

Application of novel targeting nanoparticles contrast agent combined with contrast-enhanced computed tomography during screening for early-phase gastric carcinoma

KAIMIN ZHANG¹, XIJIAN DU², KAIHU YU², KAIYU ZHANG³ and YICHENG ZHOU⁴

¹Physical Examination Center; ²Department of Radiology, Xianning Central Hospital;

³Department of Radiology, The First People's Hospital of Xianning City, Xianning, Hubei 437000; ⁴Department of Radiology, Tongji Medical College, Huazhong University of Science Tongji Hospital, Wuhan, Hubei 430030, P.R. China

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Abstract. Gastric cancer is one of the most common human tumors worldwide. The biggest bottleneck is a lack of advanced and sensitive protocols for the diagnosis of patients with early-stage gastric cancer. Therefore, more sensitive methods of diagnosing gastric cancer are urgently required to improve survival rates. In this clinical study, contrast-enhanced computed tomography (CECT) with targeting nanoparticles contrast agent (CECT-TNCA) was used to diagnose early-stage gastric cancer. The specific-targeted tyrosine kinase inhibitors of gastric cancer, including platelet-derived growth factor receptor- β , Ret and Kit, were used as TNCA. A total of 484 patients with suspected gastric cancer were voluntarily recruited to investigate the efficacy of CECT-TNCA in the diagnosis of patients with early-stage gastric cancer. Patients with suspected gastric cancer were subjected CT and CECT-TNCA to detect whether gastric tumors existed. TNCA was orally administered before CT and CECT-TNCA (20 min). Our diagnostic data revealed that CECT-TNCA improved sensitivity and provided a new protocol to diagnose tumors in patients with suspected gastric cancer at the early stage. In addition, imaging using CECT-TNCA enabled the visualization of tiny nodules in the gastric area. CECT-TNCA diagnosed 182 patients with suspected gastric cancer as tumor-free. CECT-TNCA confirmed gastric cancer in 302 patients. Our novel diagnosis indicated significantly ($P < 0.01$) differential signal enhancement in the gastric nodules via CECT-TNCA compared with CT, suggesting higher accuracy and the accumulation of TNCA in tumor nodules in the

stomach. Furthermore, survival rates of patients detected by early-diagnosis of CECT-TNCA were significantly higher than the mean five-year survival ($P < 0.01$). In conclusion, our investigations demonstrate that the sensibility and accuracy of CT is improved through combination with liposome-encapsulated nanoparticle contrast agent for the diagnosis of early stage gastric cancer when compared with single CT detection. CECT-TNCA improves the accuracy of CT and diagnostic confidence in assessing mural enhancement in patients with suspected gastric cancer.

Introduction

Gastric cancer is one of the most common human epithelial malignancies and remains the second leading cause of cancer-associated mortality for clinical cancer (1). Clinical statistics and investigations have shown that $>70\%$ of new cases of gastric cancer-associated deaths occur in developing countries (2,3). Gastric cancer exhibits higher morbidity and mortality rates than other carcinoma derived from the digestive system as gastric cancer is more invasive (4,5). A previous study has indicated that the five-year survival rate for gastric cancer is $<80\%$ (6).

Currently, the majority of clinical patients with gastric cancer are categorized as having an advanced stage once diagnosed. In addition, although reports have shown that apoptosis-resistance of gastric cancer is inevitable in the development of cancer progression, the apoptotic resistance of gastric cancer cells in patients with gastric cancer has been indicated as the most important hurdle to overcome in clinical treatment (7,8). Furthermore, apoptotic resistance has become the greatest challenge in cancer therapy due to fierce resistance of tumor cells though various types of molecules mechanism (9-11). Although several comprehensive therapies exist that induce apoptosis, which is the most important component of treatment for patients with gastric cancer, the survival rate remains low (12). These previous reports focused on the efficacy of targeted molecular therapies, and the early diagnosis of gastric cancer is often overlooked.

