Clinical Application of Gastric Mucosal Marking Targeting Biopsy

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Additional information is available at the end of the chapter

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1. Introduction

Clinical research of gastrointestinal precancerous lesions is a key point in primary prevention of gastrointestinal cancer. It’s a long time from superficial gastritis to atrophic gastritis and to the gastric carcinoma finally. So how to effectively carry on the long-term follow-up of the gastric precancerous lesions becomes a perplexing medical problem [8; 2; 3; 7]. The most accurate ways to diagnose the gastric precancerous lesions and cancer are endoscopy and endoscopic biopsy. In order to identify lesions and perform accurate biopsies, endoscopic technique keeps on improving, and dye endoscope and high definition amplification endoscope have been widely used in diagnosis and instructing biopsy. Pre- and post-comparison of original lesion is needed in the monitoring and follow-up of pre-cancerous lesions. Usually the pathological changes of chronic atrophic gastritis is inhomogeneous, the severity of atrophy in different part, the severity and the type of intestinal metaplasia differ greatly, the atypical hyperplasia is more likely to be focal. Lesion that was found in the first biopsy will be difficult to be demarcated in the next endoscopy. Pathological diagnose in different procedure will be lack of comparability since the biopsy spots are inconsistent. As a result, cancerous monitoring is impossible. Moreover, the interval of follow-up time is long, even the same endoscopic doctors can’t do the biopsy at the same point at the next endoscopy. Therefore, an accurate targeting biopsy technique is urgently needed, which could be used for the monitor and follow-up of gastric precancerous diseases and the judgment of the therapeutic effect of the gastric precancerous lesions. According to the medical requirements of the monitoring of gastric precancerous lesions and the requirements of tattooing biopsy forceps and tattooing dye, we designed and produced three kinds of mucosal tattooing biopsy forceps in this research. It took five and a half years to screen and test dilution and dosage of tattooing dye from the animal research to clinical trials. In
China, gastric cancer was the 2nd most common cancer and the incidence of gastric cancer related death was 870,000 per year, ranked as the first of all malignant tumors [16]. The retrospective analysis of malignant tumor from 1990-2002 in zhejiang province showed that gastric cancer is the 3nd most common cause of cancer related death in city and the first cause in rural area [20]. Precancerous lesions are the main risk factors of gastric cancer, and atrophic gastritis with intestinal metaplasia with or without dysplasia is the most important precancerous lesion. The chance of cancerous rate of atrophic gastritis was 8.61-13.8% to 1.27-7.1% according to different area [21]. It is believed that it takes a long time from superficial gastritis to atrophic gastritis and to the gastric carcinoma finally. At the same time, the clinical manifestation of early gastric cancer lacks specificity, so the diagnose rate of early gastric cancer is quite low, it is about 20-30% among the all gastric cancer. Although there are lots of methods to treat gastric cancer available now, including operation, chemotherapy, radiation and other new therapy, the survival rates of 5-year of advanced gastric cancer was only 20-30%. However, the 5-year survival rates of early gastric cancer was up to 90%. So early diagnose and early treatment is the key to cure gastric cancer. There are plenty of methods to follow up gastric precancerous lesions including periodic screening with gastroscopy together with biopsy, Pepsinogen I/II, Gastrin 17, tumor marker and so on. Among them, the most effective way is still gastroscopy with biopsy. Taking gastroscopy as the base, doctors can further looking for the biochemistry or molecular markers of early gastric cancer, demonstrating the effect of medicine in prevention gastric cancer or reverse of atrophic gastritis and furthermore to treat the early cancer by gastroscopy, for example endoscopic mucosa resection or endoscopic submucosa dissection. In conclusion, accurate biopsy with periodic pathological screening is most important to detect precancerous lesions prognosis. The essentiality of MTB: Nowadays patients expect medical services of high quality. In east Asia or other high incidence of gastric cancer, Atrophic gastritis patients often come to hospital and ask for monitoring gastric precancerous lesions. How to effectively monitor gastric precancerous lesions is also a tough problem of medicine and a key point of cancer prevention. Although there are lots of methods by which we can detect early gastric cancer, endoscopic examination with pathological diagnose is believed to be the principle way to monitoring gastric precancerouslesions. Precisely follow-up of precancerous lesions is also the only way leading to the understanding the prognosis of the target lesions. MTB can be used in many cases. Physicians use MTB to get target samples to monitor the precancerous lesions. How to evaluate medicines for preventing precancerous lesions, for example atrophic gastritis? Random biopsy samples sometimes are difficult to be compared in the multifocal atrophic gastritis. Only multiple marking targeting biopsy can offer the right tissue to be compared with after the long time treatment. Furthermore, in case of dysplasia lesions of gastric mucosa detected only in microscopy on first gastroscopy without MTB, the next gastroscopy sometimes couldn’t find the lesion even by the same endoscopist. In order to ensure the quality of pathological exam in monitoring precancerous lesions, MTB should be done in every biopsy.
2. Research and development of MTB

