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DOES DIFFERENT APPROACH DURING PANCREATODUODENECTOMY INFLUENCE INTESTINAL MIGRATING MYOELECTRICAL COMPLEX RECOVERY? STUDY IN EXPERIMENTAL PIG MODEL

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It is said that leaving intact the functional motor unit of the pylorus leads to better gastric emptying and reduces postoperative upper gut motility disturbances. However, despite obvious different surgical approach, both major pancreatoduodenectomies lead to substantial myoelectrical dysfunctions. The latter are not efficiently recognized. We compared Whipple and Longmire-Traverso procedures in terms of electromyography patterns of the upper jejunum musculature and the density of Cajal cells network. Twelve male weaned pigs underwent surgery first to implant bipolar electrodes and telemetry transmitters for continuous electromyography recordings and then, after 1 week recovery, to create Whipple (*n*=6) and Longmire-Traverso (n=6) pancreatoduodenectomies. The first myoelectric activity was already registered 1–2 hours after both operations. Time to first regular patterns of migrating myoelectrical complex activity was significantly longer in the Whipple than in the Longmire-Traverso group (68.2±12.9 versus 27.8±51 hours, p=0.002). However, the restored patterns were substantially disturbed in both groups. Namely, after Longmire-Traverso operation, migrating myoelectrical complex cycles were very often and significantly shorter versus control ones, with reverse migration in the area of anastomosis while after Whipple procedure migrating myoelectrical complex cycles were less frequent and of short duration, significantly shorter in comparison even with Longmire-Traverso group. Cajal cells network in the vicinity of anastomosis, and distally from it, presented greater destruction after the Whipple operation. In conclusion, the advantage of one of two major pancreatoduodenectomies in terms of myoelectrical activity correctness in upper gut has not been proved in the study.

Key words: *Cajal cells, gastric emptying, jejunum, migrating motor complex, myoelectrical activity, pancreatoduodenectomy, pylorus*

INTRODUCTION

Many postoperative upper gut motility disturbances are observed in patients operated due to diseases of the pancreas and the periampullar region. Depending on the method of pancreatoduodenectomy introduced, disturbances of both gastric emptying and intestinal content passage in the postoperative period are observed with different intensities; the latter may last for a long time after operation. Nowadays, the most frequent treatment techniques selected in the above-mentioned diseases, are Whipple (standard) and Longmire-Traverso (pylorus-preserving) pancreatoduodenectomies. The Whipple procedure covers the removal of the head of the pancreas, duodenum, 1/3 part of the stomach, the distal part of the common bile duct and gallbladder. Afterwards the restoration of the gastrointestinal tract by the creation of the choledochojejuno- pancreaticojejuno- and gastrojejuno-anastomoses, is carried out (1). The Longmire-Traverso modification consists of the transection of the duodenum 2–3 cm below the *antrum* and the creation of duodenojejunoanastomosis, resulting in the preservation of the stomach.

Although it is said that leaving intact the functional motor unit of the pylorus leads to better gastric emptying and reduces the amount of postoperative upper gut motility disturbances (2-5), in the literature due to many contradicting reports to be found, there is no dominating opinion of the clinical advantages of preserving operation over the standard one or otherwise. Some clinical observations report increasing incidence of upper gut motility disturbances following the Longmire-Traverso procedure (6-8) - even higher than after standard surgery (9-11). Further, in a prospective, randomized, multicenter analysis of 170 patients were shown that both operations were associated with comparable operation time, blood loss, hospital stay, mortality, morbidity and incidence of delayed gastric emptying (12), concluding that there was little clinical difference between the techniques. Similar conclusions have been drawn by Diener *et al.* (13) who, however, emphasized presence of major clinically relevant heterogeneities, which might considerably affect the meta-analysis outcome. Thus the present study aiming at comparison of gut motility was performed on animal models that can be better standardized than the clinical studies.

As any disturbances of contractile activity are an effect of myoelectric activity disorders (14), analysis of changes in the characteristics of the migrating myoelectrical complex (which is the electrical component of the migrating motor complex, MMC) enables the conclusion of motility disturbances in the upper gut. Migrating myoelectrical complex is a basic pattern of the interdigestive period and its regular presence is a sign of the "well-being" of the upper gut (15). Furthermore, spiking activity, being a crucial element of MMC, cannot exist without slow wave activity. This rhythm is produced by the interstitial cells of Cajal (ICC), which are responsible for generating and propagating electrical slow waves, and determine the motor activity of the upper gut (14, 16, 17). An operative modification of the upper gut, which requires a transection of the jejunum and thus disrupts the ICC network continuity, leads to the interruption of the propagation of pacemaker potentials (18).

Previous gastrointestinal electromyography (EMG) studies have shown the usefulness of pigs as an excellent substitution model for humans, particularly when the telemetry technique of recording was employed (19-21).

The first aim of the study was to compare the electromyography patterns of the upper jejunum following the conventional Whipple pancreatoduodenectomy with the pylorus-preserving Longmire-Traverso procedure using pigs. The second aim was to compare the density of the Cajal cell net across the intestine wall with its anticipating contribution to return of regular upper jejunum migrating myoelectrical complex after both of the surgical procedures. The interstitial cells of Cajal are positive for the protooncogene c-kit, which encodes the transmembrane tyrosine kinase receptor (CD-117), can be detected by immunochemical techniques (22) and visualized microscopically.

MATERIALS AND METHODS

Ethical approval

The experiments and procedures were conducted according to regulations of the European Community concerning the welfare of experimental animals and the Local Ethics Committee additionally approved all the procedures administered to the animals (Opinion no 24/2002, 26.06.2002).

Animals

Twelve male weaned pigs (Polish landrace Pietrain) with an initial body weight between 10 and 13 kg were used. The animals were kept in individual cages (0.8×1.2 m) in a temperature-controlled room with a light on between 07:00 and 21:00 h, and were fed with a commercial feed at 09:00 h and 20:00 h. The feed was given at a dose of 2% of body weight (b. wt.) for each meal; water was allowed *ad libitum*. The morning before the main surgery, food was reduced by half and then totally withdrawn on the evening before the surgery as well as during the next two postoperative days. Before surgery, the animals were randomized into one of two groups: A – operated with the Longmire-Traverso procedure and W – operated on with the Whipple procedure. Postoperatively, the pigs were rehydrated and fed parenteral (Kabiven Peripheral, Fresenius Kabi AB, Uppsala, Sweden). Starting from the 3rd postoperative day, the animals received water orally; and from the fourth postoperative day, oral feeding was introduced. All clinical aspects evaluated in the postoperative period, including body weight, were monitored regularly during the whole experiment.

