Risk factors for long-term survival in patients with ypN+M0 rectal cancer after radical anterior resection

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Abstract

Background: Regional lymph node metastases are the main adverse prognostic factor in patients with rectal cancer without distant metastases. There are discrepancies, however, regarding additional risk factors in the group of ypN+M0 patients. The purpose of the study was to assess clinical and pathological factors affecting long-term oncological outcomes in the group of ypN+M0 patients after radical rectal anterior resection.

Methods: 112 patients with ypN+M0 rectal cancer after neoadjuvant therapy and radical anterior resection were subject to a retrospective analysis. The effect of potential factors on survival was assessed with the use of Kaplan–Meier curves together with a log-rank test and multiple factor Cox proportional hazards model.

Results: In the multiple factor Cox analysis, adverse factors affecting disease-free survival (DFS) were: the use of angiotensin-converting enzyme inhibitors (ACEIs) (hazard ratio HR: 3.11, 95% CI 1.01–9.56, $p = 0.047$), presence of perineural invasion (HR: 7.27, 95% CI 2.74–19.3, $p < 0.001$) and occurrence of postoperative complications (HR: 6.79, 95% CI 2.09–22.11, $p = 0.001$), while a positive factor was the negative lymph node (NLN) count > 7 (HR: 0.33, 95% CI 0.12–0.88, $p = 0.026$). In the disease-specific survival (DSS) analysis, an adverse factor was the use of ACEIs (HR: 4.275, 95% CI 1.44–12.694, $p = 0.009$), while a positive effect was caused by NLN > 5 (HR: 0.22, 95% CI 0.082–0.586, $p = 0.002$).

Conclusions: The use of ACEIs may have a negative effect on long-term treatment outcomes in patients with ypN+M0 rectal cancer. In this group of patients, the NLN count seems to be an important prognostic factor, as well.

Keywords: Angiotensin-converting enzyme inhibitors, Lymph node metastasis, Lymph node yield, Negative lymph node count, Rectal cancer, Renin-angiotensin system

Background

Regional lymph node metastases are the main adverse prognostic factor in patients with colorectal cancer without distant metastases [1]. However, reports on the risk factor of long-term survival in the group ypN+ are not consistent. So far, the effect of comorbidities and the medication used therein, such as metformin or renin-angiotensin system inhibitors (RASIs), on the treatment outcomes has not been clarified [2, 3]. There are few publications on this issue. It has also been shown that postoperative complications, especially anastomotic leakage (AL) after rectal anterior resection (AR) may have a significant effect on survival, The results of analyses, however, are not conclusive [4, 5]. Knowing the abovementioned, at least partially modifiable, factors could create a relatively easy possibility of affecting long-term treatment outcomes of patients with rectal cancer.

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It turns out that the assessment of nodal staging according to the TNM classification does not clearly stratify subjects after neoadjuvant therapy with regard to long-term survival [6]. Therefore, attempts have been made to assess other factors, such as lymph node ratio (LNR), log odds of positive nodes (LODDS), positive lymph nodes (PLN), or negative lymph nodes (NLN). Their prognostic value, however, has not been finally established [1, 7]. A separate issue is the minimum lymph node yield (LNY), owing to which underestimation of nodal staging may be avoided. Some authors deny the adverse effect of low LNY on survival, and even suggest that it is related to a good response to neoadjuvant therapy [8].

The purpose of the study was a retrospective assessment of clinical and pathological factors affecting long-term oncological outcomes in patients with rectal cancer at ypN + M0, after neoadjuvant therapy and radical (R0) AR.

Methods

Patients

A retrospective analysis was performed on 112 patients with ypN + M0 rectal cancer post neoadjuvant therapy and radical (R0) AR, treated at the National Research Institute of Oncology in Gliwice in 2008–2016. Patients meeting the following criteria were included in the analysis: histopathological diagnosis of adenocarcinoma (up to 15 cm from the rectal margin), past neoadjuvant treatment, past radical (R0) rectal anterior resection, presence of regional lymph node metastases in a postoperative histopathological examination (ypN+), no synchronous distant metastases. Exclusion criteria: no neoadjuvant treatment, abdominoperineal rectal resection, Hartmann's procedure, local resection of tumour, non-radical resection (R1 or R2), no regional lymph node metastases in a postoperative histopathological examination (ypN0), presence of synchronous distant metastases, death in the postoperative period (within 30 days). In addition, subjects with yN1c staging were excluded from the part of analysis regarding the assessment of nodal staging parameters, where it was necessary to provide the metastatic nodal count (LNR, LODDS, PLN), since it was not possible to perform a retrospective assessment of the tumour deposits count in all findings of the histopathological examinations. The process of the study group formation is presented on the chart in Fig. 1.