2.1. Development of Marking Targeting Biopsy Forceps

2.1.1. Design and In Vitro Test

Tattooing biopsy forceps was designed according to the medical requirements. It includes three basic parts like head, body and hand shank. From the first generation of tattooing biopsy forceps to the third generation, we have made dozens of trials. The final generation forceps include: a guiding and tractive wire and an paralleled injection pipe, which can improve the patency of injection pipe and the flexibility of the open / close operation of biopsies bowl to the maximum. Injection fitting is fixed in the distal hand shank and linked to the injection needle by the injection pipe. Needle doesn’t expose when the biopsy bowl of forceps clip is closed, but exposes when the bowel is opened. The relative motion of hand shank and handlebar, by towing wire, make biopsy forceps mouth open and close, so that a biopsy can be done.

2.1.1.1. Design and In Vitro Experiment of the 1st Generation Tattooing Biopsy Forceps to the 3rd Generation Tattooing Biopsy Forceps

Design: There was a hollow hand shank in the 1st generation mucosal tattooing biopsy forceps. The proximal end of hand shank was equipped with handlebar. The hand shank was linked with two steel wires and connected with biopsy forceps jointing partly through the hose. On the lateral side of the hand shank there was a slippery groove. The gun-bolt like and pushable flared fitting interlinked with a hollow pushable needle bar in the slippery groove. With an injection pipe inside, the hollow pushable needle bar was fixed in the handlebar. The distal end of injection pipe connected with the injection needle which was in the middle of the two-bowel like biopsy forceps through the forceps head. Connected to the handlebar, the injection fitting moved with the mobility of handlebar. Therefore, Injection needle moved forward when the biopsy forceps opened and backward when the biopsy forceps closed. What’s more, the forceps body was surrounded with a metal hose; the outer diameter is 2.6 mm. Within the biopsy forceps, there were two guidance wires and an injection pipe. The guidance wires were made of medical stainless steel materials. Their diameter was 0.28 mm. The outer diameter of plastic pipe was 0.75 mm, and inner diameter was 0.25 mm. The distal end of biopsy forceps was like a bowl with a gap. Result of experiment: After many in vitro and simulation lumen injection experiments, we found the friction between the 2 guidance wires and the injection pipe was excessive when they moved in the narrow hose. As a result, the biopsy forceps became inflexible when they were opened and closed. Meanwhile, the fitting of injection pipe would easily fall off, if the resistance of infusion was too large. Design: In order to improve the open/close inflexibility, resistance of the injection pipe and capricious shedding of fitting of the 1st generation biopsy forceps, we assumed
that we could change the former two traction wires into one in the new type. So we put the injection fitting and the slippery groove from handlebar to the distal cone part of hand shank, the place where the flared injection fitting connected to the hose. A slippery groove was equipped in the joint of distal cone part of hand shank and hose. The other side of the hose connected with a fractal bowl-like biopsy forceps, in the middle of which there was an injection needle. The two ends of plastic injection pipe connected with a horn injection mouth and injection needle respectively. A handlebar was set in the other side of the hand shank which was linked with a steel wire. Then the wire passed through the distal cone part of hand shank and was connected to biopsy forceps head by the hose. In vitro experiment: This improved biopsy forceps solved the problems of resistance force of injection and easily falling off of fitting. But in the vertical connection between the hose joint and the flared injection fitting that went through slippery groove, the fitting was still needed to be pushed front and back so that the injection needle could be push forward when the biopsy pliers opened. However, the operating flexibility of biopsy forceps still could not meet medical requirements. Design: Based on the design of 2nd generation tattooing biopsy forceps, we made some improvements by fixing the injection fitting on the distal cone part of the hand shank. And there would be no slippery groove and push bar needle. Injection needle was fixed on forceps head. These two parts were linked by a plastic tube. And the injection needle would not advance and retreat with the opening and close of biopsy forceps. This design ensured the patency of the injection pipe. The relative motion of the hand shank and handlebar made the biopsy forceps bowl open and close, and exposed the injection needle as well. The steel wire that connected the hand shank with biopsy forceps joint still existed.