First surgery: implantation of electrodes, telemetry transmitter and jugular vein's catheter

After 7 days of adaptation, the pigs were operated on in order to implant three pairs of silver bipolar silicone base electrodes on the jejunum (5, 15 and 45 cm distal to the Treitz ligament) according to Gacsalyi *et al*. (19) and to insert a silicone catheter in the right external jugular vein for postoperative parenteral nutrition. Azaperone (4 mg/kg b.wt. Stressnil, I.M., Janssen & Cilag Pharma, Vienna, Austria) was given for premedication. General anaesthesia was induced with 4% halothane (Narcotan 0.01%, Leciva, Czech Republic) mixed with oxygen (2 dm^3/min gas flow) given *via* an endotracheal tube, and was maintained with halothane at a concentration of 1.5–2% during the entire surgical procedure. Right flank laparotomy was performed. The electrodes were sutured on the jejunum, and a three-channel telemetry transmitter implant (D70EEE, DSI, Oregon, MN, USA) was fixed extraperitoneally in a pocket between the abdominal muscles. A stainless steel ground electrode was fixed to the abdominal muscles. The operated pigs were allowed 7 days for recovery, during which they were intramuscularly injected with antibiotics once daily (15 mg/kg b. wt., amoxycilin, Clamoxyl™ L.A., Pfizer, England).

Second surgery: Whipple or Longmire-Traverso procedure

The second operation was performed 7 days after the implantation of electrodes and transmitter. The animals randomized into the W group, underwent the Whipple procedure; and the animals randomized into A group, the Longmire-Traverso surgery.

The Whipple pancreatoduodenectomy consisted of excision of the right part of the pancreas, the adjacent part of the duodenum, the pylorus, 1/3 distal part of the stomach and the gallbladder, closure of the distal duodenal stump, connecting the proximal remnant of the stomach with the ileal loop by an end-to-side anastomosis between electrode I and II, and connecting the common bile duct with the side of the duodenal stump with a silicone tube. Anastomosis of the distal pancreas remnant to the jejunum was not necessary due to the presence, in the pig's anatomy, of the additional pancreatic duct joining the duodenum 25 cm beyond pylorus (*Fig. 1A*).

The Longmire-Traverso procedure involved, the removal of the right part of the pancreas with the adjacent part of the duodenum (with a cutting-line 2 cm beyond the pylorus) and gallbladder, closure of the distal duodenal stump; connecting the proximal remnant of the duodenum with the jejunal loop by an end-to-side anastomosis between electrode I and II, and connecting the common bile duct with the side of the duodenal stump with a silicone tube (*Fig. 1B*).

The pigs were given antibiotics (Clamoxyl 1 mg/kg i.m.) for up to 48 hours, if no risk factors occurred. If the surgery lasted longer than 3 hours, or contamination of the peritoneal cavity with the bile or intestinal contents occurred, regular antibiotic treatment was introduced (Clamoxyl 1×1 mg/kg i.m.).

Electromyography recording

All measurements were blinded. A 24-h electromyography recording session was performed 2–3 days before the second surgery to obtain the daily control of MMC characteristics. In separate recordings of 3 animals, the effects of anaesthesia, as well as of total parenteral nutrition (150 ml i.v., Kabiven Peripheral), on the MMC was examined. No changes in myoelectric activity were observed following the anaesthesia, and total parenteral nutrition, as compared with the control.

The electromyography recordings began 2–3 days before the second surgery to obtain control EMG characteristics and were continued immediately after the second surgery for 3 weeks. After that, the animals were sacrificed by pentobarbiturate (Thiopental, Biochemie GMbH, Austria) overdose; the position of the electrodes was verified, whole-tissue intestine samples were harvested for microscopy study, and the telemetry implants were removed.

The EMG signals, modulated into radio waves, had been received by a receiver antenna (RMC-1, DSI) placed under the animal's cage. The receiver was coupled to an analogue output (DL10, DSI); each of the three signal channels were filtered (high cut-off 50 Hz, low cut-off 10 Hz) and amplified (BioAmp, ADInstruments, Melbourne, Australia) as described previously (19). A four-channel PowerLab/4e (ADInstruments) and a PC computer were used to record, display and analyse the EMG data. The 24-hour consecutive recordings were analysed off-line using Chart v4.1 (ADInstruments) software. The recordings were inspected visually for MMC. The duration(s) of the spikes, duration of MMC phases (min), the root mean square (RMS) [mV] that expresses the "power" of the electromyography signal (Application Notes, ADInstruments), the migration velocity of the spikes and phase III of MMC (cm/min) along the jejunum were analysed (19, 23). The MMC phases were classified according to Code and Marlett (15). Phase IV was short in the pig proximal small intestine (several seconds) and thus was neglected in our analysis. In pigs fed twice a day, after each feeding, the MMC was replaced or masked by a "feeding pattern" of 130±15 min duration (20).

ICC analysis

Whole-tissue samples for immunofluorescence and biochemical analyses were taken two times: during the pancreatoduodenectomy (control samples) and, after completion of the study protocol, with a post mortal abdomen cavity inspection (study samples).

During the Whipple procedure, the specimens from the pylorus, the duodenum 2 cm beyond the pylorus: and the descendent duodenum, 20 cm beyond the pylorus, were taken. During the Longmire-Traverso procedure, only one specimen from the descendent duodenum, 20 cm beyond the pylorus, was taken. During the post mortal inspection, the whole-tissue samples from the three electrode sites, as well as, the gastrojejunal or duodenojejunal anastomosis area, were taken. All tissue samples were fixed in 4% formalin, embedded in paraffin blocks, and sectioned. Prior to the immunofluorescence, tissue sections were subjected to heat-induced epitope retrieval and adhered to silanized slides.

The Cajal cell staining procedure included, the incubation protocol provided with the DAKO® Retrieval Solution and polyclonal rabbit anti-human CD117, the c-kit application directions in the Staining Procedure section (DAKO® NP-Series, DAKO Corporation, USA) and the 7-AAD (7-aminoactimycin-D) staining procedure (DAKO Cytomation). The anti-c-kit was used to quantitatively assess, by light microscope, the presence of Cajal cells due to their specific morphology and location. The anti-c-kit and 7-AAD staining (simultaneously with red and green fluorescence) were used to quantitatively assess the presence of CD-117-positive Cajal cells by confocal microscopy LSM5 PASCAL (Zeiss, Germany). All measurements were blinded. A minimum of four slides were analysed from each tissue sample by confocal microscopy, and the number of ICC associated with Auerbach's plexus (ICC-AP) were counted by field of vision.

