

Correlation between quantitative dynamic contrast-enhanced MRI parameters and molecular typing and related immune proteins in gastric cancer

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Research article

Keywords: Magnetic resonance imaging, Molecular typing, HER-2, Stomach neoplasms

Posted Date: May 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26912/v1>

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Abstract

Background

To analyze the correlation between quantitative dynamic contrast-enhanced MRI (DCE-MRI) parameters and molecular typing and related immune proteins in gastric cancer.

Methods

Forty-three patients confirmed as gastric adenocarcinoma by histopathology were enrolled in this prospective study. DCE-MRI were performed before surgery, and quantitative DCE parameters (K^{trans} , K_{ep} , V_e) were measured. The specimens were stained with biomarkers EBER-ISH, MLH1, PMS2, E-Cadherin, P53 and HER-2. According to the different dyeing results, they were divided into five molecular types and four HER-2 expression grades. The quantitative DCE parameters among five molecular types was compared using the ANOVA or Kruskal-Wallis test, and pairwise comparison was performed using the LSD test. The quantitative DCE parameters between positive and negative expressions of related immune proteins were compared using the Mann-Whitney U test. The correlations between quantitative DCE parameters and HER-2 expression grades were evaluated using Spearman rank correlation test.

Results

Among 43 cases, 3 cases were EBV-positive groups, 9 cases were MSI groups, 2 cases were aberrant E-Cadherin groups, 23 cases were aberrant P53 groups, and 6 cases were normal P53 groups. There were significant differences of quantitative DCE parameters in K^{trans} and K_{ep} among five molecular types ($P < 0.05$). The K^{trans} value of Aberrant P53 group was lower than that of EBV-positive group, MSI group and Normal P53 group, the K_{ep} value of E-Cadherin group was lower than that of EBV-positive group, and the K_{ep} value of Aberrant P53 group was lower than that of EBV-positive group and MSI group, and all differences were statistically significant (all $P < 0.05$). Aberrant E-Cadherin group has the lowest K^{trans} value (0.28/min) and K_{ep} value (0.26/min) in five groups, respectively. There were no significant differences in quantitative DCE parameters between positive and negative expressions of all related immune proteins (all $P > 0.05$). There were no significant correlation between HER-2 expression grades and K^{trans} , K_{ep} , V_e values ($r = -0.08, -0.03, -0.16, P = 0.63, 0.84, 0.31$, respectively).

Conclusion

Quantitative DCE-MRI parameters can assess some molecular types of gastric cancer in a certain extent, and not assess related immune protein expressions, and not related to HER-2 expression grades.

Background

Currently, the gastric cancer classification that based on morphological and cytological features (Lauren classification and WHO classification) can't sufficient for clinical individualized treatment. With the development of genetic technology, the research on gastric cancer has penetrated into its molecular lever. Only

by classifying the essential characteristics of gastric cancer at the molecular level can we make early diagnosis and prognosis of tumors more rationally and accurately, and also can we apply molecular targeted drugs to individualized treatment more accurately [1].

At present, there are three types of gastric cancer molecular classification is widely accepted: (a) Singapore classification: proliferative, metabolic, mesenchymal [2]; (b) International Cancer Genome Atlas (TCGA) classification: Epstein-Barr virus (EBV) gastric adenocarcinomas, microsatellite-unstable (MSI) high gastric adenocarcinomas, genomically stable group, and chromosomal instability group [3]; (c) Asian Cancer Research Group (ACRG) classification: MSI type, microsatellite-stable (MSS) / epithelial mesenchymal transition (EMT) type, MSS / TP53 inactive type, MSS / TP53 active type [4].

The above-mentioned gastric cancer molecular typing uses a variety of detection methods such as gene sequencing and immune protein detection, which are relatively expensive, and difficult to carry out in clinical as a routine detection method. In 2016, by referring to the above molecular typing, Setia N et al divided gastric cancer into five molecular types: EBV-positive gastric cancer, MSI gastric cancer, gastric cancer with aberrant E-Cadherin expression, gastric cancer with aberrant P53 expression, and gastric cancer with normal P53 expression [1]. This molecular typing method only measure five related immune proteins (EBER-ISH, MLH1, PMS2, E-Cadherin, and P53) and easy to promote in clinical.