In recent years, contrast-enhanced ultrasound, computed tomography, fl tomography, ont-positron emission, and

Correspondence to: Professor Xijian Du, Department of Radiology, Xianning Central Hospital, 228 Jingui Road, Xianning, Hubei 437000, P.R. China
E-mail: duxijiansci@163.com

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tomography (FDG-PET) has been widely used in the diagnosis of human cancer (13). Although many advantages of contrast-enhanced ultrasound have been presented, its relatively reduced resolution compared with CT means that it cannot confirm the final diagnosis for patients with suspected gastric cancer (14). However, nanoscale microbubbles have been used to improve the resolution of ultrasound, as they resonate when exposed to ultrasound waves (14,15). In addition, computerized tomography and chip technology are the most common methods used to diagnose patients with suspected cancer (16). However, the efficacy of single computerized tomography is limited to diagnosing patients with early phase tumors. Furthermore, contrast agent combined with CT for the analysis of tumor biology has been studied and achieved adequate efficacy (17). Therefore, we hypothesized that specific-targeted nanoscale microbubbles may contribute to the efficacy and resolution of CT in the diagnosis of patients with suspected gastric cancer.

In the present study, CECT combined with targeting nanoscale microbubble contrast agent (CECT-TNCA) was introduced to detect the early stage of patients with suspected gastric cancer. Notably, CECT in conjunction with ultrasound contrast further expanded its application in the field of primary diagnosis and confirmed diagnosis (18). This clinical analysis demonstrated the potential application of CECT-TNCA for imaging modality and sensitivity improvements in the diagnosis of gastric cancer. Our outcomes indicated many advantages of CECT-TNCA in both early diagnosis and final confirmation of suspected cases when compared to single CT detection.

Materials and methods

Ethics statement. The design of this clinical study was carried out in strict accordance with the approval and recommendations in the Guide for the Care and Use of clinical study of Xianning Central Hospital (XNCH: 20091108A4). All surgery and euthanasia protocols were standardized. All patients provided written informed consent.

Patients. A total of 484 patients with suspected gastric cancer aged 14.8-65.2 years were recruited for this prospective analysis, in which the follow-up period was 60 months. The number of male (222) and female (264) patients was approximately equal. Furthermore, 236 healthy subjects (male, 124; female, 112) aged 24.0-62.6 years were recruited. Biochemical parameters of patients with suspected NSCLC and healthy subjects recruited between May 2012 and June 2015 were eligible for further analysis. All patients were subjected to scanning for the detection of early-stage gastric cancer by CECT and CECT-TNCA. All healthy subjects had no cancer history or gastric diseases. Patients with cancer history were excluded in the present study.

Nanoparticles contrast agent. A novel liposome-encapsulated nanoparticles contrast agent containing multiple targets was introduced for diagnosing patients with early-stage gastric cancer. Platelet-derived growth factor receptor- β (PDGFR- β), Ret, and Kit bound with the nanoparticles of superparamagnetic iron oxide particles via covalent bonds described in a

Table I. Characteristics of patients with suspected gastric cancer.

Characteristics	Male	Female
Patients (n)	222	264
Age (years)	14.8-65.2	21.6-62.2
Medical history of cancer (n)	3	5
Blood pressure (mmHg)	110.2 \pm 12.8	113.4 \pm 10.3
Blood glucose (mmol/l)	7.7 \pm 3.6	8.2 \pm 3.2
Diagnosis (n)		
CECT-TNCA	222	264
CECT	222	264

CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

Table II. Confirmation of the dosage of targeting nanoparticles contrast agent required for diagnosis.

Variable	1-10 mg/kg (n=16)	11-20 mg/kg (n=24)	21-30 mg/kg (n=20)
Signal intensity (HU)	76.5 \pm 7.2	92.5 \pm 5.8	93.4 \pm 6.4
Sensitive (%)	64.4 \pm 17.3	86.3 \pm 10.4	83.8 \pm 9.5

previous study (19). Nanoparticles contrast agent and Optison (GE Healthcare, Chicago, IL, USA) were orally administered to ensure that they covered each corner of the stomach prior to CECT and CECT-TNCA (30 min). Following administration with CECT-TNCA, the TNCA was distributed in the stomach. Microbubbles contained targeting nanoparticles contrast agent with the capacity to target travelling tumor cells, which acted as an accurate tracer for tumor cells (20). TNCA was located in the lesion following administration with CECT-TNCA. After 30 min, the TNCA was visualized via CECT. No side effects were observed in patients exposed to TNCA.