Patient characteristics is presented in Table 1. Comorbidities were assessed separately, as well as on the basis of the Charlson comorbidity index (CCI) [9]. Distant metastases were considered synchronous if occurred up to 3 months after surgery.
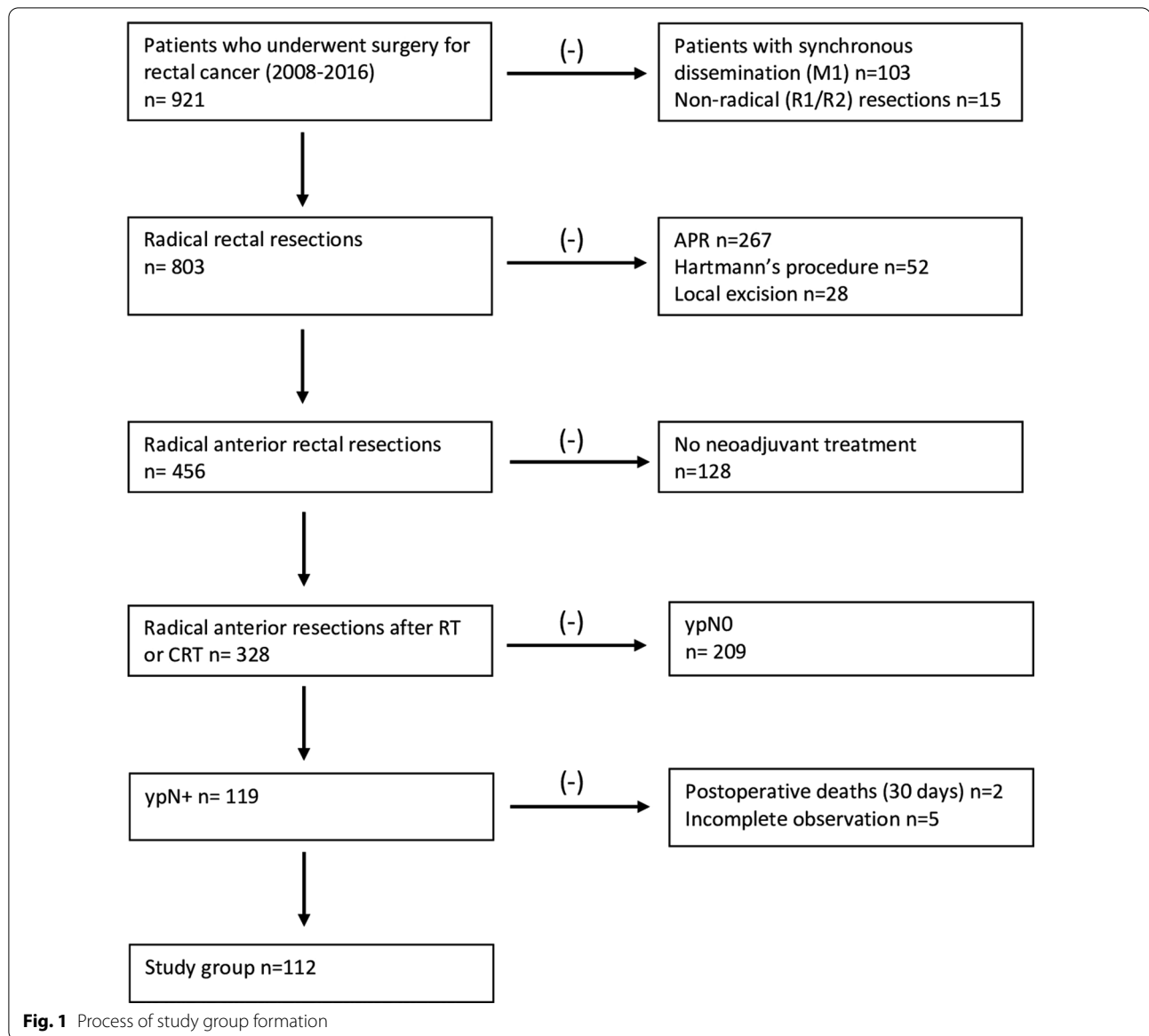
Procedures

All the patients received neoadjuvant therapy: radiotherapy (RT) at a total dose of 25–42 Gy or chemotherapy (CRT) at a dose of 42–54 Gy combined with one or two cycles of chemotherapy based on 5-fluorouracil. Before surgery, mechanical bowel preparation was performed with the administration of an oral antibiotic and perioperative intravenous antibiotic prophylaxis. AR was performed using laparotomy with total mesorectal excision. End-to-end anastomosis of bowel was performed with a circular stapler. AL, in accordance with the International Study Group of Rectal Cancer, was defined as a deficit at the anastomotic site leading to a communication between the intra- and extraluminal compartments and/or presence of pelvic abscess near the anastomosis [10]. AL was qualified as early if diagnosed within 30 days of surgery, and as late if occurred after that time. Adjuvant treatment was based on 5-fluorouracil. The histopathological examination was based on standard methods of searching for lymph nodes in the surgical specimen. Tumour regression grade (TRG) was based on the assessment of the degree of fibrosis compared to the residual tumor tissue and ranged from 0 to 3, i.e. 0 (complete response), 1 (< 10% residual tumor), 2 (10–50%) and 3 (> 50%).

Variables

Staging was assessed on the basis of the American Joint Committee on Cancer, TNM Staging System, 8th edition, 2017. LNR was calculated as the PLN to LNY ratio, while LODDS was calculated with the formula ln[(PLN count)/(NLN count)]. In the LODDS and LNR analysis in ypN1c patients, the PLN count was treated as no data and was excluded from this part of the analysis. Additional potential risk factors subject to analysis included: age, sex, body mass index (BMI), presence of comorbidities, CCI, medications used, type of neoadjuvant therapy (RT vs. CRT), time from RT completion to surgery, clinical staging of the disease before treatment, tumour distance from the anal verge, presence of loop ileostomy, occurrence of postoperative complications, TRG, perineural invasion (PNI), lymphovascular invasion (LVI), extranodal extension (ENE), width of distal margin, adjuvant chemotherapy.

Statistical methods

The effect of potential factors on survival was assessed with the use of Kaplan–Meier curves together with a log-rank test and Cox proportional hazards model. The estimation of cut-off points for the parameters related to nodal staging was based on the analysis of Kaplan–Meier curve difference significance for iteratively

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Fig. 1 Process of study group formation

increased cut-off thresholds. All calculations were made using the statistical package R version 3.5.3.

Results

In the study group, 3- and 5-year disease-free survival (DFS) was 71.9% and 59.7%, and disease-specific survival (DSS) was 85.5% and 74.4%, respectively. The mean follow-up period in the study group was 57 months. Loop ileostomy during the primary procedure was created in 23/112 (20.5%) of patients. Postoperative complications were observed in 39/112 (34.8%) of patients. AL was observed in 23/112 (20.5%) of patients, including 16/23 (69.6%) early and 7/23 (30.4%)

late ALs. In 15/23 (65.2%) cases of AL, anastomosis was separated by performing the Hartmann's procedure. Aside to the above, abnormal wound healing was observed in 6 patients, and there were 3 cases of urinary tract infection, 3 cases of pneumonia, 3 cases of bleeding and 1 case of mechanical obstruction. Postoperative complications are presented in the Additional file 1. 19 (17%) patients used angiotensin-converting enzyme inhibitors (ACEIs) at the time of treatment initiation, with 5 patients using ramipril, 5 perindopril, 3 enalapril, 2 lisinopril, 2 trandolapril, 1 cilazapril and 1 imidapril. 9 (8%) patients used angiotensin receptor blockers (ARBs).

