International Journal of General Medicine

Dovepress

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ORIGINAL RESEARCH

High Expression of CLDN 18.2 is Associated with Poor Disease-Free Survival of HER-2 Positive Gastric Cancer

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Background: Anti-claudin (CLDN) 18.2 therapy has been proven to be effective in treating advanced gastric cancer with negative human epidermal growth factor receptor 2 (HER-2). This study purposed to investigate the relationship of CLDN 18.2 expression with prognosis of HER-2-positive gastric cancer patients.

Objective: To investigate the expression of claudin (CLDN) 18.2 in Human epidermal growth factor receptor 2 (HER-2) positive gastric cancer patients after radical resection and its relationship with gastric cancer prognosis.

Methods: A total of 55 postoperative HER-2-positive gastric cancer patients were included in this study. CLDN 18.2 protein was detected by immunohistochemistry, and detailed clinical and pathological information was collected. Factors considered potentially important in the univariate analysis were included in the multivariate analysis, which involved COX regression to find the independent prognostic factors affecting disease-free survival (DFS).

Results: Immunohistochemistry showed that different levels of CLDN 18.2 protein were expressed in HER-2 positive gastric cancer tissues, and the Chi-square analysis showed that the expression level of CLDN 18.2 was significantly correlated with the lymph node stage. Higher expression levels of CLDN 18.2 were found in patients with lymph node positivity and were associated with poor prognosis in HER-2-positive gastric cancer patients. Gastric cancer patients with low and high expressions of CLDN 18.2 had postoperative median DFS of 38.5 months (95% confidence interval (CI) 28.8–48.2 months) and 12.1 months (95% CI, 11.7–41.0 months), respectively.

Conclusion: High expression of CLDN 18.2 in HER-2 positive gastric cancer is associated with poor prognosis, and the optimal treatment mode for this population is worth exploring after the approval of anti-CLDN 18.2 drugs.

Keywords: gastric cancer, HER2, claudin 18.2, prognosis

Introduction

Gastric cancer is one of the most common malignant tumors worldwide,[1] with nearly 50% of the global cases occurring in China.[2] Drug therapy, including chemotherapy, target therapy, and immunotherapy, is the key treatment for gastric cancer, which can significantly improve overall survival. Combining targeted therapy with chemotherapy and immunotherapy can further improve their efficacy. Human epidermal growth factor receptor 2 (HER-2) was the first therapeutic target to be confirmed for gastric cancer, and several clinical trials have confirmed the efficacy of anti-HER-2 therapy in treating gastric cancer.[3–5] Tight junctions are usually located at the tip of epithelial cells and have the function of intercellular adhesion. Recently, the dysfunction and alteration of tight junction proteins in a variety of cancer cells has attracted attention. Tight junctions are mainly composed of occludin and claudin (CLDN) proteins. CLDNs are transmembrane proteins involved in maintaining intercellular integrity and regulating paracellular ion transport.[6] Currently, there are 27 members of the CLDN protein family, of which CLDN 1[7] and CLDN 10[8] have been shown to

Received: 5 January 2024
Accepted: 18 April 2024
Published: 29 April 2024

have prognostic value in colon cancer and hepatocellular carcinoma, respectively, and CLDN 18[9] has prognostic value in gastric cancer. In 2008, a study identified the splice variant 2 of CLDN 18, CLDN 18.2, as a highly selective biomarker that is limited in normal tissues but highly expressed in the initiation and progression of various primary malignancies[10], and in addition, CLDN18.2 is exposed on the cell surface in cancer cells, which enables its binding to monoclonal antibodies. More and more studies have shown that Claudin 18.2 can be used as a new molecular biomarker and immunotherapy for cancer.[11] However, CLDN 18.2 has recently been identified as a new therapeutic target for gastric cancer.[12,13] Combining monoclonal antibodies and chemotherapy against CLDN 18.2 has been proven to be more effective than chemotherapy alone in treating advanced gastric cancer with negative HER-2. However, the expression of CLDN 18.2 and its effect on the prognosis of HER-2-positive people have not been fully elucidated. This study purposed to investigate the expression of CLDN 18.2 in HER-2-positive gastric cancer patients after radical resection and its relationship with gastric cancer prognosis.

