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Circulating miR-19, miR-27a, and miR-200c as novel biomarkers for resistance to neoadjuvant chemotherapy in gastric cancer patients: a pilot study

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Background Neoadjuvant chemotherapy (NAC) is a standard approach for treating locally advanced gastric cancer (GC), but resistance in some patients can result in treatment toxicity and surgical delays without therapeutic benefit. Identifying biomarkers predictive of NAC response is crucial for personalized treatment strategies. This study evaluated circulating microRNAs (miRNAs) as potential biomarkers for NAC response. Materials and methods Plasma samples from 39 GC patients undergoing NAC followed by gastrectomy (NCT04223401) were collected before treatment. Four miRNAs (miR-19a, miR-21, miR-27a, miR-200c) were analyzed via quantitative real-time polymerase chain reaction. NAC response was assessed in histological specimens using the Becker tumor regression grade (TRG), which classifies patients as Responders (TRG 1–2) or Non-responders (TRG 3). Results Among 39 patients, 20 (51%) were Responders, and 19 (49%) were Non-responders. miR-19a, miR-21, and miR-200c were significantly upregulated in Non-responders ($p < 0.05$). ROC analysis revealed miR-19a (AUC: 0.693), miR-21 (AUC: 0.700), and miR-200c (AUC: 0.772) as predictive of resistance. Univariate analysis revealed a correlation between higher levels of miR-19a, miR-21, and miR-200c and a low neutrophil count, with increased resistance risk. Multivariate analysis confirmed miR-200c as an independent predictor of resistance (OR: 20.90; 95% CI: 1.54-283.73). Conclusions This pilot study identifies circulating miR-19a, miR-21, and miR-200c as novel biomarkers for poor NAC response in GC, providing a foundation for personalized treatment strategies.

Gastric cancer (GC) continues to pose a significant global health challenge, with over one million new cases and approximately 769,000 deaths reported annually, ranking it as the fifth most common and fourth deadliest cancer worldwide[1]. Despite advancements in treatment modalities, the prognosis for patients, particularly those in advanced stages, remains poor. Neoadjuvant chemotherapy (NAC) has emerged as a promising therapeutic strategy, aiming to shrink tumors, eliminate micrometastases, and enhance surgical outcomes[2,3]. Presently, the combination of docetaxel, oxaliplatin, leucovorin, and 5-fluorouracil (FLOT) stands out as the most effective NAC regimen[4]. However, treatment efficacy varies widely among patients, with up to 40% exhibiting minimal or no tumor regression upon histopathological examination[5], leading to compromised long-term outcomes[6]. The absence of reliable predictive markers poses a considerable challenge in guiding treatment decisions and stratifying patients according to their likelihood of responding to NAC. Consequently, a substantial proportion of patients may undergo ineffective treatment, enduring unnecessary toxicity and potentially delaying radical surgery. Therefore, there is an urgent need for personalized treatment approaches facilitated by the development of biomarkers.

MicroRNAs (miRNAs) are short noncoding transcripts that post-transcriptionally regulate gene expression *via* partial base-pairing complementarity to mRNA[7]. Their regulatory reach encompasses genes implicated in

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pivotal mechanisms driving chemoresistance, including epithelial-mesenchymal transition, hypoxia, autophagy, apoptosis, and drug efflux[8]. Remarkably, circulating miRNAs exhibit exceptional stability and can be detected in various bodily fluids such as tissue, urine, saliva, stool, sputum, serum/plasma, and others, positioning them as prime candidates for cancer biomarkers[9]. Consequently, miRNAs have emerged as promising biomarkers for chemoresistance across different types of cancer[10–13]. In the context of GC, miR-19a[14], miR-21[15], miR-27a[16], and miR-200c[17] have been implicated in chemoresistance based on findings from both experimental in vitro and in vivo studies, as well as clinical investigations involving patients with metastatic GC. However, it remains uncertain whether these miRNAs could serve as biomarkers for resistance to NAC in GC patients. This pilot study was designed to address this knowledge gap and explore the potential role of miR-19a, miR-21, miR-27a, and miR-200c in predicting NAC efficacy through histological tumor regression assessment.