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Table 1 Patient characteristics

Variable	n (%) mean (SD)*
Sex	
Females	42 (37.5)
Males	70 (62.5)
Age (years)	62.21 (10.32)*
BMI (kg/m^2)	26.64 (5.05)*
CAD	5 (4.5)
AH	45 (40.2)
Diabetes mellitus	18 (16.1)
CCI > 2	25 (22.3)
Alpha blockers	3 (2.7)
Beta blockers	26 (23.2)
ACEIs	19 (17)
Calcium channel blockers	14 (12.5)
Diuretics	10 (8.9)
ARBs	9 (8)
Metformin	7 (6.2)
Glimepiride	7 (6.2)
Gliclazide	5 (4.5)
cT	
1	1 (0.9)
2	10 (8.9)
3	99 (88.4)
4	2 (1.8)
cN+	91 (81.2)
Distance to the anal verge (cm)	
0–5	42 (37.5)
6–10	52 (46.4)
11–15	18 (16.1)
Neoadjuvant	
CRT	27 (24.1)
RT	85 (75.9)
Time RT-S > 6 weeks	45 (40.2)
Loop ileostomy	23 (20.5)
ypG	
1	5 (4.5)
2	82 (73.2)
3	8 (7.1)
x	17 (15.2)
ypT	
0	1 (0.9)
2	20 (17.9)
3	91 (81.2)
TRG	
0–1	27 (24.1)
2–3	85 (75.9)
Mucinous component	6 (5.4)
Tumour deposits	29 (25.9)
PLN count	3.59 (3.75)*
NLN count	10.69 (6.16)*

Table 1 (continued)

Variable	n (%) mean (SD)*
LNY	13.99 (6.79)*
ypN	
1	79 (70.5)
2	33 (29.5)
LNR	0.26 (0.21)*
LODDS	− 1.3 (1.18)*
ENE	14 (12.5)
LVI	10 (8.9)
PNI	13 (11.6)
Distal margin (cm)	2.11 (1.61)*
Adjuvant CT	89 (79.5)
Adjuvant CT > 3 cycles	78 (69.6)

BMI body mass index, *CAD* coronary artery disease, *AH* arterial hypertension, *CCI* Charlson comorbidity index, *ACEIs* angiotensin-converting enzyme inhibitors, *ARBs* angiotensin receptor blockers, *CRT* chemoradiotherapy, *RT* radiotherapy, *Time RT-S* time from radiotherapy completion to surgery, *G* histological tumour grade, *TRG* tumour regression grade, *PLN* positive lymph nodes, *NLN* negative lymph nodes, *LNY* lymph node yield, *LNR* lymph node ratio, *LODDS* log odds of positive lymph nodes, *ENE* extranodal extension, *LVI* lymphovascular invasion, *PNI* perineural invasion, *CT* chemotherapy, *SD* standard deviation
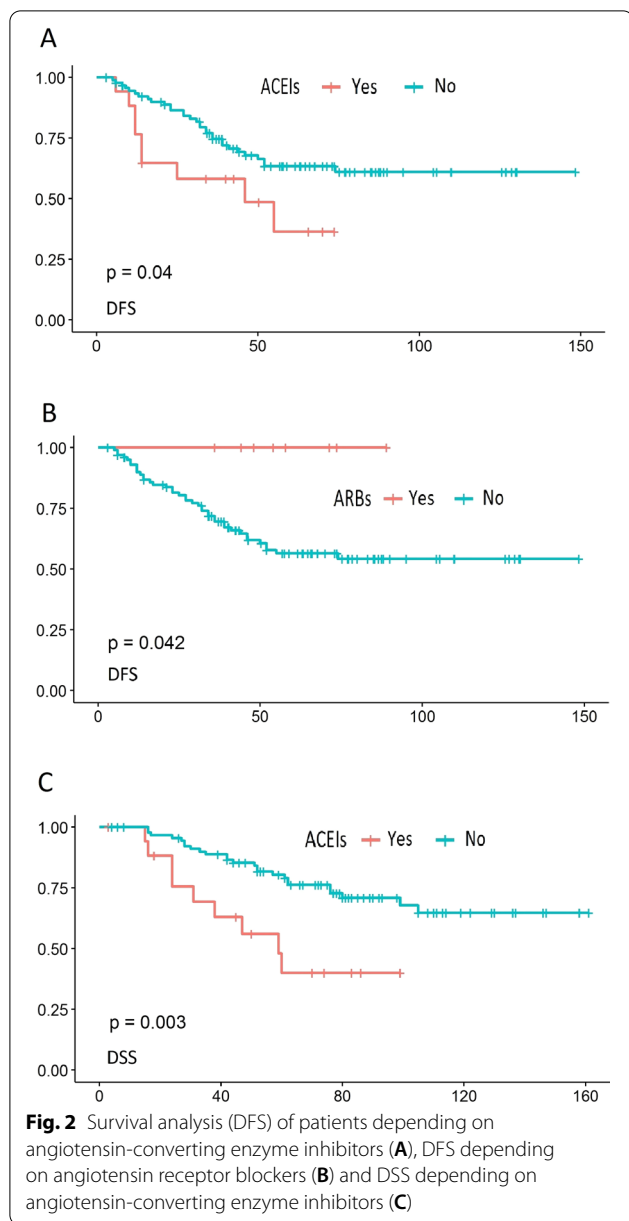
* Continuous variable

Analysis of nodal staging parameters
For the LNY variable, no cut-off point for which Kaplan–Meier curves would significantly differ was found. Similarly, no differences in survival were found while comparing nodal staging (ypN1 vs. ypN2) according to the TNM classification. For the NLN count, significant differences in survival were achieved for the cut-off point 5 (≤ 5 vs. > 5) for DSS ($p = 0.0045$) and the cut-off point 7 (≤ 7 vs. > 7) for DFS ($p = 0.029$). Results of the analysis of nodal staging parameters are presented in the Additional file 2.

Analysis of survival
Based on Kaplan–Meier curves and a log-rank test, we did not reveal an effect of comorbidities on survival, both with regard to separate analysis, and that based on CCI.

A negative effect of the use of ACEIs ($p = 0.04$) (Fig. 2a) and metformin ($p = 0.048$), and a positive effect of the use of ARBs ($p = 0.042$) (Fig. 2b) on DFS was shown. In addition, a negative effect of the occurrence of complications, regardless of the degree in the Clavien–Dindo classification ($p = 0.012$), occurrence of AL ($p = 0.024$), and after dividing AL into early and late, of early AL ($p = 0.0095$) on DFS was shown. Histological grade G3 ($p = 0.02$) and the presence of PNI ($p = 0.00015$) had a negative effect on DFS, as well.