Materials and Methods

Study Participants and Follow-Up

The patients who underwent radical gastrectomy for gastric cancer in the Affiliated Hospital of Jiangnan University from January 2018 to March 2019 were enrolled in the study. The inclusion criteria were as follows: (1) patients underwent standard D2 radical resection; (2) postoperative pathological results were HER-2 positive (immunohistochemical score of 3+ or a positive HER-2 fluorescence in situ hybridization test); (3) complete postoperative follow-up data. The last follow-up time was in September 2023. Postoperative recurrence was defined as the definitive imaging data (CT and/or PET-CT and/or MRI) and/or biopsy pathology findings. Disease-free survival (DFS) was defined as the interval from surgery to the date of postoperative recurrence. Ethical permission was obtained from the Ethics Committee of the Affiliated Hospital of Jiangnan University and conformed to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). Informed consent was obtained from all participants, and the specimens were anonymous.

Immunohistochemistry

HER-2-positive gastric cancer tissues were collected, and fixed gastric cancer specimens were embedded in a paraffin-embedding machine. Expression of CLDN 18. 2 in gastric cancer tissues was determined using the immunohistochemical method. Paraffin-embedded sections were deparaffinized and rehydrated with gradient ethanol, and endogenous peroxidase activity was blocked by incubating the gastric cancer tissues in 3% hydrogen peroxide (H_2O_2). Gastric cancer specimens were incubated in a boiled citrate buffer (pH = 6.0) to restore antigenic activity, and the activity was blocked with 5% bovine serum albumin. The samples were subsequently incubated overnight with a primary anti-CLDN 18.2 antibody (#ab222512, Abcam,1:500) at 4°C. Thereafter, the specimens were incubated with HRP-labeled secondary antibodies, and the immunoreactive proteins were detected by 3,3'-diaminobenzidine (DAB) staining (#abs996, absin). Five fields were selected for each slide, and each field was stained to different degrees (no staining = 0; weak staining = 1, moderate staining = 2, strong staining = 3), and the staining intensity (0% = 0, 1%–24% = 1, 25%–49% = 2, 50%–74% = 3, 75%–100% = 4) was evaluated. The final immune response score was determined by multiplying the intensity score with the lesion size.

Statistical Analysis

SPSS 19 software (IBM, Endicott, NY) was used for the statistical analysis via Student's *t*-test and Pearson chi-square test. The optimal cut-off value for the CLDN 18.2 score in human gastric cancer tissue sections was obtained by drawing the receiver operating characteristic (ROC) curve. The relationship between CLDN 18.2 expression and the clinicopathological data was analyzed. Moreover, factors considered potentially important in the univariate analysis were included in the multivariate analysis, which involved COX regression to find the independent prognostic factors affecting DFS. The DFS curves were plotted using the Kaplan–Meier method, and the Log rank test was used to determine whether the curves were statistically significant. Results were presented as mean ± standard error, and $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 55 gastric cancer patients were included in this study (detailed information is listed in Table 1), among which 47 underwent 1–6 cycles of fluorouracil-based systemic adjuvant chemotherapy after surgery. All patients did not receive radiotherapy. All patients did not receive anti-HER-2 treatment and/or programmed death-1/programmed cell death ligand 1(PD-1/PD-L1) antibodies treatment and/or anti- CLDN 18.2 treatment, and 20 cases experienced recurrence in the last follow-up.

Table 1 Clinical and Tumor Characteristics of the 55 GC Patients

Characteristics	All Patients (n=55)	
	n	%
Age(years)		
Mean	64.3	
SD	9.4	
≤60	15	27.3
>60	40	72.7
Sex		
Male	46	83.6
Female	9	16.4
Tumor location		
Cardia	9	16.4
Body	4	7.3
Antrum	17	30.9
Full	4	7.3
T stage		
T1+2	20	36.4
T3+4	35	63.6
N stage		
N0	17	30.9
N1-3	38	69.1
TNM stage		
I–II	28	50.9
III	27	49.1
Tumor differentiation		
Well or moderately	17	30.9
Poorly	38	69.1