Setia N's five gastric cancer molecular types have different characteristics at the molecular level, clinical manifestations and prognosis. EBV-positive gastric cancer mostly occurs in males, mostly in the cardia, and has a good prognosis, which is closely related to PD-L1. MSI gastric cancer mostly occurs in elderly women, and is associated with intestinal type in Lauren classification. The lesion is usually large, but less prone to lymph node metastasis. The prognosis has a good survival rate. Gastric cancer with aberrant E-Cadherin expression is associated with diffuse type in Lauren classification, similar to genomic stability type in TCGA, and prone to distant metastasis. Gastric cancer with aberrant P53 expression is associated with intestinal type and has a higher lymph node metastasis rate, it's HER-2 expression usually high. Gastric cancer with normal P53 expression is associated with intestinal type in Lauren classification.

Quantitative DCE parameters is volume transfer coefficient (K^{trans}), reverse reflux rate constant (K_{ep}), extracellular extravascular volume fraction (V_e), they can reflect the permeability of tumor blood vessels and can be used to predict tumor malignancy, treatment outcome and prognosis. Ma L [6] and Joo I [7] found that quantitative DCE parameters can be used to predict T stage, EGFR classification, Laurens classification, and VEGF classification of gastric cancer, but there is no literature to evaluate the correlation between quantitative DCE parameters and molecular typing, related immune proteins, HER-2 expression grade in gastric cancer.

This study was designed to explore the feasibility of quantitative DCE-MRI parameters to assess the molecular typing of gastric adenocarcinoma and expression of related immune proteins, and correlation between quantitative DCE parameters and HER-2 expression grade.

Methods

Patients

This prospective study was conducted from April 2018 to October 2019 after approved by our institutional review board. Informed consent was signed for all patients. A total of 70 patients with suspected gastric cancer who underwent DCE-MRI in our institution were collected. All results were confirmed by histopathology within two weeks after MR examination. Among them, 27 patients were excluded for the following reasons: proven special histopathological type, including squamous cell carcinoma (n = 2), stromal tumor (n = 2), leiomyomas (n = 1), polyps (n = 1), high grade intraepithelial neoplasia (n = 2); receiving preoperative chemotherapy (n = 17); Poor image quality cannot be analyzed (n = 2).

Mri Examination

Data was performed using a 3.0T MR scanner (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany) with an 18-channel body coil and a built-in 32-channel spine coil. All patients were placed in a feet-first orientation, supine position. An automatic double-tube high-pressure pressure syringe (Spectris Solaris EP, Medrad, Indianola, PA) was used to inject gadopentetate dimeglumine (Consun Pharmaceutical, Guangzhou, China) through the anterior elbow vein with metering 0.2 ml/kg, and the flow rate was 2.5 ml/s, and then inject 20 ml saline at the same flow rate. All patients were fasted for 6 hours, and 10 mg anisodamine was injected intramuscular approximately 10 minutes before the examination. To prevent ignoring small lesions, the patient did not drink water before the examination. The dynamic enhanced scan was performed using Radial Volumetric Interpolated Breath-hold Examination (StarVIBE, Siemens Healthcare, Erlangen, Germany) for a total of 42 Phases. The detail parameters of the protocols were listed in Table 1.

