Double contrast-enhanced ultrasonography in preoperative Borrmann classification of advanced gastric carcinoma: comparison with histopathology

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The purpose of this study was to investigate the accuracy of double contrast-enhanced ultrasonography (DCEUS) for assessing the Borrmann classification of advanced gastric carcinoma (AGC) preoperatively. Three hundred twenty nine patients with proved AGC were enrolled. DCEUS (intravenous microbubbles combined with oral contrast-enhanced ultrasound) was performed preoperatively. The diagnostic accuracy of DCEUS in determining Borrmann classification was compared with postoperative pathological findings. The reliability of DCEUS was analyzed. The accuracy of DCEUS in determining the Borrmann classification of AGC was 91.49%. The intra- and inter-observer reproducibility was both almost perfect for assessing the Borrmann classification of AGC by DCEUS. DCEUS could be considered as an accurate, non-invasive, and reliable diagnostic method for preoperative Borrmann classification of advanced gastric carcinoma.

The classification of advanced gastric cancer (AGC), one of the crucial factors of therapeutic strategy, proposed by Borrmann in 1926 according to the macroscopic aspect1, is still widely used by surgeons, pathologists and endoscopists around the world2. Many modalities, such as computed tomography (CT) and endoscopy have been used for assessing the Borrmann classification of AGC. Particularly, multi-detector row CT (MDCT) with multi-planar reformatted views is a powerful test for non-invasive evaluation of gastric cancer3. However, it carries a burden on ionizing radiation which may be a disadvantage4.

Upper digestive tract endoscopy is the gold standard in the diagnosis of gastric tumors. With the advent of endoscopy, accuracy in the diagnosis of gastric carcinoma has undoubtedly improved5. When combined with biopsy and brush cytology, endoscopy has an overall sensitivity of 95%–98% in the detection of gastric cancer6,7. Unfortunately, endoscopy has a wide range of accuracy according to the gross tumor growth pattern and anatomic tumor location8, and additionally which is rather invasive and traumatic. In patients with linitis plastica, endoscopy has been reported to have a sensitivity of only 33%–73%8,9. Endoscopists often have difficulty recognizing these lesions, as the tumors are located predominantly in the submucosa; therefore, the overlying mucosa appears normal. Furthermore, the tumor cells of scirrhous gastric carcinoma are often dispersed within a dense fibrous matrix and tend to be widely separated8–10.

There is an obvious tendency in modern medicine to identify widely accessible, well-tolerated, non invasive, precise diagnostic procedures. Our previous studies and other reports had shown that intravenous contrast-enhanced ultrasound combined with oral contrast-enhanced ultrasound (DCEUS) is an accurate, non-invasive, and reliable diagnostic method for preoperative assessment and detection of AGC11–16. The aim of this study was to assess the accuracy of the Borrmann classification in patients with AGC compared with postoperative pathology, using ultrasonography with DCEUS, in which an oral ultrasonic contrast agent is combined with an intravenous contrast agent.
**Table 1 | Results of Borrmann classification by DCEUS compared with pathological findings (by case)**

| Pathology          | Borrmann I | Borrmann II | Borrmann III | Borrmann IV | Accuracy |
|--------------------|------------|-------------|--------------|-------------|----------|
| DCEUS              | 20         | 3           | 0            | 0           | 86.96%   |
| Borrnman II        | 1          | 62          | 6            | 0           | 89.86%   |
| Borrnman III       | 0          | 5           | 148          | 8           | 91.93%   |
| Borrnman IV        | 0          | 0           | 5            | 71          | 93.42%   |

DCEUS = double contrast-enhanced ultrasound.

The overall accuracy of DCEUS in Borrmann classification was 91.49% (301 of 329 patients).

**Results**

Three hundred and twenty nine patients with AGC were enrolled in this study and treated with surgery. Among them, the pathological Borrmann classification in 23 cases (6.70%) was type I; in 69 cases (20.97%) was type II; in 161 cases (48.94%) was type III, and in 76 cases (23.10%) was type IV. The overall accuracy of DCEUS in determining the Borrmann classification of AGC was 91.49%, the accuracy of DCEUS in determining Borrmann I, II, III and IV was 86.96%, 89.86%, 91.93% and 93.42% respectively. A total of 17 cases were overstaged (3 patients with Borrmann I were overstaged as II; 6 patients with I were overstaged as III; 8 patients with III were overstaged as IV) and 11 cases were understaged (1 patient with II was understaged as I; 5 patients with III were understaged as II; 5 patients with IV were understaged as III) (Table 1). The sensitivity, specificity, PPV, and NPV of DCEUS in determining Borrmann classification were listed in Table 2.