In vitro experiment: The in vitro and simulation lumen test showed that the biopsy forceps bowl turned out to be opened and closed flexibly, the infusion of injection needle was free, and the movement was easy.

Figure 1. Photos of 3rd generation marking targeting biopsy forcep.
2.1.2. Establishment of Technical Standard

The establishment of targeting biopsy forceps was developed according to general technical specifications YY91076-1999 (now YY/T 1076-2004) of endoscopic hose-type biopsy forceps of People’s Republic of China, professional standards of disposable sterile needle GB15811-2001 (ISO 7864:1993) and soft PVC plastic (1995) used for transfusing (blood) liquid instruments GB15593, also combined with our design requirements. Technical performances: The main materials of marking targeting biopsy forceps are conformed to the regulations in table 1.

<table>
<thead>
<tr>
<th>Components</th>
<th>Materials Brand</th>
<th>Product Standard Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>forceps clip, injection needle</td>
<td>3Cr13Mo</td>
<td>GB1220-84</td>
</tr>
<tr>
<td>forceps core, supporting frame, hose, core bushings, joints, bushings, inner steel cable</td>
<td>1Cr18Ni9</td>
<td>« Stainless steel »</td>
</tr>
<tr>
<td>Clamp handle, clamping ring, sleeve, ring</td>
<td>ABS</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Material Standards of Marking Targeting Biopsy Forceps.

Figure 2. The construction of 3rd Generation Tattooing Biopsy Forcep.
2.1.3. Engineering Test

According to the design requirements and technical standards, we co-operated with the engineering technicians in Hangzhou advanced medical equipment Co.LTD, and developed some samples for the following experiments.

2.1.3.1. Test of Open and Shut Flexibility of Forceps Clip and Rigidity of Hose

Open and shut flexibility of forceps clip: In the special gastroscopic forceps tester, the clip of sampling forceps inserted along the simulation clamp crossings of the tester and stretched out and above turntable about 80mm, then rotated the turntable counterclockwise to 180° with a constant speed, and then counterclockwise rotated forceps head to press the hand shank by 30° (the second degree). Forceps clip should open and shut flexibly, with no stuck and obstruction phenomenon. Rigidity inspection of hose: After rotating the turntable to 180°, the sampling forceps clip inserted along the simulation clamp crossings of the tester. This process should be smooth, without any resistance. Then rotate forceps clip to press hand shank counterclockwise by 60° (the third degree). The hose should move with no obvious resistance or stretching.

2.1.3.2. Test of Anastomose Sharpness of Forceps Clip

We tested with the specialized gastric forceps tensile machine, which, in radial direction, bite a layer of NO.2 cap paper QB29-73 (regulated in the CAP PAPER and was about 30g) in the two-thirds place of forceps clip. Then we pulled the paper with clip until it was torn up. Paper bited by the forceps clip should not be slipping. The tension of sampling forceps with needles should be not more than 35N. The hose around marking targeting biopsy forceps should be closed, flat, with no apparent gaps and flex, and its appearance should be smooth, with even color but with no sharp edges and burrs.

2.1.3.3. Length of Injection Needle and Test of Tube Patency

When the clip of sampling forceps with needle opened to 90°, the two forceps clips touched the paper and then the positioning needle should be able to impale the paper. The whole device should meet the requirements that the marking targeting biopsy forceps could get in and out of the gastroscopic forceps tester freely and it wouldn’t cause obvious heaviness, and the hose wouldn’t be stretched, forceps would open and close flexibly, injection pipe would be unobstructed and the injecta flew out smoothly into a straight line.