Scan protocol. A CECT diagnosis system was used to analyze CECT clinical trials using preprogrammed settings. Preprogrammed settings were optimized to achieve optimal image formation. CECT was performed on the stomachs of all patients according to manufacturers instructions (Philips Medical Systems, Inc., Bothell, WA, USA). Details of the principles and settings of contrast-enhanced ultrasound were described in a previous study (21). In addition, CECT-TNCA imaging was performed in all patients with suspected gastric cancer.

Data analysis. Data from CECT-TNCA image sets was analyzed using the ADMIRE CECT system (version 3.10; Siemens Healthineers, Erlangen, Germany). Volume of tumors was measured by CECT-TNCA imaging. All patients with suspected early stage gastric cancer were analyzed by CECT-TNCA and CT. Gastric tumor nodules were

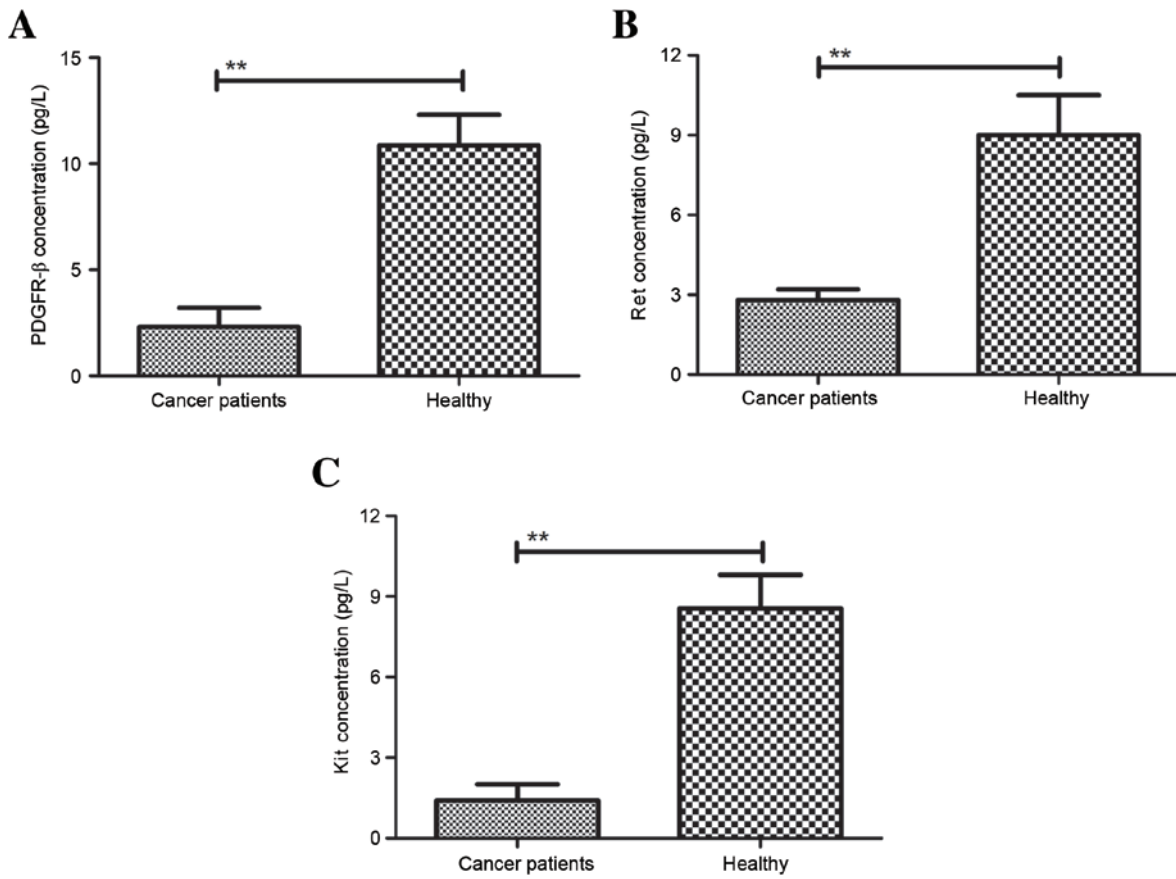


Figure 1. Analysis of plasma concentrations of (A) PDGFR-β, (B) Ret and (C) Kit between patients with gastric cancer and healthy subjects. **P<0.01 vs. control (Student's t-test). PDGFR-β, platelet-derived growth factor receptor-β.