Methods

Patients and sample collection

The samples were collected from patients who had participated in a randomized controlled trial (RCT) examining the effects of home-based prehabilitation on postoperative complications. Both the protocol[18] and the outcomes of the RCT[19] have been published previously. For this observational side-study, patients' clinicopathological characteristics were documented, and baseline blood samples were collected for further miRNA profiling before the patients had received NAC followed by gastrectomy at the National Cancer Institute, Vilnius, Lithuania (2020–2022). Before enrollment in the study, all patients provided written informed consent. Ethical approval of the statement was obtained by "the Vilnius University Regional Bioethics Committee (2020/1–1185-675)". Ethical guidelines delineated in the Helsinki Declaration of 2013 were followed throughout the course of this study.

NAC treatment with the FLOT (5-fluorouracil, oxaliplatin, leucovorin, and docetaxel) scheme was the predominant treatment method for GC patients. Patients underwent four cycles of FLOT every two weeks before surgery. Following NAC, radical gastrectomy was planned within 4 to 6 weeks. The type and approach of the surgery (open or laparoscopic) were determined at the discretion of the individual surgeon.

Evaluation of response to NAC

Pathological evaluation was conducted at the National Center of Pathology, Vilnius, Lithuania. Final tumor histology was determined, and tumors were staged according to the American Joint Committee on Cancer Staging, 8th edition, providing ypTNM staging. The histological type of tumors was classified in accordance with the WHO Classification of Tumors of the Digestive Tract (2010) and the Lauren classification of gastric carcinoma. All slides were stained with hematoxylin-eosin, with additional immunostaining performed as needed. Immunohistochemistry for HER2 status was conducted concurrently with evaluation for poorly cohesive gastric carcinoma type and signet ring cells. Expert gastrointestinal pathologists reviewed all specimens to assess histologic TRG by Becker[8] after NAC in the primary tumor. Based on the Becker TRG score, the patients were grouped into Responders (TRG 1–2) and Non-responders (TRG 3) groups.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Blood sample preparation and qRT-PCR

Peripheral venous blood samples were collected before NAC using (Lind-Vac, OÜ InterVacTechnology, Narva, Estonia) vacutainer tubes, centrifuged at 3000 g for 10 min at 4 °C. Subsequently, plasma was separated, aliquoted, and stored at −80 °C until further analysis.

RNA extraction from plasma was carried out using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Specifically, after the addition of Qiazol reagent and incubation, all samples were supplemented with cel-miR-39-3p exogenous control. The subsequent steps followed the manufacturer's protocol.

cDNA synthesis was performed using the TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, Waltham, Massachusetts, USA) according to the manufacturer's instructions. The cDNA reactions were performed using a ProFlex PCR System thermal cycler (Applied Biosystems, Waltham, Massachusetts, USA).

The levels of selected miRNAs (hsa-miR-19a-3p, hsa-miR-21-5p, hsa-miR-27a-3p, and hsa-miR-200c-3p) were determined by qRT-PCR using TaqMan MicroRNA Assays (Applied Biosystems, Waltham, Massachusetts, USA) with specific probe IDs: 479,228, 477,875, 478,384, 4,427,975. The qRT-PCR amplification was performed in 96-well plates using the QuantStudio™ 5 instrument (Applied Biosystems, Waltham, Massachusetts, USA) following the manufacturer's protocol. An initial analysis of Ct values was conducted on QuantStudio Design and Analysis Software v. 2.5.0. Based on good qRT-PCR practice, each assay was performed with two identical replicates, and the resulting Ct values were subjected to statistical analysis. Additionally, a no-template control was included for each assay to assess the possibility of reaction contamination.

Statistical analysis

Statistical analyses were performed using SPSS version 28.0 software (SPSS, Chicago, IL, USA) and GraphPad Prism version 9.5.1 for MacOS. SPSS was primarily used to analyze all baseline characteristics data, while GraphPad Prism was employed for analyzing miRNA data and creating graphs related to miRNA levels. ROC curves were created using MedCalc Software version 18.2.1. Continuous variables between groups were compared by the Mann-Whitney U test and expressed as median with first (Q1) and third (Q3) quartiles. Categorical variables were compared using Fisher's exact test or Chi-square test as appropriate and expressed as proportions and percentages.

ROC curves were created to depict the prognostic value of possible resistance to NAC markers. When calculating the Youden index for each miRNA and baseline neutrophil count, the identified cut-off values were used to define higher or lower levels of these markers. While creating ROC curves, different combinations of data were tested to improve model performance. To determine the prognostic factors of resistance to NAC, all potential factors were included in univariate analyses using binary logistic regression. Variables that showed significance in these univariate analyses were then included in subsequent multivariate analysis, also employing binary logistic regression.