The intra- and inter-observer reproducibilities were both almost perfect for assessing the Borrmann classification of AGC with a Kappa value of 0.88 (<P = 0.001) for intra-observer (Table 3) and 0.834 (<P = 0.001) for inter-observer (Table 4) by DCEUS.

**Discussion**

Conventionally, the role of transabdominal ultrasonography in the evaluation of gastric carcinoma has been to assess the presence of distant metastases, especially to regional nodes and the liver. However, it is difficult to visualize gastric cancers of small size and deep location because of interference by intragastric gas. Oral contrast enhanced ultrasound improves imaging by displacing the air in the stomach and by distending the gastric lumen, thus helping to display mucosal lesions. Intravenous contrast agent. Modern multipulse imaging methods, such as the CPS we performed with CPS mode, which uses phase and amplitude modulation to separate the microbubble signals the echoes from tissue and from the oral contrast agent within the gastric lumen, which does not appear on microbubble-specific images since it behaves like tissue with minimal non-linear properties. Because such microbubbles flow with red blood cells, the injected microbubbles act as markers for gastric cancers that are densely vascularized, thus complementing the improved visualization of the gastric wall provided by the oral contrast agent. Its advantage is the high-contrast resolution between tumor and normal tissue. Because of the use of harmonic technologies at low emission frequencies, there is some loss of spatial resolution and overall image quality, typically resulting in a grainy appearance of the CEUS classification of AGC. The intra- and inter-observer reproducibilities were both almost perfect for assessing the Borrmann classification of AGC with a Kappa value of 0.88 (<P = 0.000) for intra-observer and 0.83 (<P = 0.000) for inter-observer of DCEUS, showing that the consistency was very good and that the criteria could be learned and applied easily.

Oral contrast enhanced ultrasound is restricted by many factors, such as the limit of resolution, the small difference of the acoustic impedance among different tissues (making for low contrast), and necrotic tissue persisting in the surface of gastric ulcer causing posterior acoustic attenuation. It is difficult to distinguish tumor infiltrate from inflammation adjacent to the lesion and from peritumoral fibrosis on B-mode, so that overestimation or underestimation of the Borrmann classification may occur when using oral contrast enhanced ultrasound. A total of 27 (12.3%) of our patients were overestimated, and 24 (30.2%) patients were underestimated using oral contrast enhanced ultrasound.

Angiogenesis is essential for the growth of solid tumors. Intravenous contrast-enhanced ultrasonography (CEUS) is a useful modality to assess the angiogenesis of cancers. In term of the difference from CT contrast agents such as lipiodol, microbubble-based contrast agent does not penetrate the vessel walls and so behaves as a blood pool agent. Its distribution in the lesion represents the distribution of the microvessels so the intensity of the enhancement represents the density of these vessels. Microvascular assessment is more sensitive for the evaluation of tumor stage, lesion size and sonographic appearance. Therefore, DCEUS can be used to evaluate the morphology of gastric lesions. In this study, the CEUS was performed with CPS mode, which uses phase and amplitude modulation to separate the microbubble signals the echoes from tissue and from the oral contrast agent within the gastric lumen, which does not appear on microbubble-specific images since it behaves like tissue with minimal non-linear properties. Because such microbubbles flow with red blood cells, the injected microbubbles act as markers for gastric cancers that are densely vascularized, thus complementing the improved visualization of the gastric wall provided by the oral contrast agent. Its advantage is the high-contrast resolution between tumors and normal tissues, making it sensitive for lesion detection, characterization, and staging. More importantly, DCEUS can show the relationship of the lesion’s vasculature and the gastric wall as well as their contours.