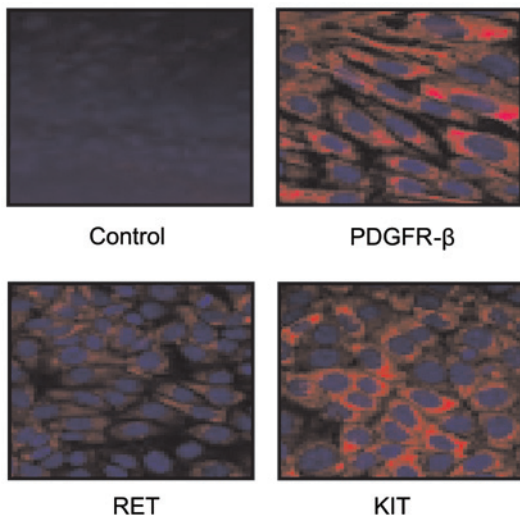


Figure 2. Immunostaining assay of PDGFR-β, Ret and Kit for gastric cancer cells from patients with gastric cancer. PDGFR-β, platelet-derived growth factor receptor-β (magnification, x40).

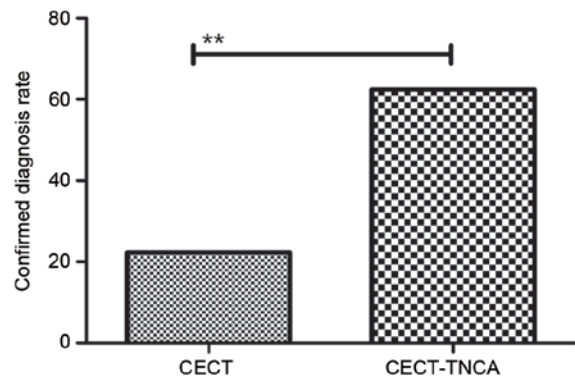


Figure 3. Comparison of the confirmed diagnosis rate determined by CECT and CECT-TNCA. **P<0.01 vs. control (Student's t-test). CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

observed and tumor size was automatically calculated using Sante CT Viewer (version 2.0; Santesoft, Ltd., Athens, Greece).

Treatment of patients with gastric cancer diagnosed by CECT-TNCA. Patients with early stage gastric cancer diagnosed by CECT-TNCA received various different treatments

including radiotherapy (n=39), chemotherapy (n=49), Chinese medicine (n=58), biological therapy (n=35), and comprehensive therapy (n=53). Median overall survival and median progression-free survival were analyzed as previously described (22).

Immunofluorescence and histological staining. Following diagnostic confirmation via CECT-TNCA, tumor cells from patients with gastric cancer were cultured *in vitro* with Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated FBS (Invitrogen; Thermo Fisher