The immunohistochemistry showed that CLDN 18.2 protein was mostly localized near the cell surface (Figure 1). CLDN 18.2 protein is expressed in different levels in HER-2-positive gastric cancer tissues. Immunohistochemistry showed that 17 gastric cancer tissues had no CLDN 18.2 expression, while the remaining 38 gastric cancer groups had different expression levels of CLDN 18.2. According to ROC analysis (Figure 2A and Table 2), a score of 2.8 was considered the optimal cut-off value. Thus, tissues with a comprehensive score less than 2.8 were classified as a low expression group, while those with a comprehensive score greater than or equal to 2.8 were classified as a high expression group. Chi-square analysis showed

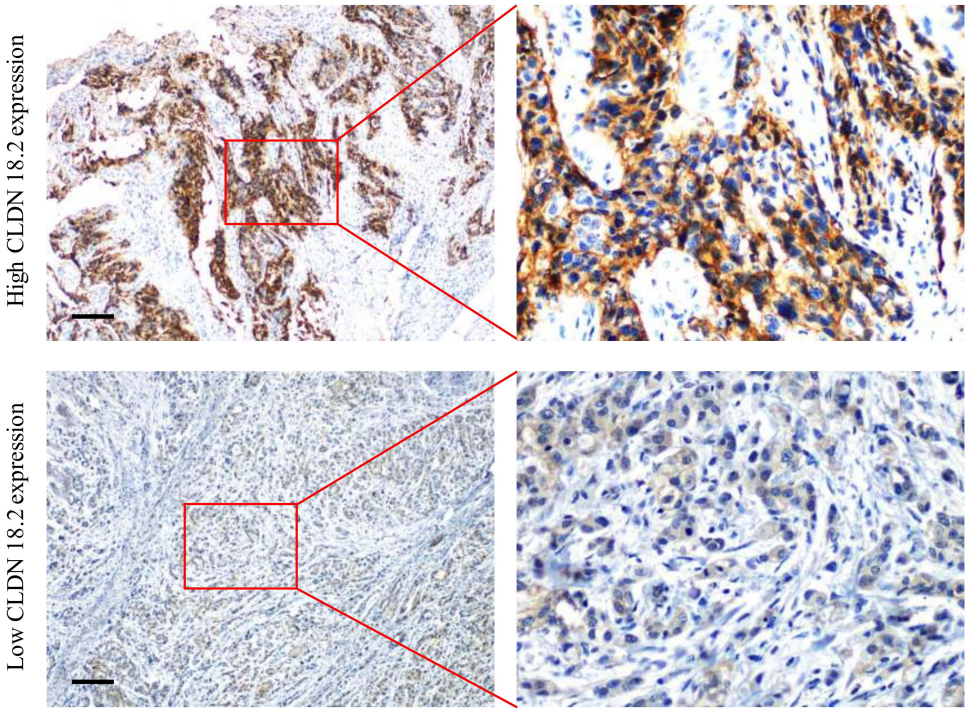

Figure 1 Representative images from immunohistochemical staining of CLDN 18.2 expression in human GC tissues. Scale bars, 100 μm.

