Brain derived neurotrophic factor declines after complete curative resection in gastrointestinal cancer

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Background. Brain derived neurotrophic factor (BDNF) is a neurotrophin involved in neural and metabolic diseases, but it is also one of the crucial factors in cancer development and metastases. In the current study, we investigated serum BDNF concentrations in patients that underwent surgical treatment for colorectal cancer or pancreatic cancer. **Methods**. Serum BDNF concentrations were measured with standard enzyme-linked immunosorbent assays, before and on the third day after the operation, in 50 consecutive patients with colorectal cancer and 25 patients with pancreatic cancer (tumours in the head of pancreas). We compared pre- and postoperative BDNF levels, according to the subsequent TNM stage, histologic stage, lymph node involvement, neuro- or angio-invasion, and resection range.**Results**. In the pancreatic cancer group, BDNF concentrations fell significantly postoperatively (p=0.011). In patients that underwent resections, BDNF concentrations fell (p=0.0098), but not in patients that did not undergo resections (i.e., laparotomy alone). There were significant pre- and postoperative differences in BDNF levels among patients with (p=0.021) and without (p=0.034) distant metastases. Significant reductions in BDNF were observed postoperatively in patients with small tumours (i.e., below the median size; p=0.023), in patients with negative angio- or lymphatic invasion (p=0.028, p=0.011, respectively), and in patients with lymph node ratios above 0.17 (p=0.043).In the colon cancer group, the serum BDNF concentrations significantly fell postoperatively in the entire group (p=0.0076) and in subgroups of patients with or without resections (p=0.034, p=0.0179, respectively). Significant before-after differences were found in subgroups with angioinvasions (p=0.050) and in those without neuroinvasions (p=0.049). Considering the TNM stages, the postoperative BDNF concentration fell in groups with (p=0.0218) and without (p=0.034) distant metastases and in patients with tumours below the median size (p=0.018).**Conclusion**. Our results suggested that BDNF might play an important role in gastrointestinal cancer development. BDNF levels were correlated with tumour volume, and with neuro-, angio-, and lymphatic

invasions. In pancreatic cancer, BDNF concentrations varied according to the surgical procedure and they fell significantly after tumour resections. Thus, BDNF may serve as a potential marker of complete resections in underdiagnosed patients. However, this hypothesis requires further investigation. In contrast, no differences according to the procedure was made in patients with colon cancer.

1 **Brain-derived neurotrophic factor declines after complete curative resection in**
2 **gastrointestinal cancer**
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17 **Abstract**

18   **Background.** Brain derived neurotrophic factor (BDNF) is a neurotrophin involved in
19 neural and metabolic diseases, but it is also one of the crucial factors in cancer development and
20 metastases. In the current study, we investigated serum BDNF concentrations in patients that
21 underwent surgical treatment for colorectal cancer or pancreatic cancer.
22   **Methods**. Serum BDNF concentrations were measured using standard enzyme-linked
23 immunosorbent assays, before and on the third day after the operation, in 50 consecutive patients
24 with colorectal cancer and 25 patients with pancreatic cancer (tumours in the head of pancreas).
25 We compared pre- and postoperative BDNF levels according to the subsequent TNM stage,
26 histologic stage, lymph node involvement, neuro- or angio-invasion, and resection range.
27   **Results**. In the pancreatic cancer group, BDNF concentrations fell significantly
28 postoperatively ($p = 0.011$). In patients that underwent resections, BDNF concentrations fell ($p = $
29 0.0098), but they did not in patients that did not undergo resections (i.e., laparotomy alone).
30 There were significant pre- and postoperative differences in BDNF levels among patients with
31 ($p = 0.021$) and without ($p = 0.034$) distant metastases. Significant reductions in BDNF were
32 observed postoperatively in patients with small tumours (i.e., below the median size; $p = 0.023$),
33 in patients with negative angio- or lymphatic invasion ($p = 0.028$, $p = 0.011$, respectively), and in
34 patients with lymph node ratios above 0.17 ($p = 0.043$).
35   In the colorectal cancer group, the serum BDNF concentrations fell significantly
36 postoperatively in the entire group ($p = 0.0076$) and in subgroups of patients with or without
37 resections ($p = 0.034$, $p = 0.0179$, respectively). Significant before-after differences were found
38 in subgroups with angioinvasions ($p = 0.050$) and in those without neuroinvasions ($p = 0.049$).
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40 0.0218) and without ($p = 0.034$) distant metastases and in patients with tumours below the
41 median size ($p = 0.018$).