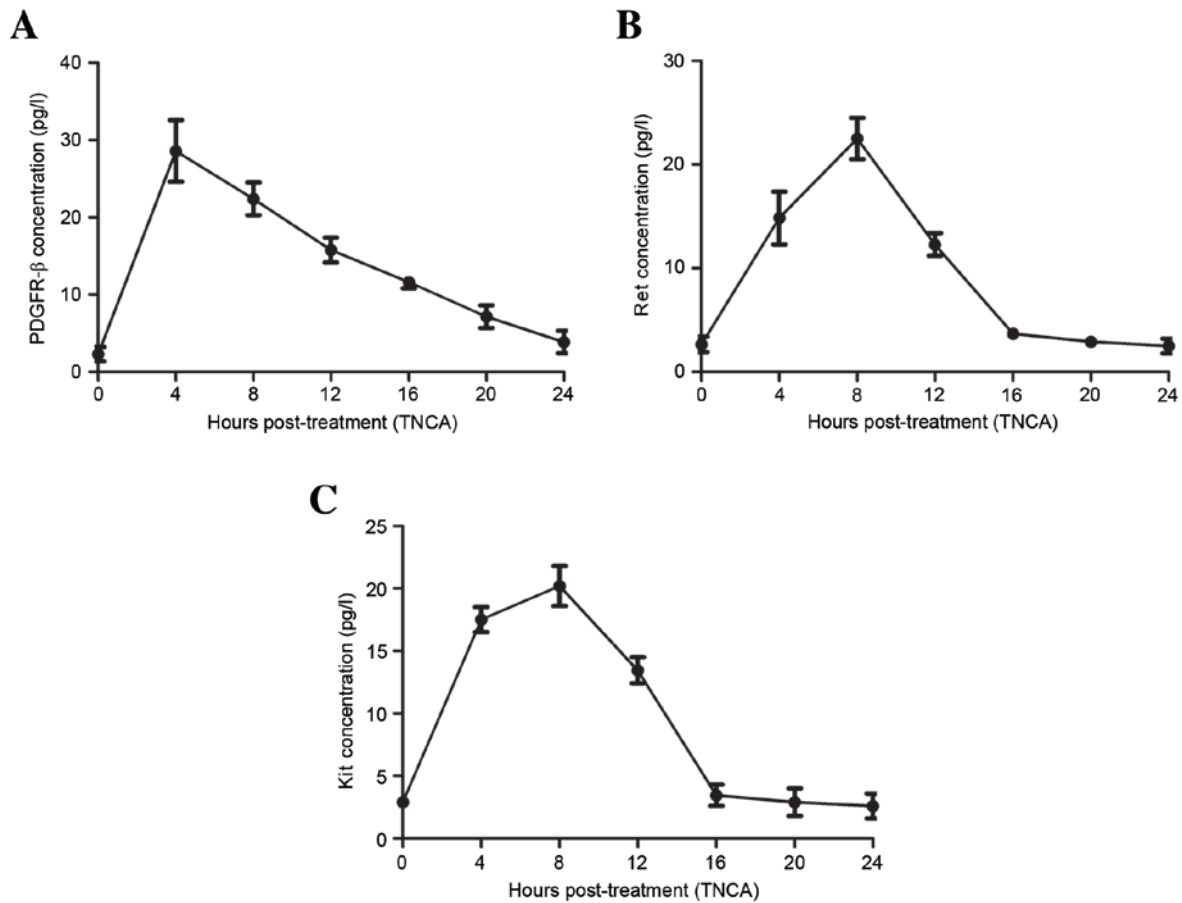


Figure 4. Metabolism of (A) PDGFR- β , (B) Ret and (C) Kit in targeting nanoparticles contrast agent in serum in patients who underwent CECT-TNCA. CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

Scientific, Inc., Waltham, MA, USA). Gastric tumor cells (1×10^6 cells/ml) were incubated with TNCA for 30 min at 37°C . Cells were observed under a fluorescence microscope. Immunofluorescence procedures were previously reported in detail (23). For histological staining, tumor sections were stained with hematoxylin and eosin staining as previously reported (24).

Statistical analysis. All data were presented as the mean and standard deviation of triplicate experiments. Unpaired data was determined by Student's t-test and comparisons of data between multiple groups were analyzed by variance. Kaplan-Meier was used to estimate the survival rate during 60-month long-term follow-up observations. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Plasma concentrations of PDGFR- β , Ret, and Kit in patients with suspected gastric cancer. In order to analyze the target characteristic of TNCA, a total of 484 patients with suspected gastric cancer were voluntarily recruited to investigate the efficacy of CECT-TNCA in the diagnosis of patients with early-stage gastric cancer. Characteristics of patients with suspected gastric cancer are summarized in Table I. The dose of TNCA to achieve the optimum efficiency was identified as 15 mg/kg (Table II). In addition, we investigated the plasma

concentration of PDGFR- β , Ret, and Kit in patients with suspected gastric cancer. As shown in Fig. 1A, PDGFR- β plasma concentration was significantly lower in patients with gastric cancer when compared with healthy subjects. We also found that plasma concentration of Ret was significantly downregulated in patients with gastric cancer when compared with healthy subjects (Fig. 1B). In addition, compared with healthy subjects, plasma concentration of Kit was significantly lower in patients with gastric cancer (Fig. 1C). Furthermore, clinical analysis also indicated that our novel TNCA containing PDGFR- β , Ret, and Kit adhered to gastric cancer cells, which contributed to the signal strength and resolution (Fig. 2).