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Fig. 2 Survival analysis (DFS) of patients depending on angiotensin-converting enzyme inhibitors (**A**), DFS depending on angiotensin receptor blockers (**B**) and DSS depending on angiotensin-converting enzyme inhibitors (**C**)

The DSS analysis showed an adverse effect of the use of ACEIs ($p = 0.003$) (Fig. 2c), metformin ($p = 0.016$), occurrence of complications, regardless of the degree in the Clavien–Dindo classification ($p = 0.025$), occurrence of AL ($p = 0.0049$), and after dividing AL into early and late, of early AL ($p = 0.00027$), and histological grade G3 ($p = 0.018$). No effect of other analysed factors on survival was revealed, including the effect of neoadjuvant treatment regimen (see Additional file 3).

Cox proportional hazards model

Results of Cox analysis are shown in Table 2. For DFS in a multiple factor analysis, significant adverse factors were: the use of ACEIs (HR: 3.11, 95% CI 1.01–9.56, $p = 0.047$), presence of PNI (HR: 7.27, 95% CI 2.74–19.3, $p < 0.001$), and the occurrence of postoperative complications (HR: 6.79, 95% CI 2.09–22.11, $p = 0.001$). And a positive factor was the NLN count > 7 (HR: 0.33, 95% CI 0.12–0.88, $p = 0.026$). In the DSS analysis, an adverse factor was the use of ACEIs (HR: 4.275, 95% CI 1.44–12.694, $p = 0.009$), while a positive effect was caused by NLN > 5 (HR: 0.22, 95% CI 0.082–0.586, $p = 0.002$). The other analysed factors were not significant in the multiple factor analysis. The above multivariate Cox analysis models were then further analyzed using the likelihood ratio test. The course and the results of this analysis are given in Additional file 4.

Discussion

The study showed a negative effect of the use of ACEIs and low NLN count on both DSS, and DFS. Additionally, DFS was negatively influenced by the presence of PNI and the occurrence of postoperative complications.

We showed a negative effect of ACEIs on survival independently of comorbidities, analysed both separately and based on CCI. The renin-angiotensin system (RAS) is one of the phylogenetically oldest endocrine systems, whose main task is to regulate water and sodium metabolism. The main component of this system is angiotensin II (AngII), which is developed from angiotensin I (AngI) with the participation of angiotensin converting enzyme (ACE) [11]. Aside to RAS in the circulatory system, a presence of all components of this system was shown in tissues (tissue RAS–tRAS), including in the neoplastic cells and tumour microenvironment cells, such as tumour associated macrophages, regulatory T cells and fibroblasts [12, 13]. This system acts in two major mechanisms opposite to each other through the AngII type 1 receptor (AT1R) and AngII type 2 receptor (AT2R). Activation of the ACE/AngII/AT1R pathway leads to vasoconstriction with consequential acidosis and hypoxia, which triggers expression of proinflammatory cytokines. Additionally, angiogenesis is intensified by an increased expression of the vascular endothelial growth factor, increase in tumour progression and its metastatic potential. And the activation of the AT2R/ACE2/Ang1-7/MasReceptor pathway triggers an antiinflammatory, antiproliferative, vasodilating and antiangiogenic effect [14]. It is estimated that about 40% AngII is formed in ACE-independent pathways [15]. Chymase, which is a serine protease, shows ACE-like activity and the ability to convert AngI to AngII. Under physiological conditions, it occurs in the form of inactive pro-chymase, and

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Table 2 Cox proportional hazards model

	Single factor analysis			Multiple factor analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Disease-specific survival (DSS)						
Sex: male	0.98	0.49–1.96	0.958			
Age > 65 years	1.41	0.72–2.77	0.321			
BMI > 30	1.32	0.60–2.93	0.495			
AH	1.63	0.83–3.21	0.154			
Diabetes mellitus	1.25	0.52–3.01	0.625			
CCI > 2	0.86	0.37–1.97	0.716			
Metformin	3.41	1.18–9.83	0.023	1.42	0.32–6.22	0.644
Alpha blockers	0.99	0.14–7.25	0.992			
Beta blockers	1.54	0.74–3.23	0.250			
ACEIs	3.05	1.41–6.60	0.005	4.28	1.44–12.69	0.009
CCBs	2.14	0.88–5.17	0.093			
Diuretics	0.95	0.29–3.11	0.930			
Noeadjuvant CRT	Ref					
Neoadjuvant RT	1.16	0.50–2.68	0.731			
RT-S ≥ 6 weeks	1.02	0.50–2.06	0.963			
Distance to the anal verge 1–5 cm	Ref					
6–10 cm	1.67	0.78–3.57	0.186			
11–15 cm	1.17	0.37–3.75	0.787			
Loop ileostomy	1.30	0.59–2.87	0.518			
Complications	2.12	1.08–4.16	0.029	2.18	0.47–10.07	0.317
ypT0-2	Ref					
ypT3	1.16	0.48–2.80	0.745			
LNY ≥ 12	0.68	0.35–1.34	0.266			
ypG3	3.01	1.14–7.94	0.026	2.82	0.69–11.52	0.149
ENE	0.98	0.35–3.75	0.968			
LVI	1.19	0.36–3.89	0.777			
PNI	1.79	0.69–4.63	0.230			
TRG 0–1	Ref					
TRG 2–3	1.34	0.58–3.08	0.496			
CT > 3cycles	0.58	0.28–1.20	0.140			
NLN > 5	0.36	0.17–0.75	0.006	0.22	0.08–0.59	0.002
PLN > 11	3.47	1.05–11.46	0.041	2.65	0.45–15.44	0.280
LNR > 0.15	2.18	1.01–4.73	0.048	1.15	0.13–10.19	0.899
Disease-free survival (DFS)						
Sex:male	1.09	0.58–2.07	0.788			
Age > 65 years	1.01	0.53–1.89	0.993			
BMI > 30	1.13	0.54–2.37	0.752			
AH	1.35	0.72–2.51	0.349			
Diabetes mellitus	1.21	0.54–2.75	0.642			
CCI > 2	0.72	0.32–1.63	0.429			
Metformin	2.76	0.97–7.83	0.006	0.85	0.22–3.26	0.808
Alpha blockers	1.13	0.16–8.24	0.904			
Beta blockers	1.59	0.81–3.12	0.181			
ACEIs	2.15	1.02–4.55	0.044	3.11	1.01–9.56	0.047
CCBs	1.98	0.87–4.48	0.103			
Diuretics	1.16	0.41–3.27	0.776			
Neoadjuvant RT	1.09	0.52–2.29	0.824			