2.1.3.4. Hardness Test

It must meet the standards of Metal Microscopic Vickers Hardness Testing Method GB4342-84.
2.2. Development of Tattooing Dye

2.2.1. Choosing the Ingredient of Tattooing Dye

According to the medical requirements, tattooing dye must be safe and non-toxic and can display for a long term, also can exceed the reviewer’s expectation of precancerous lesions (over a year). However, the presence of tattooing dye should not affect pathological analysis of tissues. According to some literatures, tattooing agents that can be used for endoscopic dying include India ink, Lugol’s ino, indigo green, methylene blue, toluidine blue, indigo, Congo red and phenol red [6]. However, most of them belong to the functional dying, contrast dying or reactive dying, all of which are just transient dying. So they do not apply to the long-term endoscopic follow-up. Only the India ink and ino indigo green may probably be used for long-term marking of mucosa and skin. Hammond DC had made a research of injecting eight different kinds of dyes to colon mucosa [5]. The result showed that only the marking with indigo green and India ink could exist clearly over 24 hours. What’s more, in their experiment, they found that indigo green could last for seven days after the injection, with no apparent inflammation and methylene blue disappeared completely in about 2 days. If we use Indigo green for preoperative marking, the marking spot can be clearly visible in 36 hours. As monitoring of pre-cancerous lesions takes months or even years to follow-up, so the short-acting tattooing dye such as indigo green etc apparently couldn’t meet the requirements. India ink as one of the most extensively used medical dyes, whose main component is carbon particles suspensoid, It has been widely used for skin tattooing and was without apparent side effects. Ponsky and King used to use India ink to mark colon lesions under endoscope, so that the lesions could be found easily during the surgery [10]. Several literatures have reported that it was safe and effective to mark the esophagus, colonic mucosa with the India ink [12; 8]. Although there are still no reports about the long-term observation of India ink that was used in gastric mucosa marking, it is relatively appropriate to choose India ink as gastric mucosa tattooing dye, considering its long-term effectiveness in skin tattooing, as well as esophageal and colon tattooing.

2.2.2. Formulating Quality Standards and Pharmaceutical Technique

2.2.2.1. The Sources of the Raw Materials and Quality Standard

The original solution of India ink (Pelikan, Hanover, Germany) was imported originally from German. Its quality conformed to European standards for drugs. Main elements were carbon, ethylene glycol, sodium tetraborate decahydrate and ammonia which was dissolved in 1:10 diluted sterile water for injection as solvent.

2.2.2.2. Configuration of the Tattooing Dye and the Formulation of Quality Standard

Configuration: The tattooing dye is configured according to the principles of the minimum concentration, the smallest dose, the longest time and the clearest image. The tattooing dye used was medical India ink. After concussed and shaked evenly, the original solution of India ink was diluted to concentrations of 1:10, 1:100, 1:1000 with sterile water for injection un-
der sterile environment, and then these liquor were loaded in small vitreous bottles. At last, the tattooing dye would be sterilized in steam with circulation (100 °C, 30 minutes) [11]. Quality standard: The appearance of tattooing dye should look like black suspensoid fluid, and be uniform, with no lump or precipitation. Moreover, pH should be neutral. Then use pH measuring device (825MP, Fisher Scientific, Pittsburgh, PA) to measure the pH value of India ink of various dilution degrees. Carbon content is measured with element analysis method (elemental analysis instrument: ThermoFinnigan, type: Flash EA1112). Pharmacal India ink –Weighed-Dried for 48h at 80 °C-Cooled in the dryer –Weighed-Element analysis. Take 1:10 diluted India ink 3ml as an example, results of analysis are as follows: Sample Wet Weight: 4.1515 g, Sample Dry Weight: 0.0555 g, Elemental Analysis Result: Carbon: 44.55%, Hydrogen: 3.62%, Nitrogen: 4.17%. Carbon Content Analysis: C % = (0.0555 x 0.4455/4.1515) by 100 = 0.60%.