Overall survival (OS) and disease-free survival (DFS) rates were analyzed using the Kaplan-Meier method and compared between the study groups using the log-rank test. The fold changes in miRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method as described by Livak et al.[20].

A summary of the overall study design and workflow is provided in Supplementary Figure S1.

Results

Patients

Between April 13, 2021, and September 22, 2022, a total of 39 GC patients were included in the study (Supplementary Figure S2). Histological analysis of TRG showed that Becker grades 1a, 1b, 2, and 3 were observed in 2 patients (5.1%), 6 patients (15.4%), 12 patients (30.8%), and 19 patients (48.7%), respectively. Based on these results, patients were categorized into Responders (TRG grades 1a, 1b, or 2; $n=20$) and Non-responders (TRG grade 3; $n=19$). Histological evaluation was performed on tumor specimens obtained at the time of surgery following completion of neoadjuvant chemotherapy. Table 1 displays the patient and disease characteristics for the study groups. Both groups were comparable in most baseline characteristics, except that patients in the Non-responders' group had lower baseline neutrophil counts and higher albumin levels.

		Responders group ($n=20$)	Non-responders group ($n=19$)	p-value
Age (years), median, (Q1; Q3)		64 (60; 69)	59 (50; 71)	0.527
Sex, n (%)	Male	14 (70%)	11 (57.9%)	0.514
	Female	6 (30%)	8 (42.1%)	
BMI (kg/m^2), median, (Q1; Q3)		24.8 (22.5; 28.1)	25.5 (22.3; 28.69)	0.989
Smoking, n (%)		5 (25%)	2 (10.5%)	0.407
CCI, n (%)	<6	19 (95%)	15 (78.9%)	0.182
	≥6	1 (5%)	4 (21.1%)	
cT, n (%)	2	2 (10%)	2 (10.5%)	0.713
	3	14 (70%)	15 (78.9%)	
	4	4 (20%)	2 (10.5%)	
cN, n (%)	0	6 (30%)	4 (21.1%)	0.716
	1–3	14 (70%)	15 (78.9%)	
cM, n (%)	0	18 (90%)	19 (100%)	0.487
	1	2 (10%)	0 (0%)	
Clinical stage by TNM, n (%)	2	15 (75%)	12 (63.2%)	0.142
	3	3 (15%)	7 (36.8%)	
	4	2 (10%)	0 (0%)	
Tumor localization, n (%)	Upper third	5 (25%)	1 (5.3%)	0.195
	Middle third	7 (35%)	7 (36.8%)	
	Lower third	8 (40%)	9 (47.4%)	
	Total	0 (0%)	2 (10.5%)	
Poorly cohesive gastric carcinoma, n (%)		6 (30%)	6 (31.6%)	0.915
Signet ring cell carcinoma, n (%)		3 (15%)	3 (15.8%)	0.999
HER2 positive gastric cancer, n (%)		0 (0%)	0 (0%)	0.999
Baseline neutrophile level (x10^9/l), median (Q1; Q3)		5.56 (4.91; 7.60)	4.16 (3.25; 5.49)	**0.008**
Baseline lymphocyte level (x10^9/l), median (Q1; Q3)		1.32 (1.08; 1.87)	2.01 (1.10; 2.35)	0.351
Baseline hemoglobin (g/l) level, median (Q1; Q3)		127 (117; 143)	131 (117; 142)	0.569
Baseline platelet level (x10^9/l), median (Q1; Q3)		238 (194; 299)	281 (212; 296)	0.667
Baseline C reactive protein level (mg/l), median (Q1; Q3)		1.0 (0.6; 3.3)	1.3 (0.6; 2.4)	0.989
Baseline serum albumin level (g/l), median (Q1; Q3)		42 (40; 44)	45 (44; 47)	**0.003**

Table 1. Baseline characteristics. Values are n (%) unless otherwise indicated.