Table 1
MRI protocols for gastric cancer

Protocols	TR / TE (ms)	Slice thickness (mm)	Slices	Flip angle (°)	Base resolution	Phase resolution (%)	FOV (mm)	Voxel size (mm)	Time acquisition
T1WI	4.34 / 1.34	3	120	9	180 × 288	90	380	0.7 × 0.7 × 2.5	18 s
T2WI	4000 ~ 8000 / 96	5.5	35	133	193 × 384	70	380	1.0 × 1.0 × 5.0	3 min18s
DWI	2600 / 51	5.5	35	—	128 × 128	100	340	2.7 × 2.7 × 7.0	30 s
Axial T1 mapping	2.33 / 1.21	5	96	8	83 × 128	80	380	3.0 × 3.0 × 7.0	18 s
StarVIBE	3.87 / 1.82	2.5	72	12	168 × 280	60	380	1.3 × 1.3 × 2.5	5 min24s

TR: Repetition time, TE: Echo time, FOV: Field of view, T1WI: T1-weighted imaging, T2WI: T2-weighted imaging, DWI: diffusion-weighted imaging, StarVIBE: Star Volumetric Interpolated Breath-hold Examination

Imaging Analysis

All DCE-MRI data was imported into the Omni-Kinetics software package in GE MITK Work bench (GE Medical, China) for post-processing. The quantitative DCE parameters were measured by two readers with more than five years of work experience without knowing the molecular typing of patients. Firstly, the abdominal aorta was selected to obtain the arterial input function (AIF), and then the Region of interest (ROI) was manually delineated in the whole tumor lesion in the most obvious enhancement phase (usually the late arterial phase or the venous phase), and the cystic necrosis, perigastric fat, intragastric air were avoided as much as possible (Fig. 1). Then the quantitative DCE parameters were generated by the Tofts model: K^{trans} , K_{ep} , V_e .

Pathological Evaluation

The specimens were fixed in 10% neutral paraformaldehyde, embedded in 4 μ m serial sections by paraffin, then stained with biomarkers EBER-ISH, MLH1, PMS2, E-Cadherin, P53 and HER-2. According to different dyeing results, they were divided into five molecular types (Figs. 2 and 3). In all cases, EBER positive cases were defined as EBV-positive group, and in the remaining cases, those with negative MLH1 or PMS2 cases were defined as MSI group. In the remaining cases, E-Cadherin negative cases or only cytoplasm positive cases were defined as aberrant E-Cadherin group. In the remaining cases, P53 positive cases were defined as aberrant P53 group, and P53 negative cases were defined as normal P53 group. The HER-2 expression grade score was based on the cell membrane staining: 0, no membrane staining; 1+, faint staining; 2+, moderate staining; and 3+, strong staining.

Statistical analysis

Statistical analysis was performed with SPSS 22.0 (Statistical Product and Service Solutions, IBM Corp, Armonk, NY, USA). The K^{trans} , K_{ep} , and V_e data were expressed as medians and interquartile range. Intraclass correlation coefficient (ICC) was used to determine the consistency of results between two readers using Bland-Altman analysis. ICC values less than 0.40 were considered poor consistency; 0.41–0.75 were considered moderate consistency; and greater than 0.75 were considered good consistency. The quantitative DCE parameters among five molecular types were compared using the ANOVA or Kruskal-Wallis test, and pairwise comparison was performed using the least significant difference (LSD) post-hoc test. The quantitative DCE parameters between different expressions of related immune proteins were compared using the Mann-Whitney U test. The correlation between quantitative DCE parameters and HER-2 expression grade was evaluated using Spearman rank correlation test. $P < 0.05$ was considered statistically significant.

Results

The 43 enrolled cases were all gastric adenocarcinoma, and clinicopathological characteristics were listed in Table 2.

Table 2
The clinicopathological characteristics of gastric cancer

Clinicopathological characteristics	Total (n = 43) (proportion)
Age (years, mean ± SD)	63.6 ± 7.5
Gender	
Male	32 (74.4%)
Female	11 (25.6%)
Tumour location	
cardia	29 (67.4%)
corpus or antrum	14 (32.6%)
molecular types	
EBV-positive gastric cancer	3 (7.0%)
MSI gastric cancer	9 (20.9%)
gastric cancer with aberrant E-Cadherin expression	2 (4.7%)
gastric cancer with aberrant P53 expression	23 (53.5%)
gastric cancer with normal P53 expression	6 (14.0%)
Tumor T-stage*	
T1	4 (9.3%)
T2	9 (20.9%)
T3	24 (55.8%)
T4	6 (14.0%)
N-stage*	
N0	13 (30.2%)
N1	9 (20.9%)
N2	7 (16.3%)
N3	14 (32.6%)
M-stage*	
M0	32 (74.4%)
M1	11 (25.6%)
*According to AJCC / UICC TNM Staging of Gastric Cancer (8th Edition)	

The consistency analysis of the quantitative DCE parameters measured by two readers was shown in Table 3. The ICC value showed good consistency.