### Dilution Degrees

<table>
<thead>
<tr>
<th>Carbon Content</th>
<th>pH Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>The original solution of India ink</td>
<td>6</td>
</tr>
<tr>
<td>1:10</td>
<td>0.6%</td>
</tr>
<tr>
<td>1:100</td>
<td>0.06%</td>
</tr>
<tr>
<td>1:1000</td>
<td>0.006%</td>
</tr>
</tbody>
</table>

### 3. Animal experiments

Aim: In order to inspect whether the properties of tattooing biopsy forceps and tattooing dye can meet medical requirements. The animal research included the function of tattooing biopsy forceps and the Character of tattooing dye. The weight, size, depth of the specimens obtained by the tattooing biopsy forceps, and whether they have met the requirements of pathological examination were explored. And also the Marking clarity and local histological response made by India ink of different concentrations, and different doses were studied.

Methods: All animal experiments were performed in animal laboratory, which has been accredited as national 3rd-level lab for animal experiments in Sir Run Run Shaw Hospital. We did MTB on New Zealand Rabbits, dogs and Pigs. The tattooing dye was India Ink in different dilution (from 1:10 to 1:1000) and different dosage (from 0.3ml to 0.7ml). The endoscopy were followed after first marking 1d, 3d, 1week, 1 months, 6 months, 12months, 15 months continously.
Results: 1d, 3d, 1 week, 1 month, 3 months, 6 months, 12 months, 15 months after the first marking, we made repeated endoscopy. Also we did biopsy on the third day, the end of the first weekend and the end of the first month. The results were showed as following. Fifteen months after endoscopic marking, we found that the clarity and the inflammatory reactions degree with India ink (concentration: 1:10, and dose: 0.5 ml) were ideal and without any necrotizing inflammation, peri-vascular inflammation, granulomatous inflammation and other severe inflammation. So clarity and inflammatory reaction degree were related to dosage and concentration. For the same concentration, the more the dosage was, the greater the inflammatory reaction would be. However, there’s only one exception. Compared with the injection liquid of 1:100 concentration and 0.5 ml dosage, inflammation reaction of the liquid of same concentration but of 0.3 ml dosage is heavier.

Figure 3. After marking 1X10 at low magnification, HE stain.
Conclusion: The animal experiments showed that marking targeting biopsy forceps had good functions and could be performed successfully and the use of marking targeting biopsy forceps was time-saving. India ink with dilution of 1:10 and dosage of 0.5 ml is the best tattooing dye, since its marker was clear, without obvious changes of local tissues.

4. Application and Obtaining of the National Patent


Figure 4. MTB forceps sample.
5. Clinical Trials

5.1. Objective

Perform marking targeting biopsy for gastric precancerous diseases; to validate clinical application value of marking targeting biopsy forceps.

5.2. Materials and Methods

5.2.1. Declaration and Authorization

In order to carry on the marking and follow-up of the gastric precancerous lesions, we got permission from Medical Hygiene Administrative Department of Zhejiang Province, China. We were also sponsored by medicine and health research funding of Zhejiang Province.
5.2.2. Inclusion Criteria

Explain the operation process and significance of marking targeting biopsy to patients who had been diagnosed with chronic atrophic gastritis in previous gastroscopy. Then they would be enrolled once they agreed to sign.

5.2.3. Exclusion Criteria

Patients who had upper gastrointestinal acute inflammation, ulcer or hemorrhagic lesions or hadn’t signed informed consent form or had the contraindications for gastroscopy.

5.2.4. Experimental Process

Preparations before gastroscopy: For patients that had met inclusion criteria or had indications of routine gastroscopy, the preparations would be made according to the ordinary gastroscopy [19]. Preparations of tattooing dye: It was made by the Pharmaceutical Department of Sir Run Run Shaw Hospital and was diluted to 1:10. Manufacturing and sterilizing methods are the same as animal experiment. Marking with marking targeting biopsy forceps: Before operation, we used moderate sterile saline to wash lumen and then flushed with 1ml tattooing dye to make sure that the infusion system was not obstructed. When doing gastroscopy, we should observe all the mucosa of esophagus, stomach and duodenal bulb first and eliminate active inflammation, ulcer, or hemorrhagic lesions. Next, we would choose five points to be the marking spots, according to Sydney System. The five points should be localized at lesser, greater curvatures of antrum, the lesser, greater curvatures of gastric corpus and gastric angle. Additional samples would be obtained if there were suspected lesions. We injected 0.3-0.5 ml tattooing dye to each spot and then did biopsy. All the samples were sent to pathological examination.
5.2.5. Follow up the patients

All the patients who underwent MTB were asked to come back to GI clinic on the day of 1 week for follow-up, and repeated the gastroscopy on the day of 13 weeks, 26 weeks, 39 weeks, 52 weeks, 78 weeks, 104 weeks, 130 weeks and even more. There are some items which should be recorded on endoscopy, including (1) The duration and clarity of marking, (2) The comparison of
pathological diagnosis for the marking point. (3) Any pathological change due to tattooing marking on mucosa. (4) Side effect of patients after the MTB procedures.