Efficacy of CECT-TNCA for the early diagnosis of patients with suspected gastric cancer. As shown in Fig. 3, clinical analysis showed that 182 patients (37.6%) were diagnosed as tumor-free and 302 patients (62.4%) were identified as having gastric cancer, as determined by CECT-TNCA. However, the positive rate of patients with gastric cancer was only 22.3% (108 patients) when evaluated via CECT alone. In addition, the investigation found that TNCA increased the plasma concentration of PDGFR- β and metabolized within 24 h (Fig. 4A). Patients who underwent CECT-TNCA exhibited increasing plasma concentrations of Ret that peaked at 12 h and attenuated by 16 h (Fig. 4B). Plasma concentrations of Kit were increased and metabolized within 20 h (Fig. 4C). These clinical data indicated that CECT-TNCA is an efficient

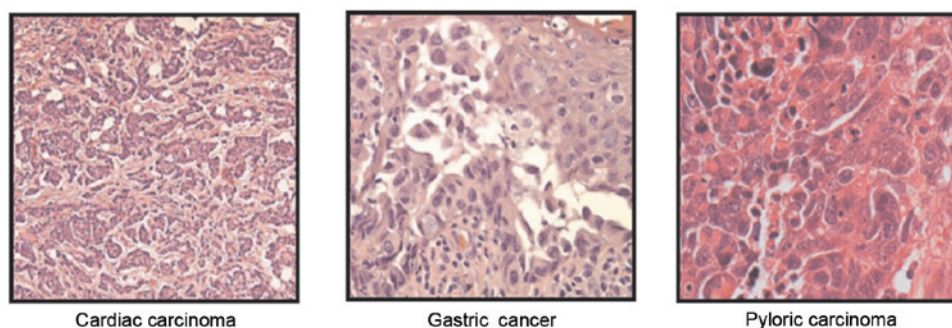


Figure 5. Histological staining analyses the types of gastric cancers in patients diagnosed by CECT-TNCA. CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent (magnification, x40).

diagnostic strategy for the early diagnosis of patients with suspected gastric cancer.

Histopathology analysis of the accuracy of CECT-TNCA-diagnosis patients with gastric cancer. After diagnosing patients with suspected gastric cancer, histopathology analysis was used to further confirm diagnosis. Representative cardiac carcinoma, gastric cancer and pylorus carcinoma incidence rates were studied in the patients who had a confirmed diagnosis of gastric cancer. As shown in Fig. 5, our data showed that histopathology analysis identified cardiac carcinoma, gastric cancer and pyloric carcinoma gastric cancer. The incidence rate of cardiac carcinoma, gastric cancer and pyloric carcinoma was 33.8% (102 cases), 47.0% (142 cases) and 19.2% (58 cases), respectively, in gastric cancer (Fig. 6). These outcomes suggest that the CECT-TNCA method is accurate and sensitive for diagnosing patients with gastric cancer.

Survival rate of patients with gastric cancer diagnosed by CECT-TNCA. Patients with early-phase gastric cancer received different treatments to inhibit tumor cell growth or eradicate gastric cancer. We analyzed the reports of the treatment methods and survival rates of patients with gastric cancer diagnosed by CECT-TNCA. Characteristics of 234 patients with early-phase gastric cancer diagnosed by CECT-TNCA are summarized in Table III. At the 60-month follow-up, we observed that 132 patients (56.4%) were tumor-free and 88 patients (37.6%) had survived and exhibited tumors. The mortality rate was 6.0% (14 patients; Table IV). Median overall survival was 45.8 months (Fig. 7; range, 30.4-63.8 months) and median progression-free survival was 36.8 months (Fig. 8; range, 24.5-52.4 months). These data indicate that patients with early-phase gastric cancer received anti-cancer treatments that prolonged the survival and progression-free survival period, and that that comprehensive therapy had notable therapeutic effects compared with the others treatments.

