 days the numbers of infiltrating mono- and polynuclear lymphocytes are observed to increase (3). Over the course of the next 8 weeks, cells of the devascularized tissue are replaced by fibrinous connective tissue, and the concentration of leukocytes decreases (3). Mature connective tissue is observed after 8 weeks (3).

The lack of any serious side-effects is predominantly explained by the circumstance that the discharge into the system of any liberated inflammatory components due to ATID can only proceed very slowly by diffusion. This is ensured by the tight ligatures and stitches. The absence of serious side-effects may also be explained by the high concentration of immune cells accumulating at the periphery of the devascularized tissue (3,6).

Safety and effects of organ devascularization. The strategy of leaving completely ligated tissue or devascularized tissue in the human or animal body is not entirely novel. Former studies, discussed in this chapter have revealed that various organs with complete devascularization involving the artery and vein blood supply can be left in the body without serious side-effects. Furthermore, replacement with fibrous tissue has been observed. Examples taken from animal studies include

transplantation of the hypophysis following total resection in the rectus muscle, bone marrow of the tibia and cortex cerebri in dogs (n=23), thereby prolonging life compared with resection alone (9), the ligation of intestines up to 180 cm in length in pigs (n=47) (3,10) without any side-effects, even following injection of pathogenic bacteria into the stomachs of pigs (n=23) (11), and the ligation of parathyroid glandules in dogs (n=14) (12), the spleen in rats (n=35) (13) and a guinea-pig (n=1) (14), kidneys in pigs (n=21) and rats (n=60) (4), testes in dogs (n=3) (15) and cocks (n=4) (16), and ovaries in dogs (n=6) (17). Following devascularization of the colon, no migration of bacteria through the ischemic wall was observed for up to 6 h (18).

Similarly in humans, the devascularization of organs has been performed without serious side-effects. In the case of hyperthyroid patients (n=225), the surgeon Charles H. Mayo, for the purpose of safety and simplicity of the operation, advocated arterial and venous thyroidal vessel ligation while leaving the devascularized part of the thyroid gland *in situ* (19). Other examples are provided by the devascularization of half the thyroid gland followed by transplantation in the abdomen (n=4) (20), preference of renal artery and vein ligation (n=1) over nephrectomy (21), devascularization with rubber bands of hyperplastic gastric polyps (n=72) (22), and *in utero* percutaneous umbilical cord ligation in complicated monochorionic multiple gestations (n=11) (23).

Safety and effects of tumor devascularization. Animal studies have been performed where tumors have been completely devascularized without any serious side-effects. Examples of primary tumor devascularization are the Lewis lung carcinoma cell line being implanted into the hind-limbs of mice with 4 to 5-fold reduced lung metastases (24), the murine osteosarcoma cell line POS-1 being implanted into the thighs of mice (n=15) with a marked reduction in lung metastases (25), and a temporary clamping of the tumor in the murine CaNT tumor model in mice, where progressively decreased tumor cell survival is observed following increasing periods of vascular occlusion (26). Notably, experiments with inoculated Flexnor-Jobling carcinoma, the Cracker sarcoma 39 and the Walker tumor in the legs of rats revealed that complete regression without recurrence was obtained after 7-9 h of temporary ligation with rubber bands (27). In addition, temporary ligation with rubber bands in dogs revealed total necrosis in 29 out of 41 benign tumors, including lipomas, angiomas, adenomas and mixed mammary tumors, and all eight malignant tumors, including mammary and perianal adenocarcinoma, fibrosarcoma, squamous cell carcinoma and melanoma (28). The effect of ATID on distant metastases has been investigated in miniature pigs (n=40) with hereditary melanoblastoma (6). The reduction in the number of metastases was observed 2 weeks following ATID, with complete elimination of all non-treated metastases over the course of 4-6 months (6).

In humans, the curative effects of ATID have been described in various medical journals. ATID was performed on a 63-year-old female with a carcinoma (15x12 cm, ~450 cc) in the left colon invading into the spleen, pancreas, mesocolon transversum, and left kidney (3). Six months later, a control laparotomy did not reveal the presence of any metastases, but

only a small amount of fibrotic tissue of the devascularized part. A 57-year-old male, with a colon carcinoma (size of a double fist, ~500 cc) with infiltration of the abdominal wall and a few liver metastases, underwent ATID (3). Control laparotomy revealed no metastases. The two patients were living in good health at the time of the publication of the studies, 7 years following ATID. ATID was performed on a 66-year-old patient, who had a carcinoma (size of a fist, ~250 cc) in the colon sigmoideum with invasion into the small intestines, a few liver metastases and a metastase in the small intestine (3). Following 5 years of living in good health, the patient died of a heart attack. A 41-year-old man with a colorectal carcinoma (~300 cc) and liver metastases, who stopped chemotherapy following the first dosage due to severe side-effects, underwent ATID (2). Nearly 3 years after the ATID intervention, the patient succumbed suddenly to myocardial infarction. Two patients, who were 79 and 82-year-old men with colorectal cancers [4x3 cm (~40 cc) and walnut-sized (~30 cc), respectively] without detectable metastases (3), died 4 and 5 years following ATID, respectively. Obduction revealed no metastases, and, in both cases, icterus (jaundice) was the cause of death. In a 65-year-old man who had a kidney adenocarcinoma with infiltration of the peritoneum, ATID was successfully performed, and the patient enjoyed 3 years of good health (4). He finally succumbed to a cerebral hemorrhage caused by high blood pressure. Additionally, a large ileal lipoma (n=1) was successfully devascularized (29).

All these results clearly demonstrate that devascularization of tissue and tumors is well tolerated in a wide variety of situations in both animal model and clinical studies. However, no attention has been given to tumor devascularization and its therapeutic potential. It is now possible to explain the therapeutic effects, and even to predict curative outcomes, on the basis of ATID theory (2).

In conclusion, the present case study has clearly indicated the critical clinical conditions for ATID that should be respected if the intention is a curative outcome. Concerning clinical safety, this case is comparable with that of standard laparotomy. Furthermore, the safety of leaving a large mass of completely devascularized tissue and/or tumor in the body has been verified in animal models and clinical studies.

The therapeutic potential of ATID should be investigated in clinical studies under optimal, well-defined conditions, ensuring maximum therapeutic benefits to patient volunteers. Future clinical data may assist in optimization of the ATID predictive model. These studies could also provide the clinical basis for an investigation of the potentiating mechanisms in the activation of the cellular immune system against all tumor antigens. Such mechanisms may involve the application of specific cytokines and immune cell growth factors. This would require their peak concentrations to be determined. The maximal activities of the specific cytokines and immune cell growth factors should be established in association with the phases of immunolysis. With the application of suitable potentiating agents at their optimal concentrations, and at the most clinically beneficial time, the presence of high systemic levels of activated cytotoxic T-lymphocytes could be sustained for longer periods. This may consequently increase the chances of a curative outcome, particularly in cases of very aggressive cancers.

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