5.3. Results

Up to 2008, there were 172 cases of gastric precancerous lesions patients enrolled in this study, 88 male, 84 female, 27~78ylo (51.98±10.03ylo). Among them, 129 cases got atrophic gastritis 75.00%; 149 cases got intestinal metaplasia (86.63%); 33 cases got 19.19% intraepithelial neoplasia, including 3 patients who underwent partial gastroectomy due to high grade intraepithelial neoplasia after the first gastroscopy.

<table>
<thead>
<tr>
<th>Degree</th>
<th>Pathological diagnosis</th>
<th>atrophy</th>
<th>Intestinal metaplasia</th>
<th>intraepithelial neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td></td>
<td>31</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>moderate</td>
<td></td>
<td>36</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>severe</td>
<td></td>
<td>62</td>
<td>92</td>
<td>5</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>129</td>
<td>49</td>
<td>33</td>
</tr>
</tbody>
</table>

Figure 8. Pathological diagnose of 172 cases.

Marking clarity: Among the 172 cases, 84 cases had repeated at least one gastroscopy after first MTB, the duration were 5 to 126 weeks. Only 8 cases marking points faded during the follow up period. 76 cases got clear marking points more than 52 weeks. The total effective rate of marking was 90.48%. At 13 week, 26 week, 39 week, 52 week, 78 week, 104 week and 130 week, the clarity of marking points was 98.81% 96.43% 92.86% 90.48% 90.48% 90.48% 90.48% respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Follow-up time (weeks)</th>
<th>Number of disappeared marking</th>
<th>Probability of disappeared initial</th>
<th>Probability of exiting marking</th>
<th>Ratio of exiting marking</th>
<th>Standard error of ratio of exiting marking</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>1</td>
<td>0.19%</td>
<td>98.81%</td>
<td>98.81%</td>
<td>1.18%</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>2</td>
<td>2.41%</td>
<td>97.59%</td>
<td>96.43%</td>
<td>2.02%</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>3</td>
<td>3.70%</td>
<td>96.30%</td>
<td>92.86%</td>
<td>2.81%</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>2</td>
<td>2.56%</td>
<td>97.44%</td>
<td>90.48%</td>
<td>3.20%</td>
</tr>
<tr>
<td>5</td>
<td>78</td>
<td>0</td>
<td>0.00%</td>
<td>100.00%</td>
<td>90.48%</td>
<td>3.20%</td>
</tr>
<tr>
<td>6</td>
<td>104</td>
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<td>90.48%</td>
<td>3.20%</td>
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<tr>
<td>7</td>
<td>130</td>
<td>0</td>
<td>0.00%</td>
<td>100.00%</td>
<td>90.48%</td>
<td>3.20%</td>
</tr>
</tbody>
</table>

Table 2. Marking points clarity during follow-up
Most of the marking points were persistently remained after 52 weeks, almost for one year, that is mean the repeated gastroscopy can follow the last gastroscopy for next biopsy after
one year duration. From the figure 12, we could see most of the marking points maintained during 52~130 weeks.

![Figure 12. Curve of marking points remained during follow-up.](image)