42   **Conclusion**. Our results suggested that BDNF might play an important role in
43   gastrointestinal cancer development. BDNF levels were correlated with tumour volume and with
44   neuro-, angio-, and lymphatic invasion. In pancreatic cancer, BDNF concentrations varied
45   according to the surgical procedure and they fell significantly after tumour resections. Thus,
46   BDNF may serve as a potential marker of complete resections in underdiagnosed patients.
47   However, this hypothesis requires further investigation. In contrast, no differences according to
48   the procedure was made in patients with colon cancer.
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50

51   **Introduction**

52   Oncologic diseases have become a main problem for national health services across the
53   world. According to the World Health Organization (WHO), in 2018, there were over 18 million
54   new cases and over 9 million deaths caused by cancer. Among these, colorectal cancer (CRC)
55   represented 10.2% of cases and 9.2% of deaths. Although pancreatic cancer (PC) was not listed
56   in the group of cancers with the most numerous new cases, it was counted in the group of
57   cancers that caused the most deaths. PC caused over 430 000 deaths per year, which represented
58   4.5% of all deaths caused by cancer (WHO, 2018). Consequently, much effort has been focused
59   on new therapies and therapeutic targets in oncologic treatment. Diagnostic methods, including
60   new tools and substances, are constantly being evaluated for efficacy in diagnoses and follow-up
61   monitoring. Among many others, neurotrophins seem to be a promising new target molecule in
62   this context.
63   Brain derived neurotrophic factor (BDNF) belongs to the family of growth factors that
64   includes nerve growth factor and neurotrophins 3, 4, and 5. BNDF plays an important role in brain
65   development and neurogenesis, taking part in memory and learning processes, and it modulates
66   energy metabolism and feeding behaviour. Serum neurotrophin concentrations have been
67   investigated in affective disorders and cognitive impairments. Low circulating BDNF is suggested
68   to play a protective role in neurodegeneration and stress responses and a regulatory role in
69   metabolism (Utami, Effendy & Amin, 2019; Hendriati et al., 2019; Munkholm, Vinberg &
70   Kessing, 2016; Krabbe et al., 2009). Cellular responses to neurotrophins are elicited via two
71   categories of cell membrane receptors: low affinity $p75^{NTR}$ receptor and high affinity and
72   specificity tyrosine kinase receptor B (TrkB). The latter one belongs to a family of cell membrane
73   receptors which are: TrkA, TrkB, TrkC for NGF, BDNF and NT3. BDNF binding to TrkB initiates
74   auto-phosphorylation of the receptor and initiates multiple signalling cascades, including pathways
75   such as: the mitogen-activated protein kinase (MAPK), phosphatidyl-inositide 3-kinase (PI3K),
76   and phospholipase C-gamma (PLC-γ). The intracellular signalling controlled by Trks and $p75^{NTR}$
77   is usually essential to the control of cell survival, proliferation, and differentiation. Phosphorylated
78   TrkB activates the growth factor receptor-bound protein leading to cell proliferation and survival,
79   metastasis formation, angiogenesis, and the inhibition of apoptosis through the MAPK and PI3K
80   pathways. BDNF increases the expression of hypoxia-induced factor (HIF-1alfa), which