		Responders ($n=20$)	Non-responders group ($n=19$)	p-value
Neoadjuvant chemotherapy cycles (number), median (Q1; Q3)		4 (4; 4)	4 (4; 4)	0.184
Chemotherapy regime, n (%)	FLOT	20 (100%)	17 (89.5%)	0.231
	Cisplatin/Carboplatin and 5-FU	0 (0%)	2 (10.5%)	
Type of surgery, n (%)	Total gastrectomy	6 (30%)	5 (26.3%)	0.803
	Esophagectomy	2 (10%)	1 (5.3%)	
	Distal gastrectomy	12 (60%)	13 (68.4%)	
Surgical approach, n (%)	Open	15 (75%)	14 (73.7%)	0.925
	Laparoscopic	5 (25%)	5 (26.3%)	
90-days postoperative morbidity, n (%)		9 (45%)	4 (21.1%)	0.301
Clavien-Dindo score, n (%)	1–2	7 (77.8%)	2 (50%)	0.530
	3–4	2 (22.2%)	2 (50%)	
ypT, n (%)	0	2 (10%)	0 (0%)	0.325
	1–2	7 (35%)	5 (26.3%)	
	3–4	11 (55%)	14 (73.7%)	
ypN, n (%)	0	8 (40%)	8 (42.1%)	0.893
	1–3	12 (60%)	11 (57.9%)	
ypM, n (%)	0	20 (100%)	19 (100%)	0.999
R0, n (%)		20 (100%)	18 (94.7%)	0.487

Table 2. Treatment characteristics. Values are n (%) unless otherwise indicated; FLOT: docetaxel, oxaliplatin, leucovorin, and 5-fluorouracil; 5-FU: 5-fluorouracil.

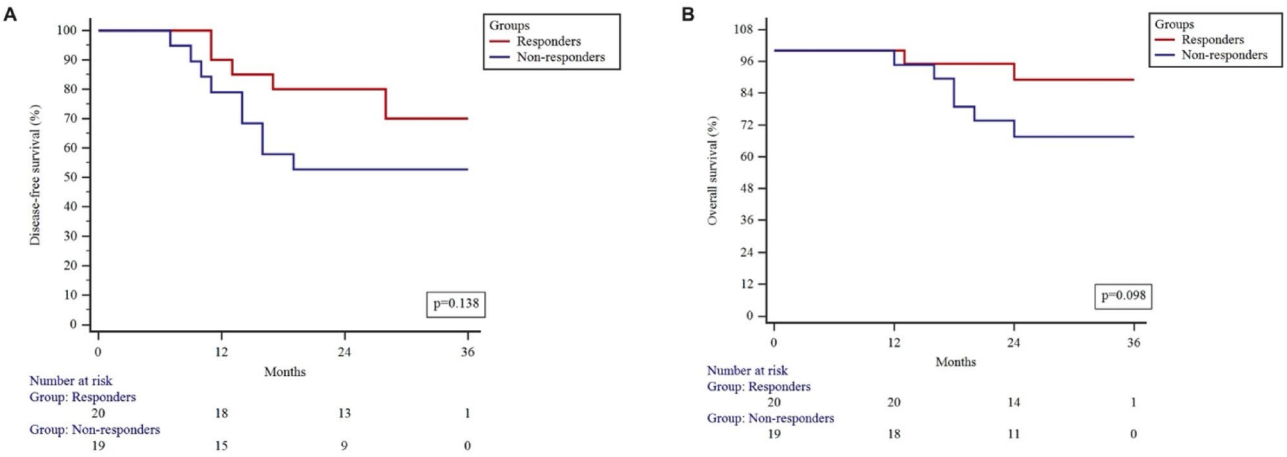

Fig. 1. Kaplan-Meier analysis showing DFS (A) and OS (B) based on resistance to NAC status.

Treatment and outcomes

Treatment characteristics are detailed in Table 2. Patients in the Responders ($n=20/20$, 100%) and Non-responders ($n=17/19$, 89.5%) groups predominantly received FLOT regimen chemotherapy ($p=0.231$) with a median number of 4 cycles in both the NAC and adjuvant settings. Both patients diagnosed with stage IV GC in the Responders' group had only positive peritoneal cytology without other distant metastases, and cytological status has converted after NAC. The type of surgery, postoperative complications and R0 resection rates were similar between the groups. The median follow-up was 26 (22; 32) months after the start of the treatment. Kaplan-Meier analysis showed a trend for higher 3-year OS (89.1% vs. 67.5%; $p=0.098$) and DFS (70.0% vs. 52.6%; $p=0.138$) for the patients in the Responders' group, although the differences failed for significance (Fig. 1).

Potential biomarkers for resistance to NAC: plasma MiRNAs level and neutrophile count

Blood samples for miRNA analysis were collected prior to the initiation of neoadjuvant chemotherapy. Relative miR-19a, miR-21, and miR-200c levels were significantly upregulated in the blood plasma of Non-responders group ($p<0.05$). Descriptive statistics of the analyzed miRNAs between groups are shown in Supplementary Table S1. There were no differences in miR-27a level between groups, thus further analyses did not include this particular microRNA. Furthermore, patients in the non-responders group exhibited decreased neutrophil count before the start of the treatment (Fig. 2).