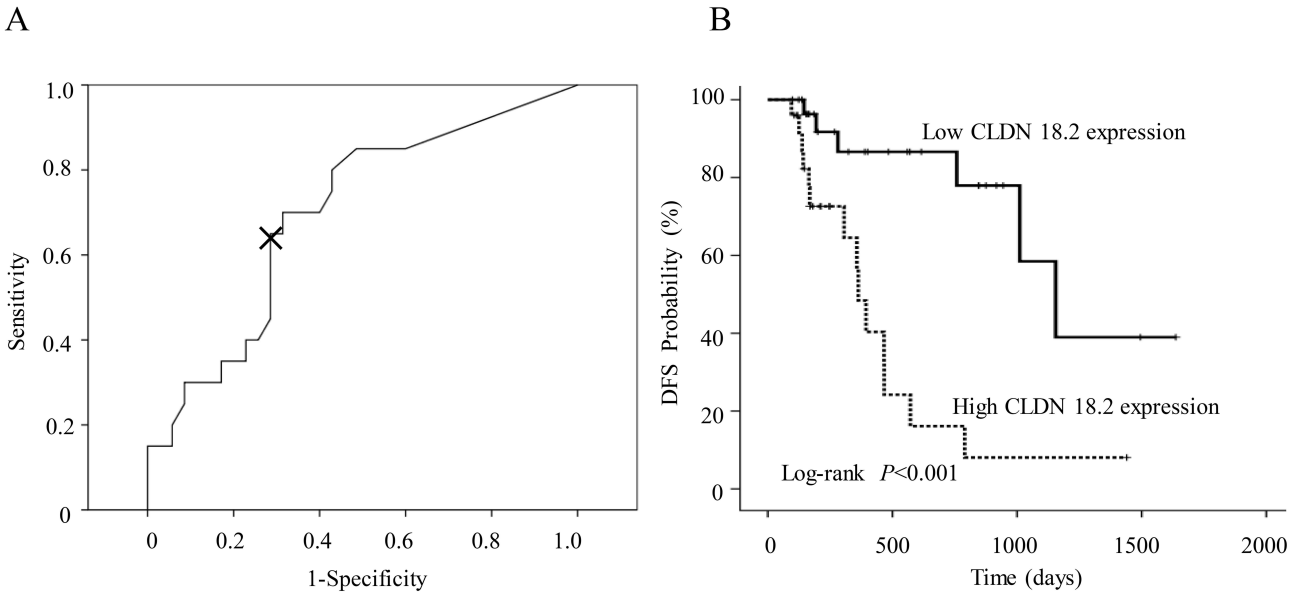

Figure 2 (**A**) ROC curve analysis identifying the appropriate cut-off value of CLDN 18.2 score with 2.8 (AUC=0.698; 95% CI=0.553–0.843). The X shown in the figure represents the position in the curve when the cut-off value is 2.8. (**B**) Kaplan-Meier survival curve showing a statistically shorter median DFS in radical resected GC patients with high CLDN 18.2 expression than with those with low CLDN 18.2 expression.

Table 2 Cutoff Values of CLDN 18.2 Score According to Disease Recurrence

Cut-Off value	Sensitivity	1-Specificity	Sensitivity+ Specificity-1-
−1	1	1	0
0.1	0.85	0.6	0.25
0.3	0.85	0.543	0.307
0.5	0.85	0.514	0.336
0.7	0.85	0.486	0.364
0.9	0.8	0.429	0.371
1.1	0.75	0.429	0.321
1.4	0.7	0.4	0.3
2	0.7	0.371	0.329
2.5	0.7	0.343	0.357
2.8	0.7	0.314	0.386
3.1	0.65	0.314	0.336
3.4	0.65	0.286	0.364
3.9	0.6	0.286	0.314
4.3	0.55	0.286	0.264
4.5	0.5	0.286	0.214
4.7	0.45	0.286	0.164
5	0.4	0.257	0.143
5.5	0.4	0.229	0.171
5.9	0.35	0.229	0.121
6.1	0.35	0.2	0.15
6.3	0.35	0.171	0.179
6.5	0.3	0.171	0.129
7.1	0.3	0.143	0.157
7.7	0.3	0.114	0.186
7.9	0.3	0.086	0.214
8.1	0.25	0.086	0.164
8.3	0.2	0.057	0.143
8.6	0.15	0.057	0.093
9.2	0.15	0.029	0.121
10.1	0.15	0	0.15
10.7	0.1	0	0.1
11	0.05	0	0.05
12.2	0	0	0

that the expression level of CLDN 18.2 was not related to age, gender, tumor location, tumor differentiation, T stage, and pathological stage but significantly correlated with the lymph node stage (Table 3). Consequently, higher levels of CLDN 18.2 expression were found in patients with lymph node positivity.

Table 3 Characteristics of GC Patients According to CLDN 18.2 Expression Level

Characteristics	CLDN 18.2 Expression		
	Low (n=30)	High (n=25)	P value
Age(years)			0.91
≤60	8	7	
>60	22	18	

(*Continued*)

Table 3 (Continued).

Characteristics	CLDN 18.2 Expression		
	Low (n=30)	High (n=25)	P value
Sex			0.42
Male	24	22	
Female	6	3	
Tumor location			0.483
Cardia	3	6	
Body	3	1	
Antrum	10	7	
Full	14	11	
Tumor differentiation			0.31
Well or moderately	11	6	
Poorly	19	19	
T stage			0.54
T1+2	12	8	
T3+4	18	17	
N stage			*0.03*
N0	13	4	
N1-3	17	21	
TNM stage			0.14
I–II	18	10	
III	12	15	

Higher Expression Levels of CLDN 18.2 are Associated with Poor Prognosis in HER-2 Positive Gastric Cancer Patients