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Table 2 (continued)

	Single factor analysis			Multiple factor analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
RT-S ≥ 6 weeks	0.79	0.41–1.49	0.463			
Distance to the anal verge 1–5 cm	Ref					
6–10 cm	1.35	0.68–2.69	0.390			
11–15 cm	0.95	0.34–2.66	0.916			
Loop ileostomy	1.21	0.58–2.54	0.614			
Complications	2.18	1.17–4.05	0.014	6.79	2.09–22.11	0.001
ypT0-2	Ref					
ypT3	1.48	0.62–3.54	0.374			
LNY ≥ 12	0.92	0.49–1.73	0.796			
ypG3	2.69	1.12–6.48	0.028	0.75	0.13–4.21	0.748
ypN1	Ref					
ypN2	1.02	0.52–2.00	0.961			
ECI	1.25	0.53–2.98	0.613			
LVI	1.79	0.70–4.57	0.225			
PNI	4.09	1.86–8.97	0.001	7.27	2.74–19.30	< 0.001
TRG 0–1	Ref					
TRG 2–3	1.27	0.59–2.76	0.545			
CT > 3cycles	0.82	0.41–1.64	0.569			
NLN > 7	0.50	0.26–0.94	0.032	0.33	0.12–0.88	0.026
PLN > 10	2.72	1.06–6.99	0.038	0.76	0.20–2.95	0.692
LNR > 0.3	1.89	1.00–3.60	0.052	1.20	0.25–5.84	0.821
LODDS > − 1	1.89	1.00–3.58	0.051	1.25	0.29–5.51	0.766

HR hazard ratio, *CI* confidence interval, *DFS* disease-free survival, *DSS* disease-specific survival, AH-arterial hypertension, *CCI* Charlson comorbidity index, *ACEIs* angiotensin-converting enzyme inhibitors, *CCBs* calcium channel blockers, *RT-S* time from completion of radiotherapy to surgery, *PLN* positive lymph nodes, *NLN* negative lymph nodes, *LNR* lymph node ratio, *LODDS* log odds of positive lymph nodes, *LNY* lymph node yield, *G* histological tumour grade, *ENE* extranodal extension, *LVI* lymphovascular invasion, *PNI* perineural invasion, *TRG* tumour regression grade, *CT* adjuvant chemotherapy

its activation is increased in the setting of local inflammation and oxidative stress [16]. Immunohistochemistry tests of tumour specimens collected from patients with colorectal cancer showed expression of chymase in the mast cells in the tumour microenvironment. It was also found that such expression was significantly higher in the group of subjects with distant metastases versus the group free of metastases [17]. Studies of HT-29 cell lines of colorectal cancer showed that these cells did not show ACE expression while showing AT1R and chymase expression. Moreover, it was shown that ACEIs did not affect AngII production in HT-29 cells [18]. Therefore, it seems that tRAS activation by AT1R in colorectal cancer may occur independently of ACE. In the light of the above data it was shown that currently there is no theoretical basis to claim that the use of ACEIs may have a significant inhibitory effect on the tRAS cascade in the colorectal tumour.

Close interactions were shown between RAS and kallikrein-kinin system (KKS) at several levels [19]. Bioactive kinins, as well as bradykinin (BK) and kallidin are formed from kininogens with the participation of plasma and tissue kallikreins. On the other hand, BK, triggered by kinases, including ACE, is converted to active des-Arg9-BK and inactive peptides. KKS affects cells with the use of two types of kinin receptors, type 1 (B1R), whose ligand is des-Arg9-BK, and type 2 (B2R), whose ligand is BK. B2R shows high expression in various tissues, while B1R is almost undetectable under physiological conditions, and its expression increases under the influence of proinflammatory cytokines, in the setting of inflammation and tissue damage. The exception are fibroblasts, which show constitutive expression of B1R [20]. There is evidence that kinins play an important role in the recruitment of proinflammatory cells in the tumour microenvironment, and stimulate neoplastic cell migration and invasion, thus increasing their metastatic potential [21, 22]. Studies of human and murine cell lines of colorectal cancer, including HT-29, revealed high expression of both B1R and B2R [23, 24]. Wang et al. showed that blocking of B2R weakens the process of invasion and migration of colorectal cancer cells [23]. Also da Costa et al. showed expression of both B1R and B2R in murine (MoCR) and human (SW-480) cell lines of colorectal cancer. In addition,

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in vivo and in vitro models revealed that blocking of kinin receptors inhibits tumour growth and reduces its metastatic potential [25]. It was also shown that kinins stimulate the process of angiogenesis, both in normal and cancerous cells [26]. ACE, aside to converting AngI to AngII, degrades BK to inactive peptides by removing two C-terminal amino acids. The use of ACEIs was shown to reduce plasma AngII and at the same time to increase the level of B2R agonists [27]. This also leads to a situation of increased level of their des-Arg metabolites, which are B1R agonists. Thus, the use of ACEIs may indirectly lead to an increased activation of KKS, both via B2R and B1R [28]. ACEIs were also shown to be allosteric enhancers of kinin receptors and to have a direct effect on the enhancement of the signal transmitted by their pathway [28]. The issue of possible AT1R/B2R heterodimer formation remains unclear, since reports on this matter are contradictory [29, 30].

The effect of using ACEIS on the interactions between tRAS and KKS in the aspect of their influence on colorectal cancer progression is poorly known. However, summing up, it may be concluded that despite the use of ACEIs, it is possible to activate the procancerous tRAS pathway via AT1R in the neoplastic tumour with the use of chymase. Additionally, local inflammatory process in the tumour microenvironment leads to an increased B1R expression, and the use of ACEIs may lead to increased KKS activation, both via B1R and B2R. This takes place in two mechanisms: inhibiting the kinin breakdown with consequential increase in the level of both receptor agonists, and allosteric enhancement of the transmitted signal. These interactions may lead to a negative effect of ACEIs on the course of the neoplastic disease, and explain our results.