Table 3
The consistency analysis of DCE quantitative parameter results measured by two readers

	Reader 1	Reader 2	ICC	P
K^{trans} (/min)	0.33 (0.28)	0.32 (0.28)	0.925	0.001
K_{ep} (/min)	0.55 (0.52)	0.48 (0.52)		
V_e	0.57 (0.27)	0.59 (0.31)		
The data were expressed as median and interquartile range, ICC: Intraclass correlation coefficient				

The differences of quantitative DCE parameters among five molecular types of gastric cancer were shown in Table 4. There were statistically differences among five molecular types in K^{trans} and K_{ep} . In Pairwise comparison, the K^{trans} value of Aberrant P53 group was lower than that of EBV-positive group, MSI group and Normal p53 group, the K_{ep} value of E-Cadherin group was lower than that of EBV-positive group, and Aberrant P53 group was lower than that of EBV-positive group and MSI group, all above groups were statistically significant (all $P < 0.05$). Aberrant E-Cadherin group has the lowest K^{trans} value (0.28/min) and K_{ep} value (0.26/min) in five groups, respectively.

Table 4
The difference of quantitative DCE parameters among five molecular types of gastric cancer

	EBV-positive group (n = 3)	MSI group (n = 9)	Aberrant E-Cadherin group (n = 2)	Aberrant P53 group (n = 23)	Normal P53 group (n = 6)	F (χ^2)	P
K^{trans} (/min)	0.46 (-)	0.48 (0.50)	0.28 (-)	0.28 (0.19)	0.48 (0.53)	2.688	0.046
K_{ep} (/min)	0.87 (-)	0.78 (0.68)	0.26 (-)	0.38 (0.37)	0.52 (0.67)	3.078	0.027
V_e	0.59 (-)	0.58 (0.28)	0.65 (-)	0.49 (0.30)	0.66 (0.46)	$\chi^2 = 2.705$	0.608

The data were expressed as median and interquartile range. Pairwise comparison: In K^{trans} values, except for EBV-positive group and Aberrant P53 group ($P = 0.036$), MSI group and Aberrant P53 group ($P = 0.036$), Aberrant P53 group and Normal P53 group ($P = 0.040$) were statistically significant, and the other groups were no statistically significant (all $P > 0.05$); In k_{ep} values, except for EBV-positive group and Aberrant E-Cadherin group ($P = 0.042$), EBV-positive group and Aberrant P53 group ($P = 0.016$), MSI group and Aberrant P53 group ($P = 0.024$) were statistically significant, and the other groups were no statistically significant (all $P > 0.05$)

The differences of quantitative DCE parameters between negative and positive groups of EBER-ISH, MLH1, PMS2, E-Cadherin, and P53 were no statistically significant (all $P > 0.05$) (Table 5).

Table 5

The differences of quantitative DCE parameters between negative and positive groups of related immune proteins expressions