**Table 2 | The sensitivity, specificity, PPV, and NPV of DCEUS for assessing each macroscopic staging of AGC**

| Pathology | Sensitivity | Specificity | PPV (%) | NPV (%) |
|-----------|-------------|-------------|---------|---------|
| Borrmann I| DCEUS 86.96 | 99.67       | 95.24   | 99.03   |
| Borrmann II| DCEUS 89.86 | 96.92       | 88.57   | 97.30   |
| Borrmann III| DCEUS 91.93 | 93.45       | 93.08   | 92.35   |
| Borrmann IV| DCEUS 93.42 | 96.84       | 89.87   | 98.00   |
images. Moreover, the depth at which the lesion resides affects the
detectability of vascularity as deep-seated lesions give poorer sig-
als. Furthermore, two-dimensional views of DCEUS do not pro-
 vide the gross appearance of macroscopic morphology of AGC. A
total of 17 cases (5.2%) of overestimation and 11 (3.3%) cases of
underestimation of Borrmann classification occurred using DCEUS.

There were some limitations to this study. First, this study was
retrospective and included only patients referred to our hospital for
surgery. Although blinded to the endoscopic, surgical and histo-
pathologic results, the observers were aware of the presence of a
tumor. Second, DCEUS examinations was paired rather than rando-
mized unpaired. Third, comparative studies between DCEUS, CT,
MRI or EUS on preoperative Borrmann classification were not car-
ried out in this study and this is the subject of ongoing research.

Conclusion
DCEUS shows promise as a new noninvasive, convenient and repeat-
able method for preoperative Borrmann classification of advanced
gastric carcinoma, which has a high accuracy.

Methods
Patients. Between October 2006 and June 2012, 390 patients were examined using
DCEUS preoperatively. The inclusion criteria were as follows: 1 adenocarcinoma of
stomach proven by endoscopic biopsy; 2 not treated with nonsteroidal
antiinflammatory drugs, chemotherapy, radiotherapy or immunotherapy previously.
Surgical resections were performed within 1 week after the DCEUS examination. The
exclusion criteria were as follows: 1 elderly patient with comorbidities for surgery (15
cases); 2 unresectable lesions with metastasis detected on preoperative evaluation (21
cases); 3 early stage tumors on postoperative pathology (25 cases). Finally, the
remaining 329 patients (203 male and 126 female; age range, 31–81 years; mean age
62.0 ± 10.5 years) were enrolled in the study. Informed consent was obtained from all
patients before their examination, and the local ethics committee and institutional
review board approved this prospective study (Ethics No.2006-02).

Ultrasonography. DCEUS of each case were performed after fasting for at least
6 hours; atropine sulfate (0.05 mg/kg) was administered via intramuscular injection
30 min before the examination to inhibit gastric peristalsis. The flowchart of DCEUS

Table 3 | Concordance of Borrmann classification by DCEUS
according to the findings performed at 2 separate time intervals
of readings

| First | Second |
|-------|--------|
| Borrmann I | Borrmann I |
| 20 | 1 |
| Borrmann II | Borrmann II |
| 0 | 64 |
| Borrmann III | Borrmann III |
| 0 | 7 |
| Borrmann IV | Borrmann IV |
| 20 | 72 |
| Total | Total |
| 21 | 70 |
| 142 | 10 |
| 71 | 79 |
| 329 | 329 |

The intra-observer reproducibility was almost perfect for Borrmann classification of advanced
gastric cancer by DCEUS with a Kappa value of 0.880 (P = 0.000, 95% CI: 0.835 – 0.925).

Table 4 | Concordance of Borrmann classification by DCEUS
according to the findings of two observers

| Observer A | Observer B |
|------------|------------|
| Borrmann I | Borrmann I |
| 18 | 1 |
| Borrmann II | Borrmann II |
| 3 | 63 |
| Borrmann III | Borrmann III |
| 0 | 6 |
| Borrmann IV | Borrmann IV |
| 0 | 0 |
| Total | Total |
| 19 | 74 |
| 157 | 79 |
| 329 | 329 |

The inter-observer reproducibility was almost perfect for Borrmann classification of advanced
gastric cancer by DCEUS with a Kappa value of 0.834 (P = 0.000, 95% CI: 0.783 – 0.885).

Figure 1 | Flowchart of DCEUS examination in patients with advanced
gastric carcinoma.

Figure 2 | Schematic diagram of Borrmann classification.
homogeneous suspension. After cooling to a comfortable temperature, the patient was asked to drink the palatable liquid as quickly as possible. It dilates the stomach and displaces the air within it so that the lumen appears as a homogenous mid-gray on B-mode imaging thus providing an acoustic window that lasts for around 60 min. It does not appear on microbubble-specific images since it behaves like tissue, with minimal non-linear properties.