81 upregulates vascular endothelial growth factor (VEGF) and TrkB expression, stimulating
82 neovascularization via recruitment of TrkB-expressing endothelial progenitor cells (Serafim
83 Junior et al., 2020).
84   The first described role of BDNF/TrkB in cancer was in neuroblastoma. That study showed
85 that BDNF/TrkB expression was preferentially elevated in aggressive tumours, and that it could
86 promote alignancy growth, invasion, and metastasis (Roesler, 2011). TrkB expression in
87 neuroblastoma was found to be associated with significantly increased expression f a subset of
88 matrix metalloproteinases (MMPs), especially MMP-1, MMP-2, and MMP-9 which take part in
89 extracellular matrix degradation (Han et al., 2007; Chopin et al., 2016).
90   The exact role of neurotrophins in oncology remains unclear. Some experimental studies
91 have shown that overexpression of hypothalamic BDNF supports natural killer and T-cell
92 cytotoxicity, which might be an essential anticancer mechanism. This echanism may be
93 responsible for the positive role that BDNF displays in cancers through immunoaugmentation.
94 Contrarily, BDNF administration resulted in changes in serum epidermal growth factor (EGF)
95 concentrations, which facilitated the migration of lung and ovarian cancer cells (Radin & Patel,
96 2017). Moreover, the synergistic inhibition of the EGF receptor (EGFR) and TrkB suppressed
97 colon cancer cell proliferation (de Farias CB et al., 2012). These findings might be explained by
98 cross-talk between TrkB and EGFR signalling, which is commonly up-regulated in other cancers
99 (Corkery et al., 2018; Qiu et al., 2006). The roles of BDNF and TrkB appear to be very important
100 in both benign and malignant diseases. To date, however, serum neurotrophin concentrations in
101 patients with cancer have not been widely investigated.
102   Here we aimed to determine the relationship between BDNF concentrations and cancer.
103 We determined serum BDNF concentrations in patients with pancreatic or colon cancer, before
104 and after surgery. We then analysed relationships between BDNF concentrations and different
105 oncological parameters, such as TNM staging, lymph nodes, neural and vascular invasions,
106 histologic grade, and tumour size.
107

108 **Materials and methods**

109 **Patients**

110   We collected serum specimens from 75 patients, 50 with CRC and 25 with PC (in the head
111 of the pancreas). We also collected serum specimens from a control group, which consisted of 20
112 patients that underwent scheduled surgery, due to an inguinal hernia. We excluded patients with
113 an inflammatory disease and patients taking immunosuppressive drugs from both the study and
114 control groups. In all cases, the platelet counts were within the normal range. We also excluded
115 patients with confirmed depression and patients taking psychoactive drugs during the study period,
116 because these conditions could influence the results, as shown by Sarabi et al (Sarabi et al., 2017).
117 All patients provided written informed consent. The study program was approved by the Ethics
118 Committee of the Medical University of Warsaw with the number KB/56/A/2014.
119

120 **Methods**

121   Each patient underwent the appropriate scheduled operation, which included laparotomy,
122 colon resection, pancreatoduodenectomy, or only a biopsy for histopathological analysis (without
123 resection). The histopathological investigation revealed adenocarcinoma in all cases. The tumour
124 volume was calculated by multiplying three diameters that we obtained from histopathological
125 reports. The tumour volumes varied from 7.5 to 810 cm^3 in the PC group and from 1 to 1650 cm^3
126 in the CRC group. The median volumes were 16 cm^3 in the PC group and 24 cm^3 in the CRC
127 group.
128   We measured serum BDNF concentrations, blood biochemistry, and morphology before
129 and on the 3rd day after the operation. BDNF concentrations were measured with an enzyme-linked
130 immunosorbent assay and standard chemical reagents (R&D Systems).
131

132 **Statistical analysis**

133   Statistical analyses were performed using Statistica 13.3 (StatSoft, Poland). P-values less
134 than 0.05 were considered significant.
135   The results were not normally distributed; therefore, we used the Mann-Whitney test for
136 comparisons between non-related data. We performed the Wilcoxon test to compare paired groups,
137 such as BDNF levels before and after the operation.
138   Patients were divided into subgroups based on the surgical procedure (resection vs. no
139 resection), lymph node and vascular involvement (vs. no involvement), tumour volume (above vs.
140 below the median), and the lymph node ratio (LNR; above vs. below 0.17). We set the LNR cut-
141 off value to 0.17, based on Rosenberg et al (Rosenberg et al., 2008).
142