There are few reports on the effect of RASIs on treatment outcomes in patients with rectal cancer. Typically, publications discuss subjects with colorectal cancer. Additionally, the effect of both groups of RASIs is assessed jointly (ACEIs/ARBs), which may significantly affect the results and cause conflicting conclusions. The effect of these drugs on the rectal cancer response to neoadjuvant treatment is unclear. Morris et al. showed that the use of ACEIs/ARBs significantly increases the frequency of complete responses after preoperative radiotherapy in a multiple factor analysis [3], while Rombouts et al. did not confirm in their study a positive effect of these drugs [31]. Similar discrepancies exist regarding the assessment of the ACEIs/ARBs effect on long-term survival. In a retrospective analysis of nearly 14,000 patients performed on the basis of SEER database, Balkrishnan et al. showed that better indices of cancer-specific mortality were shown by patients with colorectal cancer stage I–III, who received antihypertensive drugs,

as compared to patients who did not receive such drugs. A detailed analysis showed a positive effect on survival of all study drug groups (ACEIs, beta blockers, diuretics), except for ARBs. Due to data availability, the analysis excluded, however, patients below 65 years old, and those using antihypertensive drugs before the diagnosis of colorectal cancer [32]. On the other hand, Holmes et al. and Cardwell et al. in two population studies, showed no such correlation in other groups of patients with colorectal cancer (n = 3967 and n = 4762, respectively) [33, 34]. At the same time it was shown that the use of ACEIs/ARBs may increase the risk of death in the course of breast cancer and lung cancer [33]. Ozawa et al. showed a positive effect of ACEIs/ARBs on DFS in the course of left-sided colorectal cancer and stage I cancer. Both groups of drugs were analysed jointly, and the authors did not specify how many patients used ARBs, and how many used ACEIs [35]. In a retrospective analysis of 262 patients with colorectal cancer, Engineer et al. showed a positive effect of the use of RASIs + beta blockers. However, this publication does not show the ACEIs to ARBs ratio in the study group, either [36]. In an earlier study performed at the same institution, Heinzerling et al. showed a positive effect of the use of ACEIs on the occurrence of distant metastases in subjects with stage II colorectal cancer in a retrospective analysis of 55 patients. This study, however, has significant limitations. ACEIs are not directly included in the statistical analysis. Conclusions on their effect are made indirectly based on the frequency of their use by patients with arterial hypertension, which was a significant factor in the multiple factor analysis [37]. Dai et al. showed in their meta-analysis, that although the ACEIs/ARBs therapy may be related to a reduced incidence of colorectal cancer, there is no evidence that it may also affect treatment outcomes of patients with colorectal cancer [38].

To the best of our knowledge, the effect of ACEIs has not been assessed so far exclusively in the group of subjects with ypN+ rectal cancer. The analysis results point to a need of further studies in this group of patients. If our results are confirmed with larger, independent groups of patients, the exclusion of ACEIs from the therapy of comorbidities could be a simple method of improving long-term oncological outcomes in patients with rectal cancer.

We have shown a negative effect of postoperative complications on DFS, regardless of the degree in the Clavien–Dindo classification. Similar conclusions were drawn by Sprenger et al. who showed that the occurrence of any surgical complications (anastomotic leakage and/or abnormal wound healing) had a significant negative effect on OS and local recurrence free survival among the patients of the German Rectal Cancer Trial,

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as shown by a multiple factor analysis [39]. Possible mechanisms underlying the effect of complications on long-term oncological outcomes include no or delayed adjuvant therapy [40]. In a post hoc analysis we showed that the patients with complications significantly more often failed to receive > 3 cycles of adjuvant chemotherapy, which could suggest that such a claim is correct. In a multiple factor analysis, however, we confirmed the reports of no positive effect on DFS and DSS of adjuvant fluoropyrimidine-based chemotherapy used in the analysed period [41, 42].

The only parameter related to lymph nodes which in our analysis had a significant effect on survival was the NLN count in the surgical specimen, which is confirmed by observations of several authors [7, 43]. There have been promising attempts, yet requiring validation, to modify the current AJCC classification by adding the NLN count parameter [44]. This effect is explained by some authors by the presence of small (up to 2 mm) lymph nodes containing micrometastases which are not detected in standard HE staining and for that reason are assessed as NLN by pathologists. It is believed that an increased NLN count may reduce the risk of their non-removal, and, as a result, disease relapse [45]. Another possible explanation is an increase in the NLN count resulting from a stronger immune response to the tumour, with accompanying reactive lymph node enlargement. This phenomenon has a positive prognostic value and facilitates finding a higher number of lymph nodes in the specimen [46, 47].

The analysis has typical limitations of retrospective and single-centre analyses. The neoadjuvant treatment was not performed with the use of a uniform schedule. However, we have shown no effect of this factor on the treatment outcomes. Data on comorbidities and medications taken were obtained from internal and anaesthesiological consultation records prior to surgery. It was not possible to assess the duration of using the medications.

Conclusions

The use of ACEIs may have a negative effect on long-term treatment outcomes in patients with ypN + M0 rectal cancer. In this group of patients, the NLN count seems to be an important prognostic factor, as well.

Abbreviations

ACE: Angiotensin-converting enzyme; ACEIs: Angiotensin-converting enzyme inhibitors; AL: Anastomotic leakage; AngI: Angiotensin I; AngII: Angiotensin II; AR: Anterior resection; ARBs: Angiotensin receptor blockers; AT1R: Angiotensin II type 1 receptor; AT2R: Angiotensin II type 2 receptor; BK: Bradykinin; B1R: Kinin B1 receptor; B2R: Kinin B2 receptor; BMI: Body mass index; CAD: Coronary artery disease; CCI: Charlson comorbidity index; CRT: Chemoradiotherapy; CI: Confidence interval; DFS: Disease-free survival; DSS: Disease-specific survival; ENE: Extranodal extension; G: Histological tumour grade; HR: Hazard ratio;

KKS: Kallikrein-kinin system; LNR: Lymph node ratio; LNY: Lymph node yield; LODDS: Log odds of positive lymph nodes; LVI: Lymphovascular invasion; NLN: Negative lymph nodePLN- positive lymph node; PNI: Perineural invasion; RAS: Renin-angiotensin system; RASIs: Renin-angiotensin system inhibitors; RT: Radiotherapy; SD: Standard deviation; t-RAS: Tissue renin-angiotensin system; TRG: Tumour regression grade.

Supplementary Information

Additional file 1. Postoperative complications.

Additional file 2. Results of the analysis of nodal staging parameters.

Additional file 3. Survival analysis depending on neoadjuvant treatment regimen.

Additional file 4. Likelihood ratio test.

Additional file 5. Dataset.

Availability of data and materials
The dataset supporting the conclusions of this article is included within the article (Additional file 5).