The cut-off value and the clinical pathological factors of CLDN 18.2 protein were analysed. The univariate analysis showed that the N-stage, pathological stage, and CLDN 18.2 expression levels were significantly correlated with postoperative DFS in gastric cancer patients ($P < 0.05$). A further Cox multivariate regression analysis showed that the pathological stage and CLDN 18.2 expression level independently affected postoperative DFS in gastric cancer patients (Table 4). Moreover, the advanced pathological stage and higher expression levels of CLDN 18.2 were significantly positively correlated with poor prognosis. The Kaplan–Meier method was used to plot the DFS curves. Gastric cancer patients with low and high expressions of CLDN 18.2 had postoperative median DFS of 38.5 months (95% confidence interval (CI) 28.8–48.2 months) and 12.1 months (95% CI, 11.7–41.0 months), respectively (Figure 2B). The Log rank test showed that the curves had statistically significant differences ($P < 0.001$) (Table 2).

Table 4 Univariate and Multivariate Analysis of Factors Associated with DFS

Variables	Univariate Analysis		Multivariate Analysis	
	HR(95% CI)	P value	HR(95% CI)	P value
Age(years)		0.803		
≤60	1			
>60	1.18(0.33–4.22)			
Sex		0.584		
Male	1			
Female	0.66 (0.15–2.9)			
Tumor location		0.976		
Cardia	1			
Body	1.15(0.34–3.90)			
Antrum	0.83(0.16–4.37)			
Full	1.15(0.39–3.37)			
Tumor differentiation		0.879		
Well/moderately	1			
Poorly	0.93(0.37–2.35)			
T stage		0.455		
T1+2	1			
T3+4	1.43(0.56–3.61)			
N stage		0.034		
N0	1			
N1-3	4.68(1.13–19.46)			
TNM stage		0.027		0.040
I–II	1		1	
III	2.87(1.12–7.30)		2.78 (1.05–7.37)	
CLDN 18.2 expression		0.001		0.001
High expression	1		1	
Low expression	5.28(1.99–14.03)		5.24 (1.93–14.19)	

Discussion

Tight junction proteins were first described in 1998 by Shorichiro Tsukita et al as a family of integrated membrane proteins at the tight junctions of various cells. Tight junction proteins are an important component of cell junctions, which can establish small fat barriers and control the molecular flow between cells.[14–16] Different organs express different members of the CLDN family.[17] CLDN18.2 is the most abundant tight junction protein in the stomach.[18] Under normal circumstances, CLDN18.2 is expressed at lower levels in differentiated epithelial cells of the gastric mucosa. During malignant transformation and loss of cell polarity, CLDN18.2 is exposed on the surface of tumor cells

and expressed in many primary gastric cancer cells during metastasis.[10] These phenomena have drawn attention to the possible role of CLDN18.2 as a potential therapeutic target for gastric cancer.

HER-2 was the first driver gene to be identified in gastric cancer, and anti-HER-2 therapy is the primary treatment option for HER-2-positive gastric cancer. Thus, the primary consideration in formulating treatment strategies for advanced gastric cancer is to determine the HER-2 status. For HER-2 positive gastric cancer, anti-HER-2 therapy is the preferred treatment method without contraindications, while chemotherapy combined with PD-1 antibody is considered to be the recommended first-line treatment for HER-2 negative gastric cancer[3–5] because it achieves better efficacy than chemotherapy alone.[19]

In addition to HER-2, CLDN 18.2 has been identified as a new therapeutic target for gastric cancer. In recent clinical trials, combining standard chemotherapy with anti-CLDN 18.2 monoclonal antibodies for treating HER-2 negative gastric cancer could achieve better results than combining chemotherapy with placebo.[12,13] The Food and Drug Administration (FDA) has recently granted a priority review to the biologics license application seeking the approval of zolbetuximab for the first-line treatment of patients with unresectable, locally advanced or metastatic, HER-2-negative, gastric or gastroesophageal junction adenocarcinoma, and whose tumors are CLDN 18.2 positive.[20]