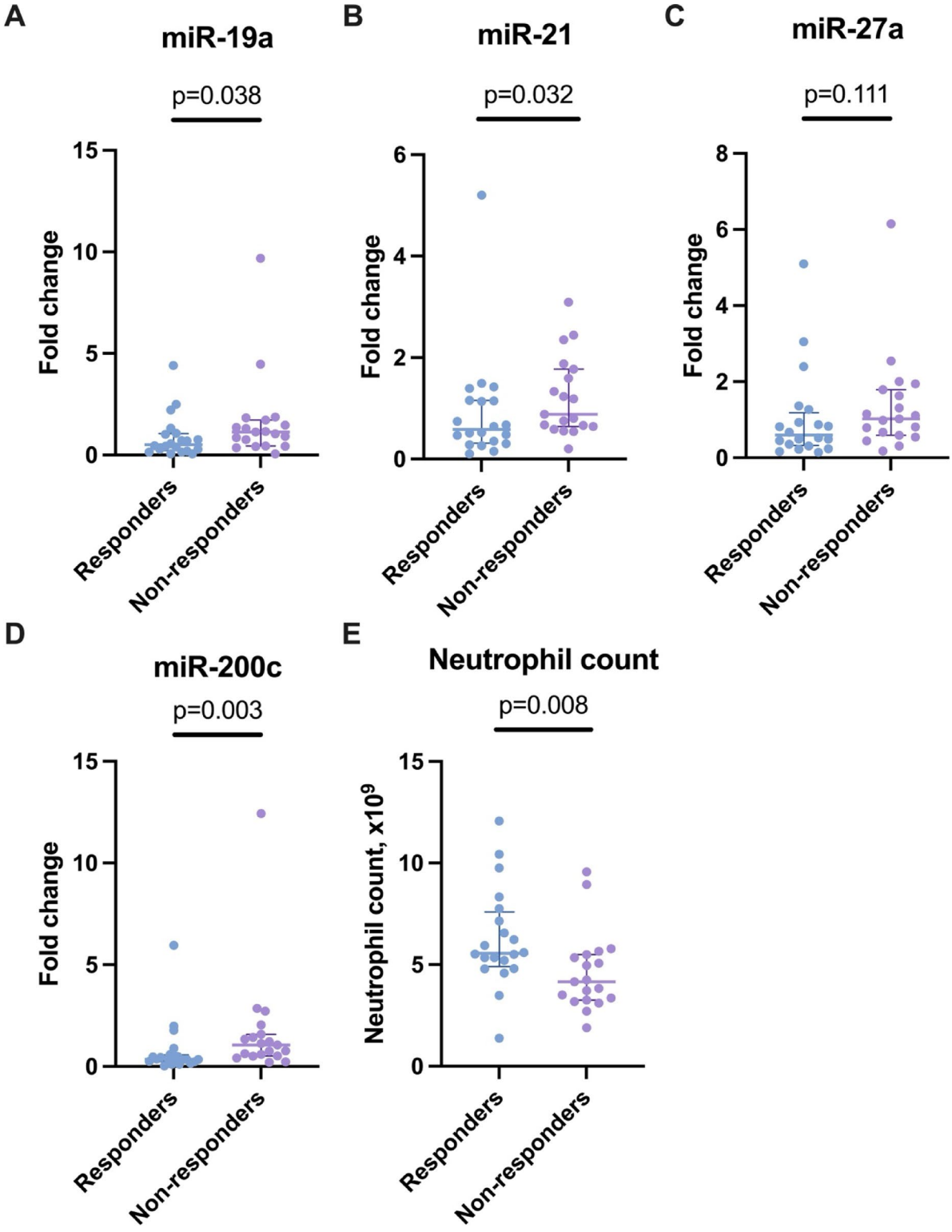

Fig. 2. Relative levels of miR-19a (A), miR-21 (B), miR-27a (C), miR-200c (D), and baseline neutrophil count (E) in the Responders and Non-responders groups.