Discussion

Cancer early diagnosis is the biggest obstacle in human cancer treatment (25,26). In recent years, contrast-enhanced ultrasound, fluorodeoxyglucose-positron emission, tomography (FDG-PET), CECT and chip technology have been widely used in the diagnosis of human cancer (27,28). In particular, CECT and chip technology present more advantages than

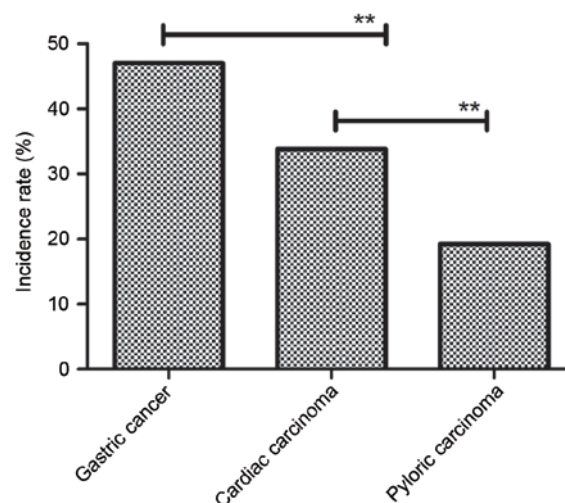


Figure 6. Analysis the incidence rate of the types of gastric cancers in patients diagnosed by CECT-TNCA. One-way analysis of variance revealed a significant effect. ** $P < 0.01$ vs. control. CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

other diagnostic methods (29,30). However, the application of gene chip technology is restricted due to expensive detection and the professional analysts required (31,32). Therefore, CECT has become the most prevalent diagnostic method in the majority of hospitals worldwide (33,34).

Though CECT has been widely applied in the diagnosis of human cancer, the accuracy and sensitivity of CECT is insufficient for the detection of early-stage tumors (35,36). Barium sulfate and iodinated contrast media are frequently used for angiography studies and the diagnosis of tumors in the digestive system (37,38). In addition, many electropositive iron and iron oxide nanoparticles are used as contrast media and have been reported to be useful in the diagnosis of human cancer in previous clinical trials (39,40). Furthermore, retroreflective-type Janus microspheres have also been reported as a novel contrast agent for enhanced optical coherence tomography (41). However, these contrast mediums only improve the partial accuracy of CT in a certain degree. Therefore, elucidating more efficient contrast mediums with targeting characteristics has attracted increasing attention from researchers and clinicians in the field of cancer research and clinical therapy.

In the present study, we introduced a comprehensive approach of CECT combined with target nanoparticles

Table III. Treatment of patients with gastric cancer diagnosed by CECT-TNCA.

Characteristics	Male	Female
Patients (n)	114	120
Age (years)	15.6-56.2	22.4-52.8
Medical history of cancer (n)	2	2
Blood pressure (mm Hg)	108.6±8.5	107.5±9.8
Blood glucose (mmol/l)	8.4±1.5	7.6±2.4
Treatments (n)		
Radiotherapy	18	21
Chemotherapy	25	24
Chinese medicine	32	26
Biological therapy	18	17
Comprehensive therapy	21	32

CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

Table IV. Survival of patients diagnosed by CECT-TNCA after 60-month follow-up.

Treatment	Survived with		
	Tumor-free	tumor(s)	DNS
Radiotherapy	12	22	5
Chemotherapy	25	20	4
Chinese medicine	37	18	3
Biological therapy	19	15	1
Comprehensive therapy	39	13	1

CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent; DNS, did not survive.

contrast agent to improve the accuracy for patients with suspected gastric cancer in the early stage. Target nanoparticles contrast mediums, containing PDGFR- β , Ret, and Kit, were encapsulated by liposome. Our clinical outcomes indicate that liposome-encapsulated TNCA presents a potential tumor-specific approach that may lead to improvements in the diagnostic accuracy of patients with early-stage gastric cancer. Targeted binding of liposome-encapsulated TNCA with gastric tumor cells enhances signal intensity in the lesions of the stomach, resulting in an improvement in the spatial resolution of CECT. Notably, pharmacokinetic tracer kinetics analysis demonstrated that the target nanoparticles contrast mediums of PDGFR- β , Ret, and Kit were metabolized within 24 h. No side effects were noted during the diagnostic period. Long-term follow-up reports showed that patients diagnosed by CECT-TNCA at the early stage present higher median overall survival (30.4-63.8 months) and median progression-free survival (24.5-52.4 months).