143 **Results**

144   The study groups consisted of 50 patients with CRC (27 males and 23 females), 25 patients
145 with PC (13 males and 12 females). The median age was 67.8 years for the entire group (CRC
146 median age of 69.2 years, PC median age 65.4 years). The control group consisted of 20 patients
147 (males and females) with a median age of 61.2 years.
148   The resection rate was 86% (n = 43) in CRC patients and 60% (n = 15) in PC patients.
149 Clinical characteristics of study participants are shown in Figure 1. The pre- and postoperative
150 BDNF serum concentrations in the investigated groups and the control group are shown in Table
151 1. The control group showed no significant differences in serum BDNF concentrations measured
152 before and after the inguinal hernia operation. In the CRC group, the BDNF concentration did not
153 differ from that in the control group both before (p = 0.5924) and after operation (*p* = 0.5399). In
154 the PC group, the BDNF concentration did not differ from that of the control group before
155 operation (*p* = 0.6428) but was lower after operation (*p* = 0.313). When the PC and CRC groups
156 were stratified by sex, the BDNF concentrations before and after operation did not differ

157 significantly between the sexes, but men in the CRC group showed a significant reduction in
158 BDNF after the operation (Table 1).
159

Pancreatic cancer

161   Table 1 and Figures 2 and 3 show BDNF concentrations before and after the operation in
162 subgroups of patients with PC.
163   We found that postoperative serum BDNF concentrations (18.490 ng/ml, SD = 7.985) were
164 significantly lower than preoperative concentrations (25.384 ng/ml, SD = 15.446, p = 0.0455).
165 Among patients that underwent curative resections, the postoperative serum BDNF concentrations
166 (16.671 ng/ml, SD = 7.612) were significantly lower than the preoperative concentrations (23.769
167 ng/ml, SD = 12.794, p = 0.0098). However, among patients that did not undergo resections, the
168 serum BNDF concentrations were not significantly different before and after the operation (27.806
169 ng/ml, SD = 19.253 vs. 21.218 ng/ml, SD = 8.130, p = 0.7518). Similar postoperative reductions
170 in BDNF were found in patients with 4th TNM stage tumours (without resections) and in patients
171 with TNM-stage I, II, and III tumours (with resections). Significant postoperative reductions were
172 also noted in patients after resection of tumours below the median size (preoperative: 17.979
173 ng/ml, SD = 7.635, postoperative: 15.218 ng/ml, SD = 4.963, p = 0.0231) and after resections in
174 patients with lymph node ratios >0.17 (preoperative: 26.474 ng/ml, SD = 7.628 vs. postoperative:
175 13.467 ng/ml, SD = 13.331, p = 0.0431).
176

Colorectal cancer

178   Table 1 and Figures 2 and 3 show BDNF concentrations before and after the operation in
179 subgroups of patients with CRC. When the entire CRC group was considered, we found that
180 postoperative serum BDNF concentrations (23.339 ng/ml, SD = 9.016) were significantly lower
181 than preoperative concentrations (26.703 ng/ml, SD = 7.969, p = 0.0076). The change in BDNF
182 concentration did not depend on the type of surgical procedure; the postoperative BDNF levels
183 were significantly lower than preoperative levels in groups with and without resections
184 (postoperative vs. preoperative BDNF: 23.607 ng/ml, SD = 9.372 vs. 26.665 ng/ml, SD = 8.290,
185 p = 0.0346, and 21.689 ng/ml, SD = 6.739 vs. 26.940 ng/ml, SD = 6.121, p = 0.0180, respectively).
186 Additionally, serum BDNF concentrations declined significantly after the operations in patients
187 with (TNM stage IV) and without (TNM stages I, II, and III) distant metastases; in patients with
188 angioinvasions (preoperative: 25.425 ng/ml, SD = 9.489 vs. postoperative: 21.203 ng/ml, SD =
189 8.199, p = 0.0505), in those without neuroinvasions (preoperative: 27.108 ng/ml, SD = 8.201 vs.
190 postoperative: 23.911 ng/ml, SD = 9.032, p = 0.049713), and in those with tumours below the
191 median size (preoperative: 27.749 ng/ml, SD = 9.287 vs. postoperative: 21.337 ng/ml, SD = 7.843,
192 p = 0.0187).
193

Discussion

Our results suggest that the role of neurotrophins in cancer development is essential. We confirmed that preoperative serum BDNF concentrations were significantly different from postoperative concentrations. Interestingly, in the PC group, BDNF levels only dropped postoperatively when a resection was performed. Thus, BDNF might serve as an indicator of complete curative resections for PC; however, further research is needed to test this hypothesis. To the best of our knowledge, no previous study has described this particular attribute of BDNF in gastrointestinal oncology, although its importance in oncology has been described (de Moraes et al., 2018; Tajbakhsh et al., 2017; Ozono et al., 2017).