		K^{trans} (/min)	Z	P	K_{ep} (/min)	Z	P	V_e	Z	P
EBER	Negative group (n = 40)	0.31 (0.29)	-1.57	0.12	0.43 (0.50)	-1.67	0.10	0.58 (0.32)	-0.72	0.48
	positive group (n = 3)	0.46 (-)			0.87 (-)			0.59 (-)		
MLH1	Negative group (n = 1)	0.66	-1.21	0.23	1.26	-1.69	0.09	0.47	-0.48	0.63
	positive group (n = 42)	0.32 (0.26)			0.46 (0.51)			0.59 (0.32)		
PMS2	Negative group (n = 9)	0.48 (0.50)	-1.13	0.26	0.78 (0.68)	-1.55	0.12	0.58 (0.28)	-0.39	0.70
	positive group (n = 34)	0.31 (0.22)			0.43 (0.38)			0.59 (0.32)		
E- Cadherin	Negative group (n = 4)	0.36 (0.36)	-0.25	0.80	0.49 (0.90)	-0.50	0.62	0.58 (0.19)	-0.21	0.83
	positive group (n = 39)	0.31 (0.28)			0.48 (0.52)			0.59 (0.32)		
P53	Negative group (n = 12)	0.42 (0.54)	-1.22	0.22	0.52 (0.63)	-1.08	0.28	0.59 (0.27)	-0.54	0.59
	positive group (n = 31)	0.29 (0.23)			0.39 (0.39)			0.58 (0.33)		
The data were expressed as median and interquartile range										

There were no significant correlations between HER-2 expression grade and K^{trans} , K_{ep} , V_e values ($r=-0.08$, -0.03 , -0.16 , $P = 0.63$, 0.84 , 0.31 , respectively) (Table 6).

Table 6

The correlation of quantitative DCE parameters of gastric adenocarcinomas with HER-2 expression grade

HER-2 expression grade	0 (n = 27)	1+ (n = 7)	2+ (n = 5)	3+ (n = 4)	r	P
K^{trans} (/min)	0.35 (0.31)	0.30 (0.29)	0.24 (0.17)	0.48 (0.42)	-0.08	0.63
K_{ep} (/min)	0.48 (0.57)	0.54 (0.72)	0.32 (0.44)	0.47 (0.29)	-0.03	0.84
V_e	0.66 (0.29)	0.47 (0.17)	0.47 (0.38)	0.73 (0.46)	-0.16	0.31
The data were expressed as median and interquartile range						

Discussion

Our research showed that the quantitative DCE parameters in K^{trans} and K_{ep} were statistical difference among five gastric cancer molecular types. The K^{trans} and K_{ep} values of Aberrant E-Cadherin group and Aberrant P53 group were lower than the other three groups, and some differences were statistically significant. The K^{trans} and K_{ep} represent the permeability of the vascular wall, indicating that the neovascularization in Aberrant E-Cadherin group and Aberrant P53 group was more abundant than the other three groups, which means that the two groups had a higher possibility of metastasis and poor prognosis, which was consistent with Setia N's study [4]. The cause of the poor prognosis in the two groups might be: decreased expression of E-Cadherin will lead to weakened adhesion between cancer cells and lead to epithelial-mesenchymal transition, and cancer cells are prone to fall off and metastasize [8]. Increased expression of P53 mediates the proliferation of cancer cells and increases the expression of vascular endothelial growth factor, resulting in increased permeability of new blood vessels and distant metastasis of cancer cells [9].

Our study showed that quantitative DCE parameters could not identify EBER, MLH1, PMS2, E-Cadherin, and P53 negative and positive groups, which might be related to the disparity in expression of related immune proteins (EBER, MLH1, E-Cadherin negative / positive ratio were 40/3, 1/42, 4/39). In Setia N's study, EBER, E-Cadherin, P53 negative / positive ratio were 139/7, 71/75, 10/136 [1], the negative / positive ratio of some related immune proteins was significantly different from our study, which might be related to the small sample size and the genetic differences between the East and the West.

Although HER-2 expression grade could be used as a prognostic indicator of malignant degree of gastric cancer, our study showed that there was no significant correlation between HER-2 expression grade and quantitative DCE parameters. Koo HR [10] also found that there was no significant correlation between HER-2 expression grade and quantitative DCE parameters in breast cancer, the reason may be related to the inconsistency between HER-2 protein expression and gene amplification.

Our research has some limitations. First, the sample size was small may lead to bias. Second, we did not classify gastric cancer according to the TCGA or ACRG classification, which is our next research direction. Third, although artifact correction was performed before the measurement of quantitative DCE parameters, there were may still occur slight displacement in lesions, and resulting in inaccurate data measurement.