Declarations

Ethics approval and consent to participate
This retrospective study involving human participants was in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Institutional Ethics Committee of National Research Institute of Oncology (KB/430-53/19). Due to the retrospective design of the study, the Institutional Ethics Committee of National Research Institute of Oncology approved informed consent waiver.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Kim HJ, Choi GS. Clinical implications of lymph node metastasis in colorectal cancer: current status and future perspectives. Ann Coloproctol. 2019;35(3):109–17.
2. Kim JM, Park JW, Lee JH, Park YH, Park SJ, Cheon JH, Kim WH, Kim TI. Survival benefit for metformin through better tumor response by neoadjuvant concurrent chemoradiotherapy in rectal cancer. Dis Colon Rectum. 2020;63(6):758–68.

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3. Morris ZS, Saha S, Magnuson WJ, Morris BA, Borkenhagen JF, Ching A, Hirose G, McMurry V, Francis DM, Harari PM, Chappell R, Tsuji S, Ritter MA. Increased tumor response to neoadjuvant therapy among rectal cancer patients taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Cancer. 2016;122(16):2487–95.

4. Allaix ME, Rebecchi F, Famiglietti F, Arolfo S, Arezzo A, Morino M. Long-term oncologic outcomes following anastomotic leak after anterior resection for rectal cancer: Does the leak severity matter? Surg Endosc. 2019. https://doi.org/10.1007/s00464-019-07189-9.

5. Jang JH, Kim HC, Huh JW, Park YA, Cho YB, Yun SH, Lee WY, Yu JI, Park HC, Park YS, Park JO. Anastomotic leak does not impact oncologic outcomes after preoperative chemoradiotherapy and resection for rectal cancer. Ann Surg. 2019;269:678–85.

6. Cui J, Zhang L, Yang L, Zhu YL, Fang H, Chen B, Ning Y, Zhang HZ. The prognostic significance of the treatment response of regional lymph nodes and the refinement of the current TNM staging system in locally advanced rectal cancer after neoadjuvant chemoradiotherapy. Cancer Med. 2020;9(24):9373–84.

7. Li X, Lu H, Xu K, Wang H, Liang X, Hu Z. Negative lymph node count is an independent prognostic factor for patients with rectal cancer who received preoperative radiotherapy. BMC Cancer. 2017;17(1):227.

8. Kim HJ, Jo JS, Lee SY, Kim CH, Kim YJ, Kim HR. Low lymph node retrieval after preoperative chemoradiation for rectal cancer is associated with improved prognosis in patients with a good tumor response. Ann Surg Oncol. 2015;22(6):2075–81.

9. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chron Dis. 1987;40(5):373–83.

10. Rahbari NN, Weitz J, Hohenberger W, Heald RJ, Moran B, Ulrich A, Holm T, Wong WD, Tiret E, Moriya Y, Laurberg S, den Dulk M, van de Velde C, Büchler MW. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the international study group of rectal cancer. Surgery. 2010;147(3):339–51.

11. Turner JM, Kodali R. Should angiotensin-converting enzyme inhibitors ever be used for the management of hypertension? Curr Cardiol Rep. 2020;22(9):95.

12. Jiang H, Tai Z, Chen Z, Zhu Q, Bao L. Clinical applicability of renin-angiotensin system inhibitors in cancer treatment. Am J Cancer Res. 2021;11(2):318–36.

13. Okwan-Duodu D, Landry J, Shen XZ, Diaz R. Angiotensin-converting enzyme and the tumor microenvironment: mechanisms beyond angiogenesis. Am J Physiol Regul Integr Comp Physiol. 2013;305(3):R205–15.

14. Simões e Silva AC, Silveira KD, Ferreira AJ, Teixeira MM. ACE2, angiotensin-(1–7) and Mas receptor axis in inflammation and fibrosis. Br J Pharmacol. 2013;169(3):477–92.

15. Dandona P, Dhindsa S, Ghanim H, Chaudhuri A. Angiotensin II and inflammation: the effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockade. J Hum Hypertens. 2007;21(1):20–7.

16. Baranowska I, Gawrys O, Roszkowska-Chojecka MM, Badzynska B, Tymecka D, Olszynski KH, Kompanowska-Jezierska E. Chymase dependent pathway of angiotensin II generation and rapeseed derived peptides for antihypertensive treatment of spontaneously hypertensive rats. Front Pharmacol. 2021;12:658805.

17. Flores de Los Rios PA, Soto Domínguez A, Arellano-Pérez Vertti RD, Castelán Maldonado EE, Velázquez Gauna SE, Morán Martínez J, Salas Treviño D, García Garza R. Differential expression of mast cell granules in samples of metastatic and non-metastatic colorectal cancer in patients. Acta Histochem. 2020;122(7):151618.

18. Kuniyasu H. Multiple roles of angiotensin in colorectal cancer. World J Clin Oncol. 2012;3(12):150–4.

19. Su JB. Different cross-talk sites between the renin-angiotensin and the kallikrein-kinin systems. J Renin Angiotensin Aldosterone Syst. 2014;15(4):319–28.

20. Dutra RC. Kinin receptors: key regulators of autoimmunity. Autoimmun Rev. 2017;16(2):192–207.

21. da Costa PL, Sirois P, Tannock IF, Chammas R. The role of kinin receptors in cancer and therapeutic opportunities. Cancer Lett. 2014;345(1):27–38.

22. Yu HS, Lin TH, Tang CH. Involvement of intercellular adhesion molecule-1 up-regulation in bradykinin promotes cell motility in human prostate cancers. Int J Mol Sci. 2013;14(7):13329–45.

23. Wang G, Ye Y, Zhang X, Song J. Bradykinin stimulates IL-6 production and cell invasion in colorectal cancer cells. Oncol Rep. 2014;32(4):1709–14.

24. Wu J, Akaike T, Hayashida K, Miyamoto Y, Nakagawa T, Miyakawa K, Müller-Esterl W, Maeda H. Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. Int J Cancer. 2002;98(1):29–35.

25. da Costa PLN, Wynne D, Fifis T, Nguyen L, Perini M, Christophi C. The kallikrein-Kinin system modulates the progression of colorectal liver metastases in a mouse model. BMC Cancer. 2018;18(1):382.

26. Ikeda Y, Hayashi I, Kamoshita E, Yamazaki A, Endo H, Ishihara K, Yamashina S, Tsutsumi Y, Matsubara H, Majima M. Host stromal bradykinin B2 receptor signaling facilitates tumor-associated angiogenesis and tumor growth. Cancer Res. 2004;64(15):5178–85.