Akil et al. showed that BDNF induces cell proliferation and has an anti-apoptotic effect mediated through TrkB, which might promote uncontrolled tumour growth. They revealed that neurotrophin and the receptor transcripts are overexpressed in tumours compared with normal tissues (Akil et al., 2011) Fan et al. confirmed the importance of TrkB in preventing CRC cells from undergoing anoikis, a type of apoptosis that is suggested to act as a barrier to metastasis. Silencing TrkB and BDNF inhibits the metastasis of CRC cells in vivo, and the downregulation of TrkB alone increases the sensitivity of CRC cells to anoikis in vitro (Fan et al., 2014). Consequently, the BDNF/TrkB pathway has been described as a potential target of anticancer therapies in patients with CRC. Radin et al. showed that cross-talk occurred between TrkB and the EGFR signalling pathway (Radin & Patel, 2017). Thus, it has been suggested that BDNF/TrkB signalling might protect CRC cells from the antitumour effects of an EGFR blockade in anticancer therapy using EGFR-targeted monoclonal antibodies (cetuximab) (Akil et al., 2016; de Farias et al., 2012).

Other authors also report the BDNF/TrkB pathway as a very promising target for cancer therapies. In a study concerning pulmonary large-cell neuroendocrine carcinoma in a murine xenograft model, the authors found that inhibition of TrkB signalling with the tyrosine kinase inhibitor k252a resulted in tumor regression and relapse prevention (Odate et al., 2013). Thomaz et al. investigated the effects of inhibiting TrkB signalling by use of the selective inhibitor ANA-12 in medulloblastoma (Thomaz et al., 2016). ANA -12 treatment reduced cancer cell viability, induced cell cycle arrest and, with human recombinant BDNF, reduced colony formation. Thus, TrkB inhibition might be have promise in medulloblastoma therapy.

BDNF may modulate cancer cells' sensitivity to standard drugs. In neuroblastoma, PI3K inhibition abrogates BDNF's ability to protect cancer cells from therap. The process is mediated by Bim, protein involved in intrinsic apoptosis. Decreases in Bim protected neuroblastoma cells from paclitaxel-indiced cell death (Li et al., 2007). BDNF might be important in maintaining resistance to apoptosis by up-regulating proteins that delay the activation of transmembrane death receptors. In breast cancer cells, production of apoptotic molecule 2 (FAIM2) through downstream of TrkB counteracts apoptosis induced by cisplatin (Radin et al., 2016).

A few studies have investigated serum BDNF concentrations in cancer. Li et al. reported that BDNF levels are lower in patients with lung cancer than in healthy controls (Li et al., 2019). Those authors found higher BDNF concentrations in patients with small cell lung cancer compared