Statistical analyses

The electromyography data was extracted from the software "Chart" into an Excel data file and statistically analysed. The data was expressed as mean and standard error (mean ±S.E.M.). The Student's *t*-test, Mann-Whitney test, one-way ANOVA (followed by the Turkey post-hoc test) or the Kruskal-Wallis test (followed by the Dunn's post-hoc test) were used to test the statistical differences between the control recordings and those following the treatments (GraphPad Prism v.4.1, GraphPad Software, San Diego, CA, USA). $P<0.05$ was taken as the level of significance.

Quantitative statistical analysis of the Cajal cells was performed using confocal microscope software Axion Vision Release 4.3 (Zeiss, Germany).

RESULTS

Clinical observations

During the adaptation period, the pigs lost on average 0.4 kg, but they recovered smoothly after the first surgery and began gaining weight between 3 and 4 postoperative day. Within 3 weeks after the second surgery, piglets from Longmire-Traverso group significantly increased their weight by 19.8% in comparison to the preoperative weight (P=0.0002). Piglets from the Whipple group did not substantially increase their weight during the whole observation period. Moreover, in the early postoperative period, their weight was significantly lower in relation to the weight of animals from the Longmire-Traverso group (10.5±0.9 *versus* 12.8±1.2 kg, P=0.009).

Table 1. Average duration, signal power (root mean square, RMS) and spike frequency of the MMC in the proximal jejunum during the day and night in weaned pigs (mean ±S.E.M.) - control recordings from electrode II site.

		Phase I	Phase II	Phase III	MMC
Duration [min]	Day	7.7±3.5	51.5±9.15	4.4±1.3	64.4±9.1
	Night	7.1±2.8	54.3±12.3	5.1±1.2	67.8±12.6
	p	0.60	0.35	0.09	0.23
RMS [mV]	Day	0.018±0.006	0.062±0.026	0.106±0.021	
	Night	0.016±0.006	0.051±0.013	0.118±0.040	
	p	0.29	0.39	0.94	
Spikes frequency [Hz]	Day	0.163±0.12	0.562±0.19	1.901±0.27	
	Night	0.132±0.11	0.407±0.13	2.274±0.53	
	p	0.452	0.026	0.516	

The unpaired t-test or Mann Whitney test were used to compare the differences in each column.

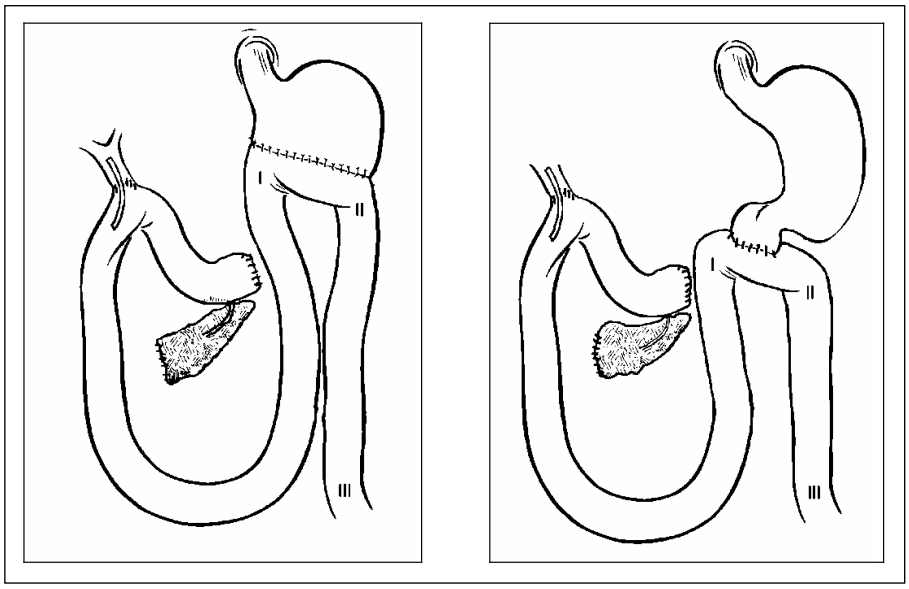

Fig. 1. Whipple (left) and Longmire-Traveso (right) pancreatoduodenectomy with schematic position of electrodes I, II, and III.

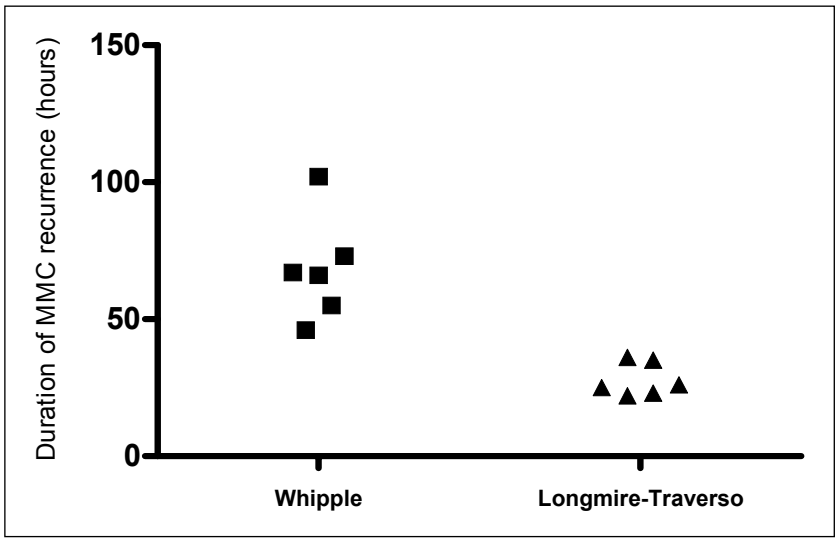

Fig. 2. Time to restore the small intestine MMC pattern (in hours) after Whipple and Longmire-Traverso surgery in examined pigs. The MMC was faster restored in the Longmire-Traverso group (P=0.002, non-parametric Mann-Whitney test).

In contrary, the animals from the Longmire-Traverso group, presented more often the clinical symptoms of upper gut motility disturbances than those after the Whipple procedure. The piglets from the Whipple group returned to their normal physical movement, felt hungry and looked for food from the first hours after operation; whereas those from the Longmire-Traverso group, presented a worse condition, were not interested in water and often vomited. Furthermore, in 50% of them, symptoms of delayed gastric emptying, according to the criteria by Noh (18), were still continuing after 3 weeks of observation.