Conclusions

This study revealed that quantitative DCE-MRI parameters can assess some molecular types of gastric cancer in a certain extent, and not assess related immune protein expressions, and not related to HER-2 expression grade.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (Ethic's committee of the Zhengzhou University, 20180416). Informed consent was signed for all patients.

Consent for publication

Not applicable.

Funding

none.

Competing interests

The first author and all coauthors confirm that there are no potential conflicts of interest to disclose.

Funding

none.

Authors' contributions

YLL, GJB and QJR design research, acquire, analyze and interpret data and draft manuscripts, YLL and LYN perform statistical analysis on the data, ZH and XQX perform pathological assessment, and ZHK performs data collection. All authors revised and approved the submitted manuscript.

Acknowledgements

None.

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Figures

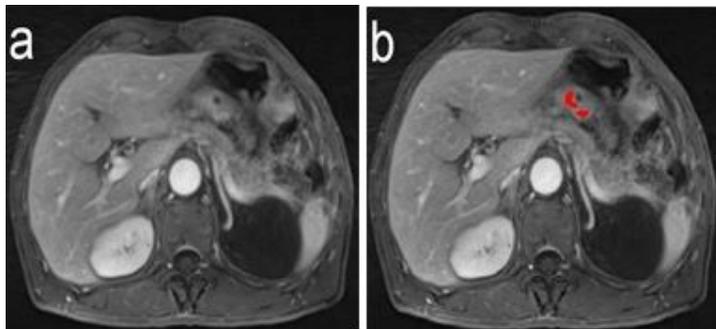


Figure 1

Male, 68Y. The lesion was located in the antrum. there were cystic necrosis areas. the ROI was manually outlined in solid part of tumor