234 with patients with non-small cell lung cancer. That finding was consistent with findings by Kimura
235 et al., who noted that BDNF and TrkB expression levels are elevated in small cell lung cancer.
236 Elevated BDNF levels are associated with cancer development and a poor prognosis (Kimura et
237 al., 2018). In acute pediatric leukemia, serum BDNF levels were found to be lower than in healthy
238 controls and patients with clinical remission (Portich et al., 2016). The authors reported that normal
239 BDNF levels were associated with better outcome, whereas low serum BDNF levels might serve
240 as a potential marker of active disease. In another cancer study, in patients with CRC, serum BDNF
241 concentrations were also found to be reduced (Brierley et al., 2013). In a retrospective study it was
242 shown that BDNF concentrations were significantly lower in two cohorts of patients with CRC
243 compared with healthy controls; however, no significant differences in BDNF concentrations
244 between patients in different Dukes stages were found. Contrary to the above results, higher serum
245 BDNF concentrations in CRC patients compared with healthy controls were reported by Wang et
246 al (Wang et al., 2021). The authors confirmed that BDNF serum levels are correlated with tumour
247 mass and higher in metastatic patients. In the current study, we found no significant differences in
248 preoperative serum BDNF concentrations between the cancer groups and the control group. In
249 contrast, we found that the postoperative reduction in BDNF concentration was significantly
250 greater in the PC group than in the control group (p = 0.031). We cannot explain this difference
251 from other authors results. Significant factors which might influence BDNF and TrkB levels are
252 cancer biology and disease advancement. In an arising tumour, due to intensive vascularisation,
253 the serum BDNF level may be higher than it is in mature tumours where the tissue neurotrophin
254 concentration is high. Our study confirms the significantly higher BDNF concentration in patients
255 with TNM stage IV tumours, but we didn't find any significant differences in neurotrophin
256 concentration related to tumour size.
257   There were no significant differences in preoperative BDNF levels between consecutive
258 TNM stages in the CRC and PC groups. In the CRC group, we noted a significantly lower
259 postoperative concentration that was not dependent on surgical procedure (i.e., there was no
260 difference between the resection and no resection groups). These results are consistent with a study
261 by Zoladz et al. Those authors investigated serum BDNF and CRP concentrations after breast
262 surgery. They showed significantly reduced BDNF concentrations in serum (but not in plasma) at
263 24 h after surgery. Thus, they concluded that a decline in BDNF level might lead to an attenuation
264 of breast cancer cell growth and metastasis (Zoladz et al., 2019). In the present study, in the PC
265 group, we found significantly lower BDNF concentrations after a resection, but not after a
266 laparotomy. No previous studies have published a similar observation. Thus, in patients with PC,
267 the serum BDNF concentration appeared to be associated with tumour removal, because it did not
268 significantly change when cancer tissue was not removed. This finding suggested that there might
269 be some kind of relationship between the BDNF concentration and the presence of cancer tissues.
270   Tanaka et al. evaluated BDNF and TrkB mRNA levels in CRC, and compared their levels
271 between primary and metastatic gastric tumours (Tanaka et al., 2014). Those authors showed that
272 BDNF mRNA was associated with liver and peritoneal metastases. Patients that displayed
273 overexpression of either BDNF or TrkB and patients that co-expressed both had significantly

274   worse prognoses. Other authors have confirmed high mRNA levels of all NTs and Trks (except
275   TrkC) in pancreatic cancer cell lines (Ketterer et al., 2003). Results showed significantly higher
276   mRNA levels in cancer tissues compared with normal pancreas for BDNF but not for TrkB
277   receptor. All NTs and their receptors were expressed at higher levels in the nerve bundles than in
278   cancer cells, in accord with previous studies (Sakamoto et al., 2001). Thus, NTs might enhance
279   cancer cells' growth and promote their proclivity to infiltrate the nerve tracts in pancreatic cancer.
280   In an experimental study, Fan et al. confirmed that TrkB gene silencing and TrkB protein
281   downregulation resulted in a slight induction of apoptosis. [Fan et al., 2014] In a mouse model, it
282   was shown that TrkB overexpression attenuated apoptotic trends in cancer cells.

283   Additionally, Okugawa et al. observed elevated BDNF expression in primary tumours
284   compared with the adjacent normal mucosa in patients with gastric cancer (Okugawa et al., 2013).
285   On the other hand, Esfandi et al. noted a tendency toward BDNF downregulation in gastric cancer
286   tumour tissues (Esfandi et al., 2019). Interestingly, they found that BDNF levels were higher in
287   tumours with lymphatic/vascular invasion, compared with tumours without lymphatic/vascular
288   invasion, consistent with results presented previously (Jiffar et al., 2017; Maehara et al., 2000). In
289   the present study, BDNF concentrations did not appear to be associated with lymph node
290   involvement. We found no substantial differences in the pre- or postoperative serum BDNF
291   concentrations between groups with and without lymphatic invasion, in either type of cancer. The
292   only significant reduction in BDNF levels after the operation was found in the PC group with no
293   lymphatic invasion. We evaluated the lymph node ratio (LNR) as a marker of completely resected
294   metastatic lymph nodes, based on Rosenberg et al (Rosenberg et al., 2008). Those authors showed
295   that the LNR is a strong independent prognostic factor for patients with CRC, with a cut off value
296   of 0.17. They showed that patients with LNRs above 0.17 have a poor prognosis. We could not
297   confirm that finding in our analyses. In our patients with CRC, the serum BNDF concentrations
298   were not different between groups with LNR values below and above 0.17, either before or after
299   the operation. Interestingly, we found a significant postoperative reduction in BDNF in the PC
300   subgroup with LNR>0.17 ($p = 0.043$). Thus, postoperative reductions in BDNF concentrations
301   most likely depended on lymph node status, as shown previously (Esfandil et al., 2019; Jiffar et
302   al., 2017; Maehara et al., 2000).