The distal esophagus and the cardia of all patients were studied in real time B-mode using conventional tissue settings while the patients ingested the oral agent. Then the remaining parts of the stomach and the duodenal bulb were examined in turn, with the patient in the supine and both decubitus positions to facilitate complete filling and visualization of the lesion. The entire movie sequence of each case was stored on magnetic optical disks for analysis. When the lesion was displayed clearly, contrast-enhanced ultrasonography (CEUS) was performed using the contrast pulse sequencing (CPS) mode. The transmit frequency of 1.5 MHz and an acoustic power of \(2^{15}\) to \(2^{21}\) dB. This resulted in a low mechanical index (0.20), which minimized microbubble disruption. A cannula with 19-gauge was inserted into an antecubital fossa vein and 2.4 mL of SonoVue (Bracco, Milan Italy) was injected as a bolus followed by 10 mL of saline flush for each contrast study. A three-way tap was used so that the saline flush could be given immediately after the microbubble injection. A timer on the sonographic unit was activated at the beginning of the injection. The entire movie sequence (at least 5 minutes) was stored on magnetic optical disks for analysis. The i.v. contrast study could be repeated a second time if necessary.

Contraindications of DCEUS were defined for patients with pregnancy, breastfeeding and severe heart diseases, including instable coronary artery disease, frequent and/or repeated angina or chest pain in the past 7 days, acute cardiac failure, severe arrhythmic disorders, patients with right-to-left shunts, acute endocarditis, prosthetic valves, severe increase in pulmonary artery blood pressure, uncontrolled systemic hypertension, and adult respiratory distress syndrome18.

**Image analysis and Borrmann classification.** The cine loops of DCEUS were reviewed by two radiologists (SL and PH, with 12 and 17 years of experience, respectively) in consensus without knowledge of the definitive diagnosis and other
imaging information at the time of the analysis. Advanced carcinomas of the stomach were classified according to the Borrmann system, into four macroscopic tumor growth patterns types (Figure 2): Borrmann type I, nodular polypoid tumor without ulceration and usually with a broad base (Figure 3); type II, tumor is an ulcerative lesion but elevated and distinct borders(Figure 4); type III, an ulcerating tumor with a penetrating, infiltrating ulcer base (Figure 5); type IV, a diffuse thickening of the gastric wall, without a discretely margined mass or ulceration (Figure 6).

For the inter-observer reliability, the image data of DCEUS were analyzed again by another two ultrasound experts (MP and YZ, with 13 years of experience, respectively) in consensus, and the results were compared with the previous findings (obtained by SL and PH) for calculating the inter-observer reliability. After three month, long enough for observer (SL) to forget his first responses, the analysis was repeated to evaluate the intra-observer reliability of DCEUS in assessing Borrmann classification of AGC. All these reviewers were blinded to the results of surgery and other reviewers’ findings.

Immediately after surgery, the gastrectomy specimens were transferred to the department of pathology. The macroscopic types of the surgical specimens were classified according to Borrmann criteria by the pathologist (SW, with 6 years of experience), who was unaware of the ultrasound findings.

Statistical analysis. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each macroscopic staging. Additionally, concordance of DCEUS within and between observers were assessed using Kappa analysis and a well reliability was set as Kappa value large than 0.75. For all analyses, P values less than 0.05 was considered statistically significant.

Figure 5 | An ulcerative lesion with larger ulcer can be shown using OCEUS (A), and the ulcerating tumor (arrow) with a penetrating, infiltrating ulcer base (large arrow) was demonstrated by DCEUS (B). This case was classified as Borrmann III by DCEUS.

Figure 6 | A diffuse thickening of the gastric wall (between the arrows), without a discretely margined mass or ulceration can be seen on OCEUS (A) and DCEUS (B). This case was classified as Borrmann IV by OCEUS and DCEUS. STO = distended lumen of stomach filled with oral contrast agent.
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**Author contributions**

P.H. designed this study. M.P. and Y.Z. acquired the data. P.H., S.L., S.W. and J.C. interpreted the data. P.H. and S.L. wrote the main manuscript text. All authors reviewed the manuscript.

**Additional information**

**Competing financial interests:** On behalf of all authors of this paper, I declare that this study will not lead to any financial or other kinds of conflicts of interest.

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