Notably, during the design of these clinical trials, HER-2-positive patients were artificially excluded due to clinical guidelines. However, the synergistic anti-tumour effect of chemotherapy against CLDN 18.2 that was confirmed based on the HER-2 negative populations may theoretically be equally effective for HER-2 positive populations. Although a phenomenon has been observed in non-small cell lung cancer where the efficacy of patients who are positive for a HER-2 positive driver gene is highly dependent on targeted therapy,[21,22] HER-2, a driver gene, does not seem to have the same critical role in gastric cancer. Previous studies on immunotherapy for gastric cancer based on the CHECKMATE 649 and ORIENT 16 trials showed that HER-2-negative gastric cancer patients could benefit from the anti-PD-1 treatment. However, the KEYNOTE 811 trial demonstrated that HER-2-positive gastric cancer patients could benefit from the combination of anti-PD-1 and anti-HER-2 treatment.[23] This suggested that inhibiting the activation of the driving gene HER-2 pathway in gastric cancer can be achieved via synergistic combination with other therapies, as demonstrated in a recent study that involved treating gastric cancer with anti-HER-2 and anti-vascular endothelial growth factor receptor (VEGFR)-2 therapies.[24]

Based on the data from previous clinical trials and the successful anti-CLDN 18.2 treatment in GLOW and SPOTLIGHT experiments, we explored whether different expression levels of CLDN 18.2 affect prognosis in HER-2 positive gastric cancer patients. Although the studies on the relationship between the expression level of CLDN 18.2 and prognosis in gastric cancer generated inconsistent conclusions, they all involved the whole gastric cancer population.[25,26] However, specific analysis of subgroups with different molecular subtypes may be more suitable since gastric cancer is considered highly heterogeneous. In this study, we found that the expression levels of CLDN 18.2 were inconsistent in the HER-2-positive gastric cancer patients. We further classified the expression levels of CLDN 18.2 through ROC analysis into high and low subgroups. The results showed that high expression of CLDN 18.2 was an independent adverse prognostic factor, and HER-2 positive gastric cancer patients with high expression of CLDN 18.2 had poor prognosis. This suggests that simple anti-HER-2 treatment may not be sufficient for treating HER-2-positive gastric cancer. Additionally, choosing between anti-HER-2 and anti-CLDN 18.2 treatments might be challenging when the anti-CLDN 18.2 drugs are approved for clinical application. Moreover, there are research reports that the high expression of CLDN18.2 in gastric cancer is associated with more infiltration of CD4+T cells and CD8+T cells,[27] which are considered more suitable for immunotherapy. This further complicates the dilemma of choosing anti-HER-2, anti-CLDN18.2, and PD-1 antibodies for treating gastric cancer patients with HER-2 positivity and high expression of CLDN18.2, thus necessitating large-scale clinical trials for informatics conclusion. Despite the findings, this study was limited by the relatively small sample size. There is a need to further expand the sample size and include the population with advanced HER-2-positive gastric cancer in the future to verify our conclusion.

The interpretation criteria for the high expression of CLDN 18.2 used in this study are consistent with those used in the clinical trials. In the previous Phase I–III clinical trials, the investigators determined the mode-to-strong CLDN18.2 expression in ≥40% tumor cells as the inclusive criteria, which is equivalent to the standard 8 points or above used in our study. We determined 2.8 points as the best cut-off value for classifying prognosis based on the ROC analysis. One

possible explanation could be that the current anti-CLDN 18.2 antibody (zolbetuximab) is not effective enough for the population with medium expression (2.8–8 points). New anti-CLDN 18.2 drugs, such as ADC drugs targeting CLDN 18.2, anti-CLDN 18.2 combined immunity, and anti-CLDN 18.2 combined with anti-HER-2, may benefit patients with 2.8–8 points.

In order to more accurately identify patients with gastric cancer who need targeted CLDN18.2 therapy, a reliable and reproducible method to detect CLDN18.2 is necessary. At present, detection of CLDN18.2 mainly relies on IHC. The Roche VENTANA CLDN18 (43–14A) Assay, which was used to detect CLDN18.2 in the previous SPOTLIGHT and GLOW studies,[28] can also be used for in vitro diagnosis. A recent study evaluated the analytical performance of three CLDN18 antibodies in terms of reproducibility, in terms of precision, accuracy, sensitivity, and specificity, through a ring study performed across an international cohort of laboratories. In this study, the VENTANA CLDN18 (43–14A) Assay performed well in all of these terms.[28] Due to the limited availability of VENTANA CLDN18 (43–14A) Assay, Abcam products were used in this study. At present, there are no studies comparing the performance of Abcam with the VENTANA CLDN18 (43–14A) Assay for the detection of CLDN18.2, but there are several studies using Abcam products for the detection of CLDN18.2.[29,30] Due to the excellent performance of VENTANA CLDN18 (43–14A) Assay, VENTANA CLDN18 (43–14A) Assay should be chose to detect CLDN18.2 when conditions permit.