303   We also divided patients into groups according to tumour size, either below or above the
304   median tumour volume. In both cancer types, the postoperative serum BDNF levels significantly
305   declined only in patients with smaller tumours, of below the median volume. We found no
306   differences in preoperative BDNF concentrations based on tumour size. In some previous studies,
307   the authors have speculated that, in CRC, larger tumours might be less aggressive than smaller
308   tumours, and thus larger tumours might indicate a lower incidence of lymph node metastases,
309   lymphatic invasion, and venous invasion. In contrast, newly arising tumours, due to their high
310   vascularization, might cause relevant changes in serum BDNF levels after a resection (Fukata et
311   al., 2019; Guzel et al., 2018; Beaten et al., 2012). That hypothesis suggested that the BDNF
312   concentration might not depend on tumour tissue mass; rather, it might depend on the
313   aggressiveness of a growing tumour. Thus, high BDNF concentrations might be associated with a

314 poor prognosis, as mentioned above. However, to our knowledge, no study has investigated the
315 relationship between BDNF levels in cancer tissue and tumour mass.

316 In patients with CRC, we found a significant postoperative decline in serum BDNF
317 concentrations when angioinvasion was present or neuroinvasion was absent. Conversely, in the
318 PC group, a significant postoperative decline in BDNF was only observed when angioinvasion
319 was absent. Although vascular and neural invasions were not included in the TNM staging, they
320 both can both influence the clinical course and prognosis. In the present study, we did not observe
321 significant differences between patients in different TNM stages, in either the CRC or PC group.
322 However, the BDNF concentrations declined significantly postoperatively, regardless of whether
323 patients displayed metastases. Due to the small numbers of patients in the angio/neuroinvasion
324 subgroups, we could not determine whether angio/neuroinvasion influenced the postoperative
325 decline in BDNF levels.

326 We did not observe any differences between men and women in pre- and postoperative
327 serum BDNF concentrations. Piccini et al. showed that peripheral BDNF varies diurnally, and that
328 men have significantly lower concentrations than women (Piccini et al., 2008). Similarly, Choi et
329 al. confirmed that plasma BDNF concentrations underwent diurnal variations in men, but not
330 women. However, those authors did not observe a significant difference between men and women
331 (Choi, Bhang & Ahn, 2011). In the present study, we observed significant declines in BDNF serum
332 concentrations after the operation in men, but not in women, in both the CRC and control groups.
333 We cannot explain this result, but it might have been caused by diurnal variations in BDNF
334 concentrations. We do not suspect that cancer had any influence on this decline.

335 The main limitation of this study was the small number of patients included. When the
336 population was divided into subgroups, the numbers of participants were insufficient to draw
337 unequivocal conclusions. Similarly, inclusion of a larger control group would make the
338 conclusions more convincing. Assigned study not assumed measurement of TrkB and BDNF in
339 cancer tissues what might additionally limit our conclusions, especially concerning BDNF/TrkB
340 as a potential target for novel anticancer therapies. However, we should underline that it is a
341 preliminary study to give a basis and direction to further research.

342 **Conclusions**

343 In summary, we observed a significant connection between serum BDNF concentrations
344 and GI cancer. Changes in serum BNDF concentrations were associated with tumour resection,
345 although this result was significant only in the PC group. In these patients, the BDNF concentration
346 declined significantly when the primary tumour was resected; thus, it might be considered a
347 potential indicator of complete resection. Changes in BDNF concentration also depended on the
348 disease stage, and they were closely related to lymph node involvement. Serum BDNF levels
349 depended on tumour aggressiveness, rather than on tumour tissue mass.

350 In conclusion, it should be emphasized that our results showed that serum BDNF
351 concentrations corresponded to the GI oncologic disease course. However, an unambiguous
352 conclusion requires further studies on larger groups of patients.