Electromyography recordings

Before the second surgery, a total of 17±1.0 MMC cycles were recorded daily, with a significant difference in the number of MMC cycles recorded during the day (7.3±1.0) and night (10.0 ±0.9) (p=0.0007). No significant day-night differences were found in the duration of phases I, II and III of the MMC. The signal power (RMS) and spike frequency were similar, except for spike frequency during phase II (*Table 1*). The velocity of phase III migration along the jejunum, registered by the subsequent electrodes, was within average during daytime: 0.6±0.12 cm/s between electrodes I and II and 0.55±0.14 cm/s between electrodes II and III, with no statistical differences noted between day and night.

Directly after the second operation, no MMC cycles were present in either animal group. The first myoelectric activity (represented by single spike activity and bursts of spikes) was already registered 1–2 hours after operation.

The length of time until the first regular patterns of MMC activity was significantly longer in the Whipple group than in the Longmire-Traverso (68.2±12.9 versus 27.8±51 hours, p=0.002) (*Fig. 2*). In both groups, however, the restored patterns were substantially disturbed.

After the Longmire-Traverso operation, MMC cycles were very often. The number of recurrent cycles was on average 22.8 ±1.4 during the daytime, which corresponds to 40.7±5.6 during the whole 24 hours. Recurrent MMC were significantly shorter than those from the controls, and characterized with diminished "power" of signal and lower spike frequency, especially for phases II and III (*Table 2*). The phase III migration speed near anastomosis (*i.e.*, between electrodes I and II) amounted to 0.36 ±0.13 cm/s, being significantly smaller as compared to the control (p=0.001). No circadian variation was observed. Moreover, a pathological reverse migration from electrode site II to I was observed in this area. The speed of reverse signal

Table 2. Characteristics of the MMC cycle restored after Longmire-Traverso pancreatoduodenectomy (average duration [min], signal power (root mean square, RMS) and spike frequency) on electrode I site during the 1 postoperative day in relation to control recordings in pigs (mean ±S.E.M.). Recordings from electrodes II and III were similar.

	Phase	I	II	III	MMC
Duration [min]	Control	7.0±3.1	51.6±8.8[a]	4.5±1.1[a]	63.1±8.2[a]
	Longmire-Traverso	7.7±3.8	31.0±19.0[b]	3.6±0.6[b]	42.4±20.8[b]
	p	0.93	<0.0001	0.0061	<0.0001
RMS [mV]	Control	0.018±0.006	0.087±0.02[a]	0.119±0.023[a]	
	Longmire-Traverso	0.014±0.001	0.021±0.004[b]	0.038±0.006[b]	
	p	>0.05	<0.0001	<0.0001	
Spikes frequency [Hz]	Control	0.066±0.03	0.485±0.14	2.013±0.34	
	Longmire-Traverso	0.119±0.023	0.047±0.03	0.475±0.21	
	p	0.008	<0.0001	<0.0001	

The one-way ANOVA followed by Turkey or Kruskal-Wallis followed by Dunn's post-hoc tests were used to compare the differences in each column. P<0.05 was regarded as the level of significance.

Table 3. Characteristics of the MMC cycle restored after Whipple pancreatoduodenectomy (average duration [min], signal power (root mean square, RMS) and spike frequency) on electrode I site during the day (3rd postoperative day) in relation to control recordings in weaned pigs (mean ±S.E.M.). Recordings from electrodes II and III were similar.

	Phase	I	II	III	MMC
Duration [min]	Control	7.0±3.1	51.6±8.8	4.5±1.1	63.1±8.2
	Whipple	4.6±3.1	26.5±13.7	4.8±0.9	36.3±13.6
	p	0.006	<0.0001	<0.0001	<0.0001
RMS [mV]	Control	0.016±0.009	0.087±0.023	0.119±0.023	
	Whipple	0.027±0.009	0.035±0.004	0.050±0.008	
	p	<0.03	<0.0001	<0.0001	
Spikes frequency [Hz]	Control	0.066±0.03	0.485±0.14	2.013±0.34	
	Whipple	0.024±0.01	0.116±0.03	0.651±0.23	
	p	0.0015	<0.0001	<0.0001	

The one-way ANOVA followed by Turkey or Kruskal-Wallis followed by Dunn's post-hoc tests were used to compare the differences in each column. p<0.05 was regarded as the level of significance.

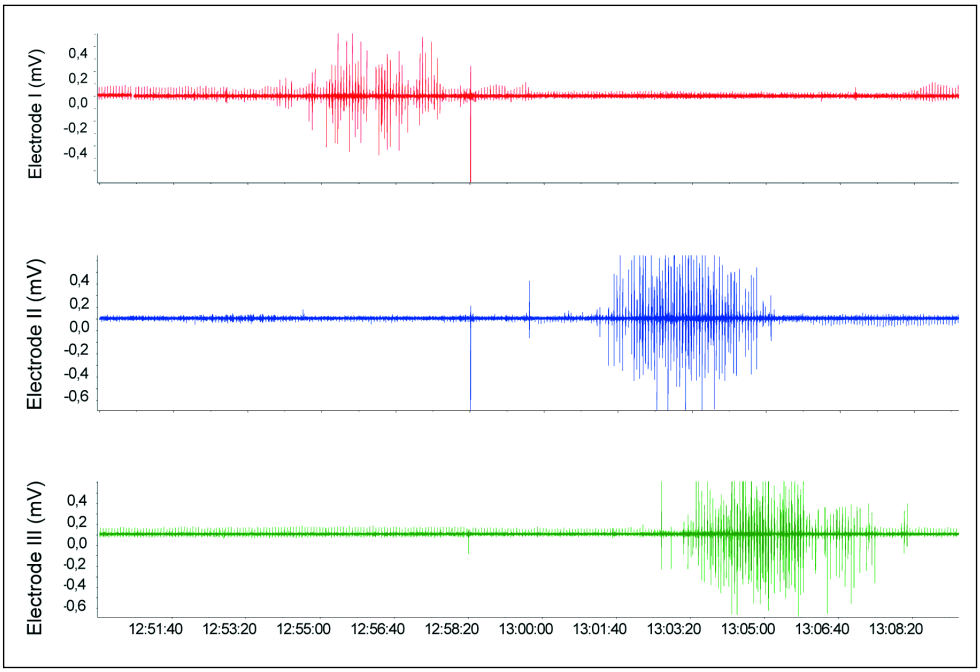

Fig. 3. Delay in migration of spike activity between electrodes I and II in electromyography recordings after Whipple procedure. Marked delay in migration speed from electrode I to electrode II is seen. Recording parameters: signal sampling speed 40/s, filters: hi-cut frequency >50 Hz, low-cut frequency <10 Hz, line filter: 50 Hz. Time scale: hh:mm:ss. A high amplitude spike in all 3-electrode sites at 12:58:20 is artifactual.