Limitations

The study had some limitations. The sample size of our clinical collection was limited, because some patients were not followed up in time, which resulted in the loss of part of the data. In addition, because our hospital had moved its address, some pathological samples were improperly stored during this process, which could not be used for subsequent experimental verification. Despite the influence of many adverse factors, we still collected 55 samples and finally reached the conclusion that supports our view, but we still believe that we can further verify our conclusion by collecting more samples if conditions permit.

Conclusion

Our study found a significant correlation between the expression level of CLDN 18.2 and the prognosis in HER-2-positive gastric cancer subgroups. Thus, we look forward to exploring the optimal drug treatment mode for this population after the approval of anti-CLDN 18.2 drugs.

Abbreviations

Her 2, Human epidermal growth factor receptor 2; DFS, disease-free survival; CI, confidence interval; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; FDA, Food and Drug Administration; VEGFR, vascular endothelial growth factor receptor; CLDN, claudin.

Ethics Statement

The experiments involving clinical samples were approved by the Ethics Committee of the Affiliated Hospital of Jiangnan University, and the study conformed to the principles outlined in the World Medical Association Declaration of Helsinki.

Acknowledgments

We would like to thank all participants for their cooperation in this study.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

This work was supported by National Natural Science Foundation of China (82172583), Wuxi Taihu Lake Talent Plan, Supports for Leading Talents in Medical and Health Profession.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *Ca a Cancer J Clinicians*. 2022;72(1):7–33. doi:10.3322/caac.21708
2. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *Ca a Cancer J Clinicians*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Wang DS, Liu ZX, Lu YX, et al. Liquid biopsies to track trastuzumab resistance in metastatic HER2-positive gastric cancer. *Gut*. 2019;68 (7):1152–1161. doi:10.1136/gutjnl-2018-316522
4. An E, Ock CY, Kim TY, et al. Quantitative proteomic analysis of HER2 expression in the selection of gastric cancer patients for trastuzumab treatment. *Ann Oncol*. 2017;28(1):110–115. doi:10.1093/annonc/mdw442
5. Van Cutsem E, Bang YJ, Feng-Yi F, et al. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. *Gastric Cancer*. 2015;18(3):476–484. doi:10.1007/s10120-014-0402-y
6. Kyuno D, Takasawa A, Takasawa K, et al. Claudin-18.2 as a therapeutic target in cancers: cumulative findings from basic research and clinical trials. *Tissue Barriers*. 2022;10(1):1967080. doi:10.1080/21688370.2021.1967080
7. Resnick MB, Konkin T, Routhier J, Sabo E, Pricolo VE. Claudin-1 is a strong prognostic indicator in stage II colonic cancer: a tissue microarray study. *Mod Pathol*. 2005;18(4):511–518. doi:10.1038/modpathol.3800301
8. Cheung ST, Leung KL, Ip YC, et al. Claudin-10 expression level is associated with recurrence of primary hepatocellular carcinoma. *Clin Cancer Res*. 2005;11(2 Pt 1):551–556.
9. Sanada Y, Oue N, Mitani Y, Yoshida K, Nakayama H, Yasui W. Down-regulation of the claudin-18 gene, identified through serial analysis of gene expression data analysis, in gastric cancer with an intestinal phenotype. *J Pathol*. 2006;208(5):633–642. doi:10.1002/path.1922
10. Sahin U, Koslowski M, Dhaene K, et al. Claudin-18 splice variant 2 is a pan-cancer target suitable for therapeutic antibody development. *Clin Cancer Res*. 2008;14(23):7624–7634. doi:10.1158/1078-0432.Ccr-08-1547