After identifying potential biomarkers of response to NAC, ROC curves were created to depict prognostic values of plasma miR-19a, miR-21, miR-200c, and baseline neutrophil count for resistance to NAC and to identify the cut-off values for further analysis. ROC analysis revealed sufficient prognostic value of all analyzed plasma miRNAs and baseline neutrophil count, with the best characteristics attributable to miR-200c (Fig. 3; Table 3). Combining biomarkers did not yield any additional prognostic value, as the ROC parameters were

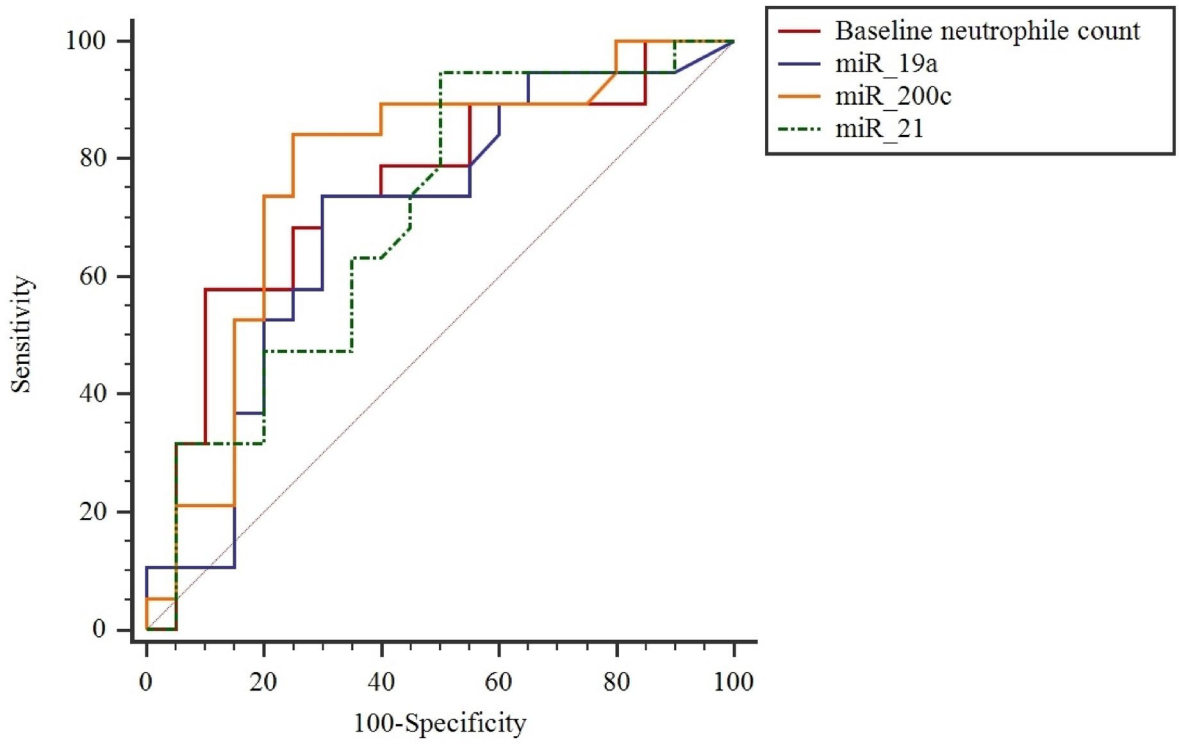

Fig. 3. ROC curves of biomarkers for predicting chemoresistance to NAC in GC.

miRNA	AUC	95% CI	*p*-value	Youden Index	Cut-off value	Sensitivity, (%)	Specificity, (%)
miR-19a	0.693	0.525–0.831	0.028	0.436	>0.75	73.6%	70.0%
miR-21	0.700	0.532–0.836	0.020	0.447	>0.52	94.7%	50.0%
miR-200c	0.772	0.610–0.891	0.001	0.592	>0.49	84.2%	75.0%
Baseline neutrophil count	0.747	0.583–0.872	0.002	0.478	≤4.24	57.8%	90.0%

Table 3. Parameters of the ROC curves predicting resistance to NAC. AUC: area under the curve; 95% CI: 95% confidence interval.

comparable to those obtained with individual biomarkers alone (Supplementary Table S2). Additionally, we explored whether circulating miRNA levels varied according to TNM cancer stage. Comparisons between stage groups are summarized in Supplementary Figure S3.