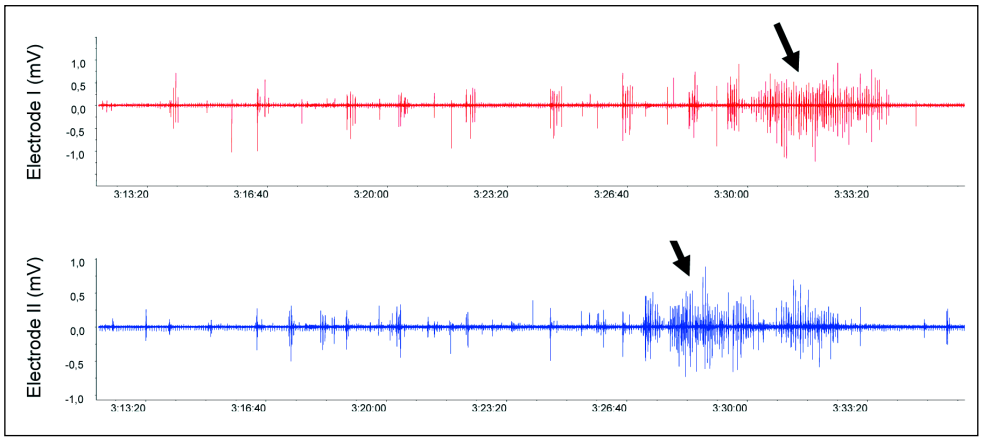

Fig. 4. Reverse migration of phase III MMC from electrode II to I (arrows) after Longmire-Traverso surgery procedure. Recording parameters: signal sampling speed 40/s, filters: hi-cut frequency >50 Hz, low-cut frequency <10 Hz, line filter: 50 Hz. Time scale: hh:mm:ss.

Table 4. Relation between the amounts of ICC located in the Auerbach's plexus in different parts of jejunum and restoration of regular MMC cycle among the animal after Longmire-Traverso and Whipple procedures.

Animal		MMC restoration (h)	Number of Cajal cells				
			Blind loop	Electrode I	Anastomosis	Electrode II	Electrode III
Longmire-Traverso	A	35	1	5	2	6	6
	B	23	2	5	3	8	5
	C	25	1	4	1	7	4
	D	36	0	4	3	6	5
	E	22	0	5	1	8	7
	F	26	1	4	4	7	7
	Mean	27.8±5.1	0.8±0.6	4.5±0.5	2.3±1.0	7.0±0.7	5.7±1.0
Whipple	R	46	1	3	4	5	5
	S	73	0	2	3	3	2
	T	55	1	5	2	4	4
	U	67	1	3	2	2	3
	W	102	0	2	1	2	2
	Z	66	1	3	2	2	5
	Mean	68.2±12.9	0.7±0.4	3.0±0.7	2.3±0.8	3.1±1.0	3.5±1.2
p		0.001	0.66	0.01	1.0	0.0001	0.02

The one-way ANOVA followed by Turkey or Kruskal-Wallis followed by Dunn's post-hoc tests were used to compare the differences in each column. P<0.05 was regarded as the level of significance.

migration was on average 0.14±0.04 cm/s, and the signal migration speed from electrode II to III was 0.54±0.16 cm/s, which was not different from the migration speed observed before second surgery (p=0.9).

MMC cycles, restored after the Whipple operation, were less frequent and of short duration, significantly shorter in comparison with both the control and pylorus-preserving groups; and had substantially reduced "power" of signal (RMS) as well as a lower spike frequency (*Table 3*). The average number of MMC cycles registered by electrode I was 18±4 (P<0.001) with the day-night distribution being, respectively, 6±2.7 and 11.5±2.5 cycles. Phase III migration was evidenced by all three electrodes. Its velocity near gastrointestinal anastomosis, between electrode's site I and II, was smaller than in the distal jejunum (*Fig. 3*), and significantly different from the control (0.135±0.03 cm/s versus 0.6±0.12 cm/s, p<0.0001). The migration speed between electrode II and III (0.63±0.16 cm/s) was similar to that in the control recordings.

After three weeks recovery, the total number of MMC cycles in the two groups was close to the control: in Longmire-Traverso group it was 25.3±1.5 and in Whipple group, 21.5±4 cycles. Recordings from the last day of observation indicated a significant shortening of MMC cycle duration in the both experimental groups (47.0±9.6 in Longmire-Traverso and 31.3±10.2 in Whipple *versus* 64.4 ±9.1 in control group, P<0.0001). Signal power and frequency of spike activity in the MMC phases showed high irregularity as compared to the control. However, in the Longmire-Traverso group, the parameters were close to the control, whereas in the Whipple group - significantly lower as compared to the control.

The modifications of migration observed after 3 weeks of the postoperative period were characteristic for each of the group. In the Longmire-Traverso group, there was a typical migration with a predominant signal at the electrode II site, which coincidentally showed little delayed activity at the