11. Cao W, Xing H, Li Y, et al. Claudin 18.2 is a novel molecular biomarker for tumor-targeted immunotherapy. *Biomark Res*. 2022;10(1):38. doi:10.1186/s40364-022-00385-1
12. Shah MA, Shitara K, Ajani JA, et al. Zolbetuximab plus CAPOX in CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma: the randomized, Phase 3 GLOW trial. *Nature Med*. 2023;29(8):2133–2141. doi:10.1038/s41591-023-02465-7
13. Shitara K, Lordick F, Bang YJ, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. *Lancet*. 2023;401(10389):1655–1668. doi:10.1016/S0140-6736(23)00620-7
14. Athauda A, Chau I. Claudin 18.2-a FAST-moving target in gastric cancer? *Ann Oncol*. 2021;32(5):584–586. doi:10.1016/j.annonc.2021.02.021
15. Singh P, Toom S, Huang Y. Anti-claudin 18.2 antibody as new targeted therapy for advanced gastric cancer. *J Hematol Oncol*. 2017;10(1):105. doi:10.1186/s13045-017-0473-4
16. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and −2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol*. 1998;141(7):1539–1550. doi:10.1083/jcb.141.7.1539
17. Markov AG, Aschenbach JR, Amasheh S. Claudin clusters as determinants of epithelial barrier function. *IUBMB Life*. 2015;67(1):29–35. doi:10.1002/iub.1347
18. Hagen SJ. Unraveling a New Role for Claudins in Gastric Tumorigenesis. *CMGH*. 2019;8(1):151–152. doi:10.1016/j.jcmgh.2019.04.004
19. Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. *Lancet*. 2021;398 (10294):27–40. doi:10.1016/S0140-6736(21)00797-2
20. Ryan C FDA Grants Priority Review to Zolbetuximab for CLDN18.2+ Advanced Gastric/GEJ Adenocarcinoma. Available from: https://www.onclive.com/view/fda-grants-priority-review-to-zolbetuximab-for-cldn18-2-advanced-gastric-gej-adenocarcinoma. Accessed April 26, 2024.
21. Ramalingam SS, Vansteenkiste J, Planchard D, et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *New Engl J Med*. 2020;382(1):41–50. doi:10.1056/NEJMoa1913662
22. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *New Engl J Med*. 2009;361(10):947–957. doi:10.1056/NEJMoa0810699
23. Janjigian YY, Kawazoe A, Yanez P, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. *Nature*. 2021;600(7890):727–730. doi:10.1038/s41586-021-04161-3
24. Kim CG, Jung M, Kim HS, et al. Trastuzumab Combined With Ramucirumab and Paclitaxel in Patients With Previously Treated Human Epidermal Growth Factor Receptor 2-Positive Advanced Gastric or Gastroesophageal Junction Cancer. *J Clin Oncol*. 2023;41(27):4394–4405. doi:10.1200/JCO.22.02122
25. Kubota Y, Kawazoe A, Mishima S, et al. Comprehensive clinical and molecular characterization of claudin 18.2 expression in advanced gastric or gastroesophageal junction cancer. *ESMO Open*. 2023;8(1):100762. doi:10.1016/j.esmoop.2022.100762
26. Ungureanu BS, Lungulescu CV, Pirici D, et al. Clinicopathologic Relevance of Claudin 18.2 Expression in Gastric Cancer: a Meta-Analysis. *Front Oncol*. 2021;11:643872. doi:10.3389/fonc.2021.643872
27. Wang C, Wang Y, Chen J, et al. CLDN18.2 expression and its impact on prognosis and the immune microenvironment in gastric cancer. *BMC Gastroenterol*. 2023;23(1):283. doi:10.1186/s12876-023-02924-y

28. Jasani B, Taniere P, Schildhaus HU, et al. Global Ring Study to Investigate the Comparability of Total Assay Performance of Commercial Claudin 18 Antibodies for Evaluation in Gastric Cancer. *Lab Invest*. 2024;104(1):100284. doi:10.1016/j.labinv.2023.100284
29. Wang X, Zhang CS, Dong XY, et al. Claudin 18.2 is a potential therapeutic target for zolbetuximab in pancreatic ductal adenocarcinoma. *World J Gastrointest Oncol*. 2022;14(7):1252–1264. doi:10.4251/wjgo.v14.i7.1252
30. Moentenich V, Gebauer F, Comut E, et al. Claudin 18.2 expression in esophageal adenocarcinoma and its potential impact on future treatment strategies. *Oncol Lett*. 2020;19(6):3665–3670. doi:10.3892/ol.2020.11520