Tyrosine kinase inhibitors of PDGFR- β , Ret, and Kit-mediated angiogenesis have been identified as key factors in the development of human cancer (42,43). PDGFR- β , Ret,

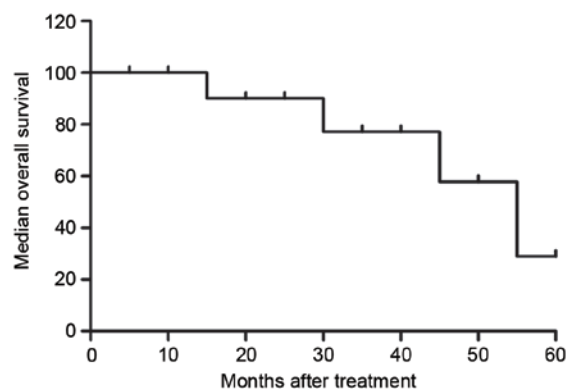


Figure 7. Evaluation the median overall survival of patients diagnosed by CECT-TNCA in early stage. Kaplan-Meier was used to estimate the survival rate during 60-months long-term observation. CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

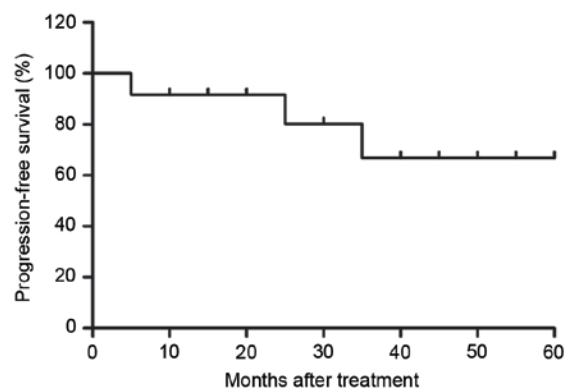


Figure 8. Analysis of the median progression-free survival of patients diagnosed by CECT-TNCA in early stage. Kaplan-Meier was used to estimate the survival rate during 60-months long-term observation. CECT-TNCA, contrast-enhanced computed tomography-targeting nanoparticles contrast agent.

and Kit have potent anti-tumor activity against a number of human tumors through binding with targets (44). Previous reports have suggested that target receptors of PDGFR- β , Ret, and Kit in gastric tumors are effective treatments (45-47). Although previous contrast media have not previously been compared to determine which media is optimal for the visualization and diagnosis of gastric cancer (48). Indeed, CECT using a different contrast agent enables non-destructive diagnosis of the biochemical and biomechanical properties of patients with various diseases (49). In addition, a previous study has evaluated dynamic CECT imaging in the differentiation of benign and malignant tumors observed by tumor vessel and permeability nodule perfusion (50). However, conventional contrast agents present lower efficacy for tumor analysis due to rapid diffusion outside the lungs, which prevents optimal imaging (51). Furthermore, previous reports have shown that iodinated contrast agents are less sensitive to changes in cells morphology (52,53). Our design showed that liposome-encapsulated target nanoparticles contrast mediums are potential nanoparticles contrast agents that may improve the accuracy of diagnosing early-stage gastric tumors. Targeted binding of target nanoparticles contrast mediums with gastric tumor cells

enhanced signal intensity in lesions in stomachs diagnosed by CECT-TNCA.

In conclusion, to the best of our knowledge, this is the first report of liposome-encapsulated targeted nanoparticles contrast mediums combined with CECT for the diagnosis of patients with suspected early stage gastric cancer. CECT-TNCA was administered orally to augment the signal intensity in the stomach, leading to a reliable and sensitive assessment of the tumor for clinical diagnosis in patients with gastric cancer.

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