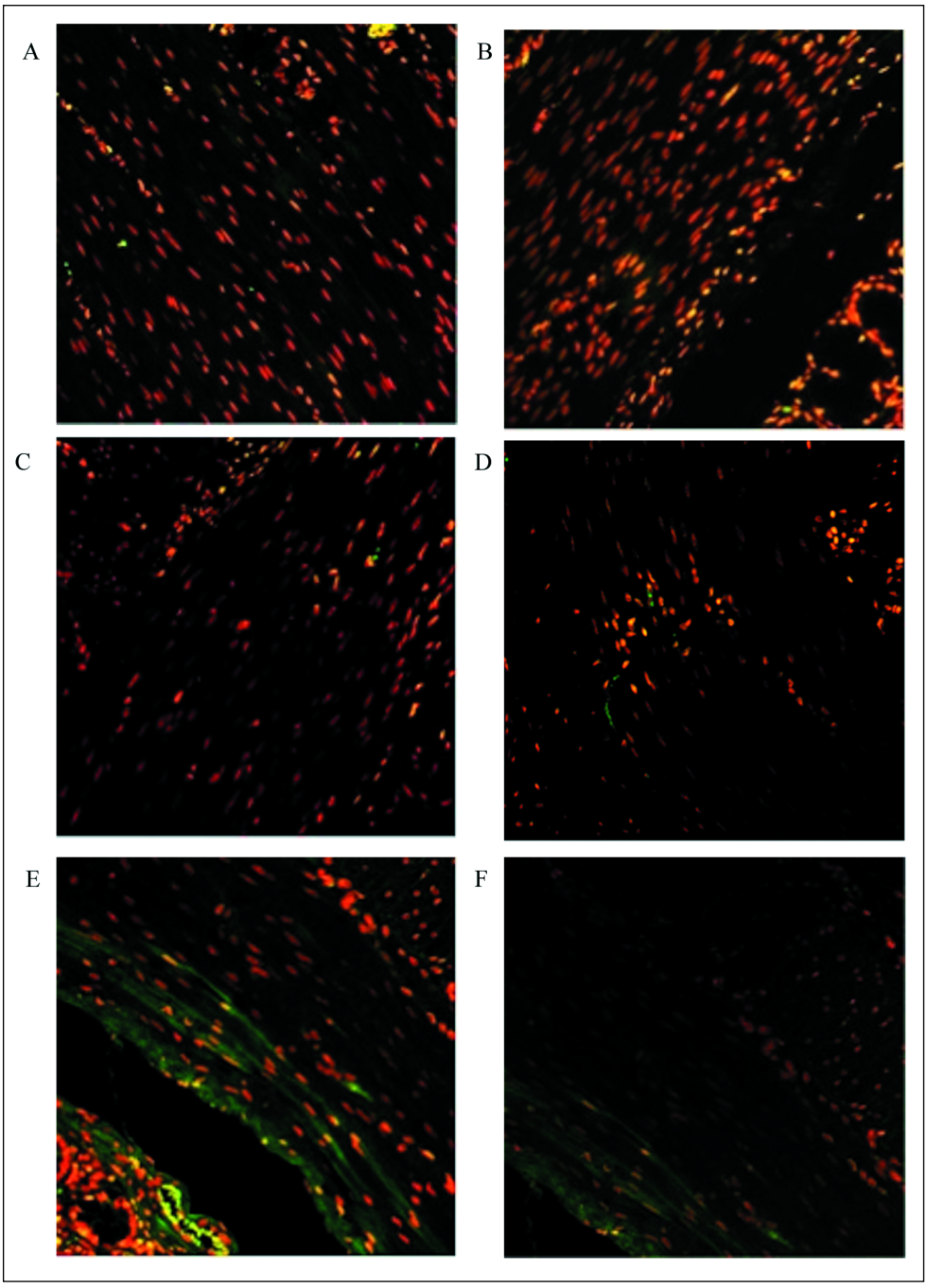

Fig. 5. Interstitial cells of Cajal associated with Auerbach's plexus in the Longmire-Traverso pig gut samples taken from: (A) The area of gastrointestinal anastomosis. (B) Area of electrode I. (C) The anastomosis area. (D). Area of electrode I. (E). Area of electrode II (F). Area of electrode III. (green fluorescence - interstinal cells of Cajal; red fluorescence - cell nuclei; objective ×40).

electrode I site. The speed of reverse migration from electrode II to I was 0.14±0.05 cm/s during the day and 0.18 ±0.1 cm/s at night (*Fig. 4*). In recordings from the Whipple group, the migration between electrodes I and II (near gastrointestinal anastomosis) remained slow in comparison with the migration of phase III both, in the control as well as in the Longmire-Traverso group. The duration of the feed pattern in group A was 54.7±11.8 min, and in Whipple group was 46.7±8.09 min which did not show any statistically significant differences from the duration found from the control group (p=0.2620). The RMS of the feed pattern registered in Longmire-Traverso group was 0.068±0.03 mV, and in Whipple group was 0.089±0.02 mV, which, additionally, did not show any statistically significant difference from the control values (p=0.23).

Identification of interstitial cells of Cajal

The number of CD117-positive cells in the upper jejunum in the Whipple group, near all the electrode sites, was lower than in the Longmire-Traverso group (*Table 4*) and the control group (*Figs. 5*, *6*). The ICC number near anastomoses after the both surgeries was significantly lower than in the distal segments of the intestine, *i.e.*, close to the electrode II and III sites, where it reached control values. However, in the closed loop area, the ICC was not observed at all. It was noticed that the network of Cajal cells in the vicinity of anastomosis, and distally from it, presented greater destruction after the Whipple operation in relation to the preserving surgery. Furthermore, a direct positive relation between the amount of ICC located in the region of the Auerbach's plexus, the restoration of regular MMC cycle