353

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355

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358

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Figure 1

Clinical characteristics of study participants.

Values are the number of patients (%) and the total number of patients analysed, unless otherwise indicated. G: histology grading; TNM: extent of invasion and/or size of tumour, regional node metastasis/involvement, metastasis; LNR: lymph node ratio, meaning the ratio of tumour-infiltrated to resected lymph nodes.

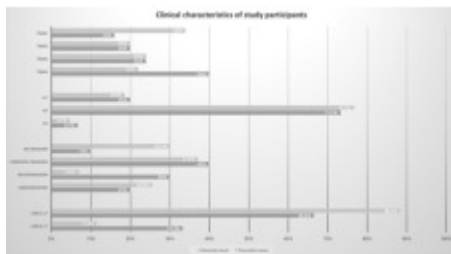



Figure 2

Pre- and postoperative serum BDNF concentrations in patients with colorectal cancer and pancreatic cancer.

Values in ng/ml. Only significant *p*-values are indicated. CRC: colorectal cancer; PC: pancreatic cancer; BDNF: brain-derived neurotrophic factor.

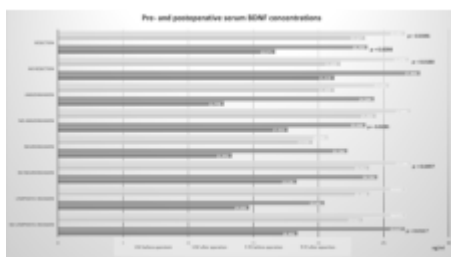
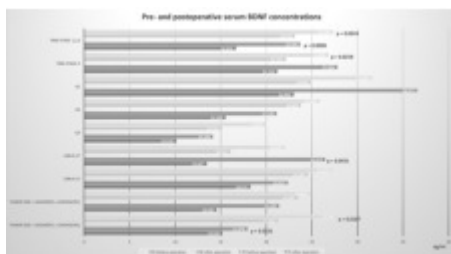



Figure 3

Pre- and postoperative serum BDNF concentrations in patients with colorectal cancer and pancreatic cancer.

Values in ng/ml. Only significant *p*-values are indicated. CRC: colorectal cancer; PC: pancreatic cancer; BDNF: brain-derived neurotrophic factor; TNM: extent of invasion and/or size of tumour, regional node metastasis/involvement, metastasis; G: histology grading; LNR: lymph node ratio, meaning the ratio of tumour-infiltrated to resected lymph nodes.

BDNF concentrations before and after the operation in patients with colorectal cancer, patients with pancreatic cancer,and the control group.

* *p*-values less than 0.05 were considered significant.Women and men did not show a statistically significant difference, both in the colorectal cancer and in the pancreatic cancer group.Values are the mean ± standard deviation unless otherwise indicated. BDNF: brain-derived neurotrophic factor; CRC: colorectal cancer; PC: pancreatic cancer.

1 **Tab. 1**
2 BDNF concentrations before and after the operation in patients with colorectal cancer, patients
3 with pancreatic cancer, and the control group.

Colorectal cancer	BDNF concentration before operation (ng/ml)	BDNF concentration on 3rd day after operation (ng/ml)	p-value*
CRC group (n = 50) Men (n = 23) Women (n = 22)	26.703±7.969 26.549±7.474 26.885±8.682	23.339±9.016 22.718±8.994 24.068±9.189	p = 0.0076 p = 0.0109 p = 0.2123
PC group (n = 25) Men (n = 13) Women (n = 12)	25.384±15,446 23.868±11.645 27.026±19.155	18.490±7.985 19.081±91.29 17.850±6.879	p = 0.0110 p = 0.0747 p = 0.0597
Control group (n = 20) Men (n = 15) Women (n = 5)	26.413±13.601 22.692±10.087 27.754±14.639	22.406±12.060 21.441±9.317 22.727±13.120	p = 0.0620 p = 0.8927 p = 0.0409

5 * p-values less than 0.05 were considered significant.
6 Women and men did not show a statistically significant difference, both in the colorectal cancer
7 and in the pancreatic cancer group.
8 Values are the mean ± standard deviation unless otherwise indicated. BDNF: brain-derived
9 neurotrophic factor; CRC: colorectal cancer; PC: pancreatic cancer.
10