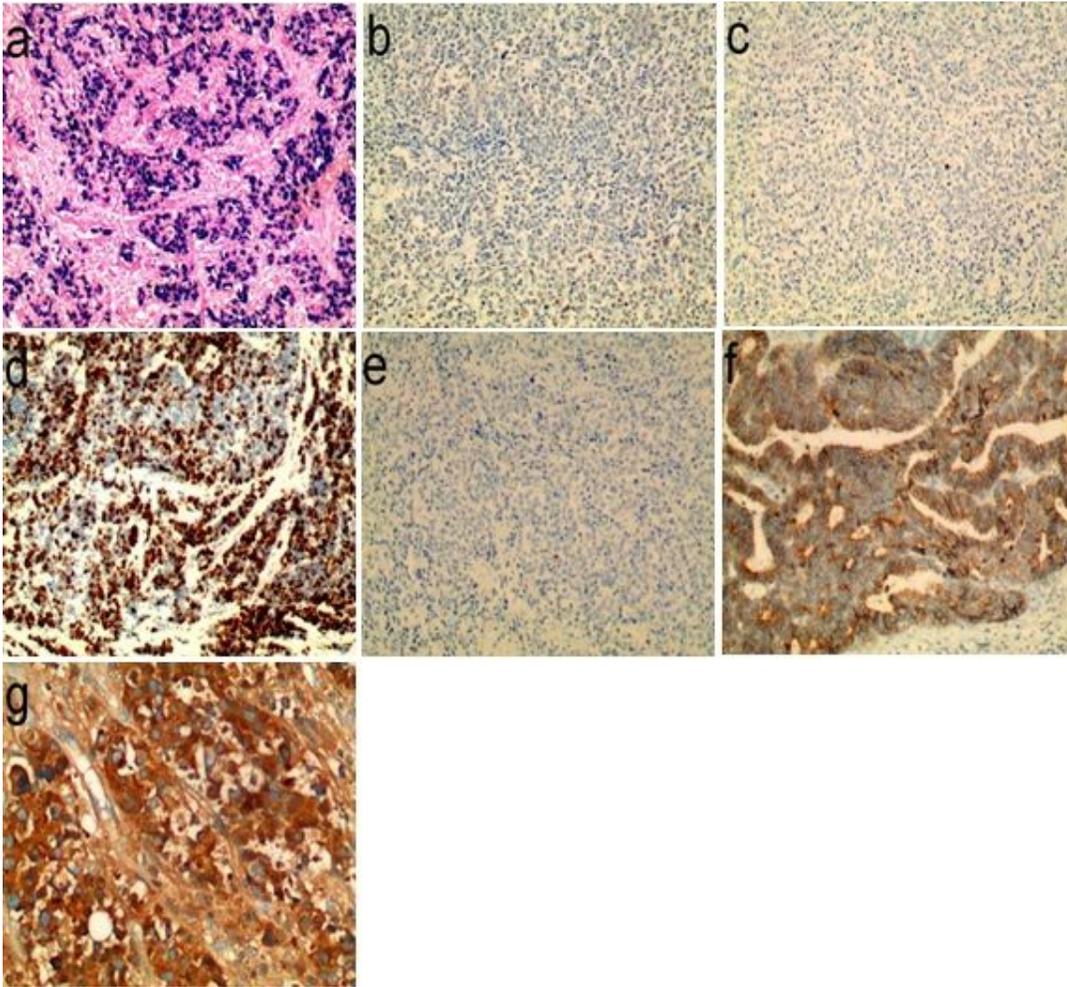


Figure 2

Staining of related immune proteins. a. EBER (+), b. MLH1 (-), c. PMS2 (-), d. P53 (+), e. E-Cadherin (-), f. E-Cadherin membrane (+), g. E-Cadherin both membrane and cytoplasm (+) (a,b,c,d,e $\times 100$; f,g $\times 400$)

a

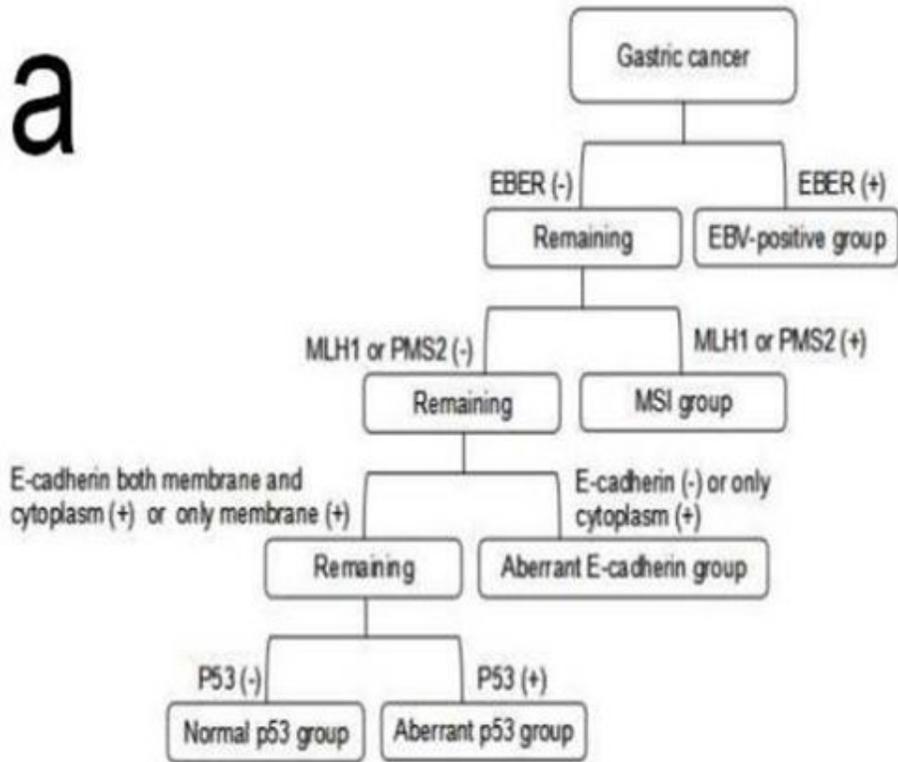


Figure 3

Flow chart for determining five molecular subtype groups based on different staining