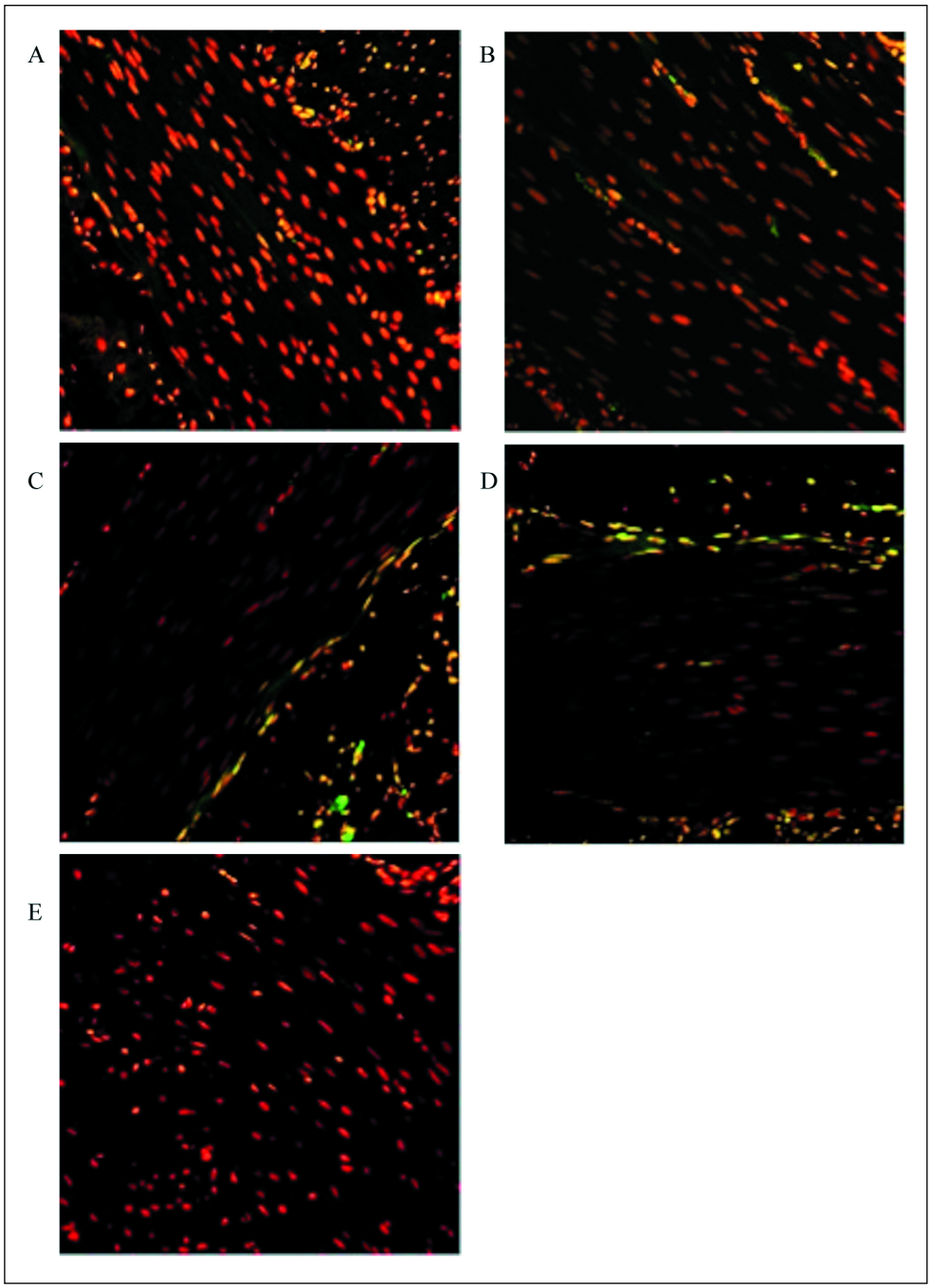

Fig. 6. Interstitial cells of Cajal associated with Auerbach's plexus in the gut samples of pigs after Whipple procedure: (A) Area of gastrointestinal anastomosis. (B) Area of electrode I. (C) Area of electrode II. (D) Area of electrode III. (E) The blind loop area (green fluorescence - interstitial cells of Cajal; red fluorescence - cell nuclei; objective ×40).

among the same group and between the groups of animals, were noted (*Table 4*).

DISCUSSION

The first single spike activity already appeared 1–2 hours after pancreatoduodenectomy. Such early myoelectric activity after operation is a very rare observation in literature (21).

In our study, the MMC cycles were restored 2.5 times faster after the Longmire-Traverso than the Whipple procedure. Most likely, this is related to the presence of pacemaker in preserved part of the stomach. However, it appears to be strongly influenced by extrinsic factor, which is the extent of surgical injury. The greater injury, the longer MMC recovery time.

Nevertheless, in our observation, the piglets after the pylorus-preserving operation more often presented clinical symptoms of upper gut motility disturbances than those after the standard one. Myoelectric activity in this group was much more intensive and showed higher incidence of qualitative and quantitative disturbances, which did not allow for fast stabilization of intestinal motor activity. The restored intestine needs, even after pylorus-preserving operation, at least 3 weeks to recover proper motility activity during the fasting period.

The most characteristic changes in EMG of animals after the Longmire-Traverso were related to the migration of MMC in phase III. Preliminary, this phase migrated distally from electrodes II to III, and proximally (reverse way) to electrode I. Around day 3, postoperatively, a new observation was detected - phase III which originated from electrode II, migrated distally to electrode III and