5.4. Discussion

From this study, it is showed that MTB technique had lots of advantages compared to common biopsy as following: MTB is accurate, standard and comparable, however, the common biopsy is blindness, spontaneity and incomparable. So the MTB technique can not only fulfill the demands of patients for comparison of lesions before and after the treatment, but also offer accurate pathological diagnosis to physicians. Furthermore with MTB technique, the risk of medical service could be reduced by the comparable evidence of endoscopic biopsy. As the important stage of the gastric precancerous lesions, chronic atrophic gastritis has always been the focus of the primary prevention of gastric cancer. Endoscopy with biopsy is considered to be the most accurate index in diagnosing gastric precancerous lesions and cancer. During the clinical monitoring and follow-up of pre-cancerous lesions, we need to compare the current lesions with previous lesions. Especially in the chronic atrophic gastritis, multi-focal atrophy is quite common. The atrophy and the intestinal metaplasia degree in different spots vary greatly. Atypical hyperplasia tends to be focal. The lesions we see in the first biopsy will be difficult to be demo demonstrated in the next review. So pathological diagnoses at different endoscopy will be lack of comparability since the biopsy spots are inconsistent. As a result, cancerous monitoring becomes very difficult. Moreover, the interval of follow-up time is long. Therefore even the same endoscopist can’t do biopsy at the same position in different time. Therefore, in clinical practices, an accurate targeting endoscopic biopsy technique is urgently needed, which could be used for the monitoring and follow-up of gastric precancerous diseases and the observation of the therapeutic effect of the gastric precancerous lesions. After the explorations of animal experiments, we declared and finally were approved to carry on the clinical marking and follow-up for the gastric precancerous...
lesions. First, all patients should sign the informed consents before included in the clinical trials. Through clinical trials, we confirmed that the specimens obtained by the marking targeting biopsy forceps which were developed by ourselves are similar to those obtained by ordinary biopsy forceps in satisfying histological examination. We chose 0.3-0.5 ml India ink diluted to 1:10 as tattooing dye. Marking were clear without apparent side effect. After 3-15 months follow-up, marking spots were still clearly visible. In brief, the marking targeting biopsy forceps are safe, reliable and effective. The history of using India ink for marking and targeting has been for decades. Besides the tattooing on the skin, colon is another organ that frequently being marked. Long-term marking is mainly used on colonic mucosa or esophageal mucosa, but we haven’t seen any reports about the long-term use of Indian ink on the human gastric mucosa. Dilution and dose of India ink that used on colon and esophagus and residue time of tattooing dye are similar to our results. Most of them did not induce any inflammation either. Fennerty MB marked 32 colorectal polyps’ excision parts of 26 patients with 0.2-0.5 ml India ink [4]. Colonoscopy was performed every 6 months after the marking and targeting. The average time of follow-up was 14 months. Up to six months, the marking spots were still clear, without inflammation. The patient didn’t complain any discomfort. McArthur CS marked the colon mucosa under colonoscopy for 195 patients with India ink. 50 cases were marked preoperatively so that lesions could be easily found during the operation. 145 cases are marked for future endoscopic follow-up [8]. Abdominal pain, fever, and other side effects did not appear in these 195 patients. Shatz BA did colonoscopic biopsy on seventy-four points with India ink in 55 patients [13]. The duration of follow-up ranged from 1.5 months to 117 months (average of 36 months). No infection, abdominal pain, fever or other performance appeared in these 55 patients. Majorities of them had no pathologic changes. Six patients had mild chronic inflammation. Proliferative changes appeared in one patient. But none of them had the neoplastic changes in the marking spots of India ink. Shaffer made marking on 19 patients with Barrett esophagitis [12]. Each spot was injected with India ink about 0.1 ml while the four circumferential injection spots were chosen in starting position of Barrett esophagus. Endoscopy was repeated every 3, 6, 9, 12 months postoperatively, that is the first, third, ninth, fifth, twenty-fourth and thirty-sixth month after first marking. Visibility of India ink is good or very good in 94.7% patients when gastroscopies were repeated. There’s only one patient (5.3 percent) getting poor visibility in the marking spot. When marking on patients, we chose 0.3 to 0.5 ml Indian ink with a dilution of 1:10 for this experiment. The reasons for not choosing the dose of 0.5 ml were that basic diseases are precancerous lesions and safety is our first choice when we made marking on gastric mucosa in patients with chronic atrophic gastritis. Although we got a conclusion from animal experiments that 1:10 diluted Indian ink of 0.5 ml might have the clearest view, but in the same concentration, the lower doses might have minor inflammation [14]. So we used lower doses for injection in our human beings [16]. Of course, the observation of the gastric precancerous lesions shall be a long-term effort. The evolution of related disease itself is unpredictable. And the long-term influences of marking targeting biopsy to the gastric precancerous lesions also calling for further studies, prospective research with large samples are expected. Gastric mucosal surface is acidic in pH value, which is totally different from neutral environment of colonic mucosa. Normally, there isn’t any bacteria in gas-
tric lumen, which greatly reduces the risk of infection caused by puncture. So it is relatively safe to mark in the stomach. Besides, there are three layers in the muscularis of gastric wall which are thicker than colon. So compared with the bowl wall, the gastric wall is less likely to be penetrated. Therefore, it is favorable for marking safely in the stomach. When performing a marking injection, needle should be inserted to the submucosa layer in tangent direction. In this way, we can get the best effect. If the needle is inserted superficially to the mucosal surface, the marker will fall off, which will affect the observation. However, if the needle is inserted deeply to the muscular layer, it will also be hard to observe and may puncture the gastric wall.