27. Pellacani A, Brunner HR, Nussberger J. Plasma kinins increase after angiotensin-converting enzyme inhibition in human subjects. Clin Sci (Lond). 1994;87(5):567–74.

28. Erdös EG, Tan F, Skidgel RA. Angiotensin I-converting enzyme inhibitors are allosteric enhancers of kinin B1 and B2 receptor function. Hypertension. 2010;55(2):214–20.

29. Hansen JL, Hansen JT, Speerschneider T, Lyngsø C, Erikstrup N, Burstein ES, Weiner DM, Walther T, Makita N, Iiri T, Merten N, Kostenis E, Sheikh SP. Lack of evidence for AT1R/B2R heterodimerization in COS-7, HEK293, and NIH3T3 cells: How common is the AT1R/B2R heterodimer? J Biol Chem. 2009;284(3):1831–9.

30. AbdAlla S, Lother H, Quitterer U. AT1-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. Nature. 2000;407(6800):94–8.

31. Rombouts AJ, Hugen N, Verhoeven RH, Kuiper JG, Poortmans PM, de Wilt JH, Nagtegaal ID. Is preoperative chemoradiation in rectal cancer patients modulated by ACE inhibitors? Results from the Dutch cancer registry. Radiother Oncol. 2019;138:86–92.

32. Balkrishnan R, Desai RP, Narayan A, Camacho FT, Flausino LE, Chammas R. Associations between initiating antihypertensive regimens on stage I-III colorectal cancer outcomes: a medicare SEER cohort analysis. Cancer Med. 2021;10(15):5347–57.

33. Holmes S, Griffith EJ, Musto G, Minuk GY. Antihypertensive medications and survival in patients with cancer: a population-based retrospective cohort study. Cancer Epidemiol. 2013;37(6):881–5.

34. Cardwell CR, Mc Menamin ÚC, Hicks BM, Hughes C, Cantwell MM, Murray LJ. Drugs affecting the renin-angiotensin system and survival from cancer: a population based study of breast, colorectal and prostate cancer patient cohorts. BMC Med. 2014;12:28.

35. Ozawa T, Hashiguchi Y, Yagi T, Fukushima Y, Shimada R, Hayama T, Tsuchiya T, Nozawa K, Iinuma H, Ishihara S, Matsuda K. Angiotensin I-converting enzyme inhibitors/angiotensin II receptor blockers may reduce tumor recurrence in left-sided and early colorectal cancers. Int J Colorectal Dis. 2019;34(10):1731–9.

36. Engineer DR, Burney BO, Hayes TG, Garcia JM. Exposure to ACEI/ARB and β-blockers is associated with improved survival and decreased tumor progression and hospitalizations in patients with advanced colon cancer. Transl Oncol. 2013;6(5):539–45.

37. Heinzerling JH, Anthony T, Livingston EH, Huerta S. Predictors of distant metastasis and mortality in patients with stage II colorectal cancer. Am Surg. 2007;73(3):230–8.

38. Dai YN, Wang JH, Zhu JZ, Lin JQ, Yu CH, Li YM. Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers therapy and colorectal cancer: a systematic review and meta-analysis. Cancer Causes Control. 2015;26(9):1245–55.

39. Sprenger T, Beißbarth T, Sauer R, Tschmelitsch J, Fietkau R, Liersch T, Hohenberger W, Staib L, Gaedcke J, Raab HR, Rödel C, Ghadimi M. Long-term prognostic impact of surgical complications in the German rectal cancer trial CAO/ARO/AIO-94. Br J Surg. 2018;105(11):1510–8.

40. Des Guetz G, Nicolas P, Perret GY, Morere JF, Uzzan B. Does delaying adjuvant chemotherapy after curative surgery for colorectal cancer impair survival? A meta-analysis. Eur J Cancer. 2010;46(6):1049–55.

41. Bosset JF, Calais G, Mineur L, Maingon P, Stojanovic-Rundic S, Bensadoun RJ, Bardet E, Beny A, Ollier JC, Bolla M, Marchal D, Van Laethem JL, Klein V, Giralt J, Clavère P, Glanzmann C, Cellier P, Collette L, EORTC Radiation Oncology Group. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomised study. Lancet Oncol. 2014;15(2):184–90.

Zeman *et al. BMC Gastroenterology*   (2022) 22:141

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42. Breugom AJ, van Gijn W, Muller EW, Berglund Å, van den Broek CBM, Fokstuen T, Gelderblom H, Kapiteijn E, Leer JWH, Marijnen CAM, Martijn H, Meershoek-Klein Kranenbarg E, Nagtegaal ID, Påhlman L, Punt CJA, Putter H, Roodvoets AGH, Rutten HJT, Steup WH, Glimelius B, van de Velde CJH. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo)radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. Ann Oncol. 2015;26(4):696–701.

43. Sun Y, Zhang Y, Huang Z, Chi P. Prognostic implication of negative lymph node count in ypN+ rectal cancer after neoadjuvant chemoradiotherapy and construction of a prediction nomogram. J Gastrointest Surg. 2019;23(5):1006–14.

44. Zhang ZY, Gao W, Luo QF, Yin XW, Basnet S, Dai ZL, Ge HY. A nomogram improves AJCC stages for colorectal cancers by introducing CEA, modified lymph node ratio and negative lymph node count. Sci Rep. 2016;6:39028.

45. Yu L, Zhang XT, Guan SH, Cheng YF, Li LX. The number of negative lymph nodes is positively associated with survival in esophageal squamous cell carcinoma patients in China. Open Med (Wars). 2020;15:152–9.

46. He WZ, Xie QK, Hu WM, Kong PF, Yang L, Yang YZ, Jiang C, Yin CX, Qiu HJ, Zhang HZ, Zhang B, Xia LP. An increased number of negative lymph nodes is associated with a higher immune response and longer survival in colon cancer patients. Cancer Manag Res. 2018;10:1597–604.

47. George S, Primrose J, Talbot R, Smith J, Mullee M, Bailey D, du Boulay C, Jordan H, Wessex Colorectal Cancer Audit Working Group. Will Rogers revisited: prospective observational study of survival of 3592 patients with colorectal cancer according to number of nodes examined by pathologists. Br J Cancer. 2006;95(7):841–7.

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