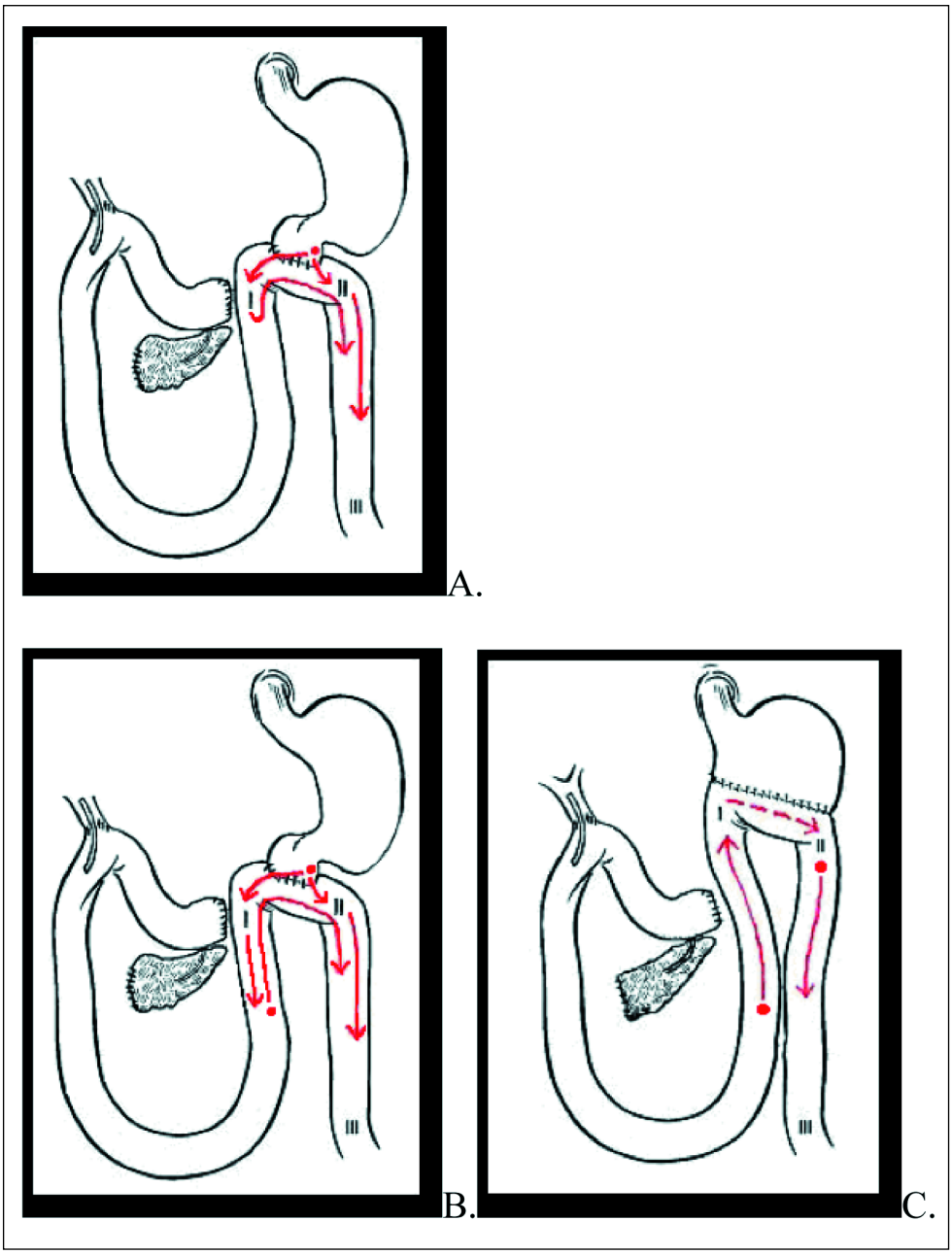

Fig. 7. Migration characteristics of phase III in postoperative period.
(A) Effect of physiological duodenal pacemaker activity (*re-entry* phenomenon) after Longmire-Traverso procedure. (B) Concomitant activities of two pacemaker centers after Longmire-Traverso procedure. (C) Ectopic pacemaker with essential delay in anastomosis region as well as another ectopic pacemaker triggered below the anastomosis.

proximally to electrode I; and then, „came back" to migrate from electrode I to electrodes II and III, respectively (*Fig. 7A*). The latter phenomenon created a picture of a "circulating phase III". A similar occurrence, known as "re-entry", plays an important role in the pathophysiology of heart arrhythmias (24). Phenomenon of "re-entry" was already recognized as a mechanism of asynchronic myoelectrical activity of ileum (25); however, this theory has never been proved. The next change in the migration feature of phase III MMC after the Longmire-Traverso was the appearance of independent activity from the region of electrode I. Starting from day 7, no more reverse migration between electrodes II and I was detected. Phase III occurred independently from electrode I and then migrated properly (*Fig. 7B*), which was maintained till the end of the observation period.

The fact of the predominance of EMG measurements from electrode II during the first postoperative days suggests that migrating active potentials are generated by the natural duodenal pacemaker still present due to 2 cm of duodenum preserved in this technique. During observation, concomitant ectopic pacemaker activation is observed in the blind loop, corresponding to independent activity registered in electrode I.

Once in slides from the proximal part of the ileal loop, no Cajal cells were shown contrary to a few ICC in the area of electrode I, thus it can be elucidated that the ectopic pacemaker is situated in the distal part of "excluded" loop. It is assumed that the final observed migration characteristic is a combination of the concomitant action of two pacemakers: physiological and ectopic. The latter, can be recognized as an adaptation of the modified ileum to the new iatrogenic condition. Possibly, the parallel activity of the physiological and ectopic pacemaker, and thus numerous reverse migrations and "storm of impulses" in the areas of anastomosis, makes it impossible for proper gastric emptying and ileal passage through the areas of anastomosis. This was manifested by clinically observed symptoms of motility disorders.

After the Whipple procedure, the migration of phase III from electrodes I to III was observed during the whole study and no

reverse migrations were detected. However, when there were some changes in the spreading of MMC in the areas of wide anastomosis observed, the MMC migration was significantly slower. Namely, the time to pass in this area for spikes was so prolonged, that phase III at electrode III appeared independently and was detected almost at the same time as that at electrode II. The modification of phase III with the later restored MMC after the Whipple procedure probably comes from the existence of the ectopic pacemaker in the distal part of the blind loop, which took over the function of the removed duodenal pacemaker and acted as the predominant pacemaker, creating the rhythm for the distal part of the small intestine. It seems that, due to essential delay of electrical impulse transmission through the anastomosis area, a new pace making area below the latter was reached and thus, distal transmission was preserved (*Fig. 7C*). The prolonged migration through the gastrointestinal anastomosis seemed to be responsible for clinically observed delayed gastric emptying (26). Finally, weaker EMG activity after the Whipple procedure resulted in rare manifestation of clinical symptoms compared to the Longmire-Traverso group.

The stress caused by inflammation within the anastomosis area (27) may have been a possible reason for the delay in migration through the gastrointestinal anastomosis, due to its known destructive impact on the Cajal cells affecting the function of slow wave generation. The macrophages, responsible for the induction of inflammation (28) are similarly localized in the vicinity of the deep muscle plexus as the Cajal cells (29); hence, it is possible that released pro-inflammatory factors destroyed ICC (30). It is proven that in humans, the production of nitric oxide, cytokines and bioactive mediators is due to the response of surgical injury (31). In mice, Yanagida *et al*. (32) showed an absolute lack of both slow wave activity and Cajal cells in the closest area of anastomosis during the period of 5 hours after ileum resection. At the same time, in the muscle layer of the anastomosis region, many macrophages and pro-inflammatory neutrophils were shown. Also, myoelectric activity was shown using *in vitro* model to contribute to host-bacteria interactions (33). In our study, the prolonged period of myoelectric activity disturbances in the anastomosis area after the pancreatoduodenectomy, especially Whipple procedure, in comparison to those observed by Yanagida *et al*. (32), may have resulted in bigger injuries to Cajal cells.

Der *et all*. (34) showed in mouse model that infection markedly altered the structural integrity of the ICC network within the small intestine, which impaired the slow wave activity and compromised the electrical coupling within the ICC network. The similar observations were made by Rumessen (35), who highlighted the changes in ICC function as a result from both the primary damage and the secondary changes, reflecting neuroimmune-mediated metabolic response.

Our study also seems to confirm, that both intensive inflammatory reaction and increased apoptosis processes in the area of surgical injury underlie to the some extend the disruption of ICC network continuity.

By analysing the relations between the amount of Cajal cells and MMC recovery after operation, both within and between the groups, a tendency towards a bigger amount of ICC, in the animals where regular cycles restored faster, was found. This relation confirms that the potential therapeutic approach to Cajal cells activity, by both limiting their destruction and promoting faster recovery, would lead to efficient treatment of motility disturbances after extensive abdominal procedures.

Conclusions

The myoelectric background of postoperative motility disorders after Whipple and Longmire-Traverso pancreatoduodenectomies is different. In the Longmire-Traverso group, the disturbances originating from intensive and poorly synchronized activity, often results in the occurrence of reverse migration and the re-entry phenomenon. In contrast, motility disorders after the Whipple procedure come from poor myoelectric activity and prolonged migration through the wide anastomosis region, the latter resulting in an intensive inflammatory reaction and extensive destruction of Cajal cell network along the anastomosis.

Despite using highly homogenous model involving healthy animals, the advantage of one of two major pancreatoduodenectomies in terms of myoelectrical activity correctness in upper gut has not been showed in the present study.

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