In the univariate analysis higher miR-19a, miR-21, miR-200c levels, and low neutrophil count were significantly associated with the resistance to NAC (Table 3) and were further analyzed and included into the multivariate analysis. Multivariate analysis showed that patients with high levels of plasma miR-200c have higher odds for resistance to NAC (OR: 20.90; 95% CI: 1.54–283.73.54.73), while other biomarkers (higher miR-19a, miR-21 levels and lower neutrophil count) were not predictive for the resistance (Table 4).

Discussion

This pilot study aimed to evaluate the potential of miR-19a, miR-21, miR-27a, and miR-200c as novel biomarkers for predicting resistance to NAC in GC patients. Present findings show that upregulated plasma levels of miR-19a, miR-21, and miR-200c can predict resistance to NAC with sensitivities ranging from 73.6% to 94.7% and specificities from 50.0% to 75.0%. Additionally, a lower neutrophil count was identified as a potential predictor of resistance to NAC as well. Multivariate analysis revealed that patients with high miR-200c plasma level have the highest odds of developing resistance to NAC.

NAC is the current standard for treating locally advanced GC, with the FLOT regimen being the most effective option[4]. This neoadjuvant approach enables in vivo chemosensitivity testing by assessing TRG in surgical specimens. Despite the use of advanced cytotoxic treatment regimens, approximately half of the patients exhibit minimal or no histological tumor regression following NAC[5,21]. These patients are known to have significantly worse long-term prognosis[6] and thus can be considered resistant to NAC. Consequently, the benefit of NAC for these patients is questionable, as the treatment poses significant toxicity and delays potentially curative surgery. Therefore, the development of novel biomarkers for resistance to NAC is crucial for enhancing GC treatment and enabling treatment personalization.

Factor	Odds ratio	95% Confidence Interval
Univariate analysis		
Age	0.98	0.93–1.04
Male sex	0.58	0.15–2.20
cT4	0.47	0.07–2.93
cN+	1.60	0.37–6.91
Poorly cohesive gastric carcinoma	1.07	0.27–4.19
Signet ring cell carcinoma	1.06	0.18–6.05
Higher miR-19a level	6.53	1.61–26.46
Higher miR-21 level	18.00	2.00–161.82.00.82
Higher miR-200c level	16.00	3.24–78.87
Lower neutrophil count	12.37	2.21–69.21
Multivariate analysis		
Higher miR-19a level	5.18	0.46–58.29
Higher miR-21 level	0.88	0.03–24.43
Higher miR-200c level	20.90	1.54–283.73.54.73
Lower neutrophil count	10.07	0.86–117.68.86.68

Table 4. Univariate and multivariate analyses of clinicopathological characteristics and biomarkers for resistance to NAC in GC patients.

Emerging evidence highlights the role of miRNAs in chemoresistance[22]. Additionally, circulating miRNAs show remarkable stability and can be detected in various body fluids, making them attractive biomarkers. The present study provides the first clinical evidence that upregulated levels of miR-19a, miR-21, and miR-200c are associated with resistance to NAC in GC patients. miR-19 is part of the miR-17–92 cluster, known as oncomir-1, one of the most potent oncogenic miRNA clusters[23,24]. Among the miR-19 family, miR-19a stands out as a prominent oncogenic miRNA. Its oncogenic activity promotes c-MYC-induced lymphomagenesis by inhibiting apoptosis and repressing the tumor suppressor phosphatase and tensin homolog (PTEN)[23,25]. Additionally, miR-19a activates the protein kinase B (AKT)-mammalian target of rapamycin (mTOR) pathway, which functionally antagonizes PTEN to enhance cell survival[23,25]. The targeting of PTEN by miR-19a, particularly in the context of PTEN/AKT signaling, also contributes to cancer cell resistance to chemotherapeutic agents[25–27]. In the context of GC, miR-19a/b has been shown to promote multidrug resistance in GC cells in vitro by accelerating the efflux of chemotherapeutic drugs and inhibiting drug-induced apoptosis[14]. These mechanisms of multidrug resistance involve the targeting of PTEN[14]. Similarly, PTEN is a target of miR-21. A recent study found that M2 macrophages had high levels of miR-21 in both their exosomes and cell lysates. These exosomes can transfer miR-21 to GC cells, leading to cisplatin resistance by suppressing PTEN expression and activating the PI3K/AKT (phosphatidylinositol 3-kinase) signaling pathway, thereby inhibiting cell apoptosis[28]. In other cancers, miR-21 down-regulates PDCD4 (programmed cell death protein 4) expression, causing paclitaxel resistance in breast cancer[29] and 5-FU resistance in colon cancer[30]. The PDCD4 loss in GC is associated with disease aggressiveness and poor prognosis[31], although it's role in the chemoresistance warrants further investigation. The evidence regarding the role of miR-200c in chemoresistance and its pro/antioncogenic mechanisms is controversial. Lower levels of miR-200c expression have been reported in gemcitabine-resistant pancreatic cancer cell lines and cisplatin-resistant breast cancer cells[17,32]. The underlying mechanisms involve miR-200c's ability to inhibit epithelial-mesenchymal transition (EMT)[33]. Contrarily, miR-200c has been shown to increase sensitivity to cisplatin in GC tissues by targeting and downregulating pr (zinc finger E-box-binding homeobox 2), a transcription factor involved in promoting EMT and drug resistance. On the other hand, miR-200c overexpression has also been reported to induce cisplatin chemoresistance by activating the AKT pathway in esophageal cancer[34]. Our pilot study showed increased levels of plasma miR-200c in resistant to NAC GC patients, suggesting that miR-200c is related to resistance to NAC in the clinical setting. Our findings are in line with those of Valladares-Ayerbes et al., who reported that circulating miR-200c levels were significantly elevated in gastric cancer patients compared to healthy controls, and that higher miR-200c expression was associated with advanced stage, increased lymph node involvement, and poorer overall and progression-free survival[43]. These converging lines of evidence strengthen the plausibility of miR-200c as both a prognostic and predictive biomarker in gastric cancer[35]. In conclusion, the present study confirms previous in vitro findings, demonstrating for the first time in a clinical setting the role of miR-19a, miR-21, and miR-200c to predict chemoresistance in GC patients undergoing NAC.

Moreover, the present study found that a lower neutrophil count at baseline serves as a prognostic marker for chemoresistance in GC patients undergoing NAC as well. To our knowledge, this is the first study to demonstrate such findings. Tumor-associated inflammation is linked with systemic inflammation, cancer development, tumor characteristics, and chemosensitivity[36–38]. While the neutrophil-to-lymphocyte ratio has previously been proposed as a marker for poor prognosis and poor response to chemotherapy in various types of cancers[39–41], this study highlights the specific association between low neutrophil count and chemoresistance. The mechanisms by which neutrophils contribute to chemoresistance are not fully understood. However, recent evidence suggests that neutrophils may play a role through extracellular trap-dependent mechanisms, involving T-cell exhaustion,

drug detoxification, angiogenesis, epithelial-to-mesenchymal transition, and extracellular matrix remodeling[42]. Although this study only investigated neutrophil count rather than their function, we are not able to explain why a lower count of neutrophils is associated with poor response to NAC.

As a future direction, our research could benefit from computational models that identify miRNA biomarkers in complex diseases. Recent reviews[43–45] highlight how network-based inference, ensemble learning, and deep learning methods are increasingly applied to integrate miRNA, clinical, and pathological data. Earlier work[46] also emphasized that such models accelerate discovery of miRNA–disease associations. Applying these approaches in gastric cancer could improve prediction of chemoresistance by combining circulating miRNA levels with clinical variables, moving biomarker research from descriptive findings toward predictive and personalized applications.

The present study has several limitations that should be considered. First, the relatively small sample size may limit the generalizability and statistical power of the findings. Particularly, we consider, that the lack of power and relatively short follow-up of patients prevented us from showing statistically significant impairment of long-term outcomes in resistant to NAC group despite the clear trend was observed. Second, as a single-center study, there may be biases that limit the applicability of the results to other populations or clinical settings. Third, although miRNA levels were correlated with chemoresistance, tumor tissue and functional experiments to elucidate the precise biological mechanisms behind this association were not included. Similarly, we did not perform experiments to address the underlying biological mechanisms explaining the relationship between low neutrophil count and chemoresistance. Another limitation of this study is that we did not perform functional wet-lab experiments to validate the biological role of the identified miRNAs. Future studies could investigate these miRNAs in gastric cancer cell lines or preclinical models, for example by modulating their expression to assess effects on chemoresistance, apoptosis, and cell proliferation. Despite these drawbacks, this study is the first to demonstrate the role of miR-19a, miR-21, and miR-200c in predicting chemoresistance in GC patients undergoing NAC.

Conclusions

This pilot study for the first time identified circulating miR-19a, miR-21, miR-200c as potential novel biomarkers to predict resistance to NAC in GC patients.

Data availability

The data are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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