6. Next aim following with MTB

In order to mark suspicious areas for follow-ups without hurting stomach, and cut down time spent on gastroscopy, we propose a computer simulated marking targeting biopsy (CSMTB) system. By constructing a three-dimensional (3D) virtual stomach model and using a tracking device, the position of gastroscope is shown in multi-view: in triplanar views, in global stomach model views, and in virtual gastroscopy view. In this way, physicians are guided to overcome disorientation so that the time spent on gastroscopy is cut down. By using a tracking device, the positions of suspicious areas are marked virtually in the 3D virtual stomach model. In this way, it will not hurt patients’ stomach [15; 17]. We have the national invention patent for the CSMTB proposal.
7. Highlight

7.1. Innovativeness of MTB

MTB is a creative technique, biopsy and marking can be done together with the MTB forceps. MTB forceps meet the demand of biopsy with marking together; marking lesions that made it easier to be found during follow-up; accurate biopsy; minimally invasive treatment of precancerous lesions and so on. MTB technique can be used not only in gastrointestinal tract mucosa, but also at respiratory tract as well as urinary tract.

7.2. Character of MTB forceps

They are easy to perform, endoscopist can use the MTB forceps to do biopsy and marking in one step. Furthermore the depth and size of biopsy sample can be adjusted by the endoscopist. The MTB forceps can pass through the channel of ordinary gastroscope, so there is no extra fee for the biopsy procedure. There are different forceps lengths (50-230cm) available which is suitable for the different endoscope. The Maximum depth of injection needle is 3.2mm, so it is safe to use the MTB forceps on any part of human lumen tract. The MTB forceps is not expensive for single use that can avoid cross-infection.

7.3. Character of MTB dying solution

The main ingredients are carbon particles suspensoid. It has stable physicochemical properties. There is no need to store them in dark place. There is no carcinogenic compounds and no risk to induce inflammatory, granuloma or any other pathological change (already widely used in cosmesis). Our former research has showed that there is no influence on pathological or cytological diagnose. The best dilution of India ink is 1:10 for MTB, and the best dose for each marking spot is 0.3~0.5ml high clarity, long duration and less pathological reaction.

7.4. Indications and contraindications of MTB

Indications: All the patients who is proper to undergo EGD or colonoscopy

Contraindications: (1) pregnant and lactation women. (2) patient who is against MTB. (3) all contraindications of ordinary EGD or colonoscopy.

7.5. Preoperative preparations

Endoscopist should review the former endoscopy reports carefully. An information consent form should be obtained from the patient. Nurse should test the MTB forceps in advance, and also rinse the MTB forceps with normal saline followed with India ink to make sure it is ready to use.

7.6. Instruction for MTB operation

When perform MTB, inject first, followed with biopsy. It is better to properly push while injection. We can increase the dosage of marking on posterior wall, which is easy to fade.
7.7. Points for attention postoperation and side-effect of MTB

The nurse should rinse the MTB forceps as soon as possible after the operation. The position and endoscopic findings of all the biopsy samples should be recorded in detail, pathologist should be notified that they are MTB samples with India ink.

Some patients will suffer from the transmitted upper abdominal pain, usually recovered within one week. No fever or GI bleeding reports.

7.8. Application of MTB

In 2006, MTB was enrolled into the State technological and engineering projects, the 863 Plan, as a new technology for the diagnosis and treatment of major diseases by Chinese Ministry of Science and Technology. Up to the end of 2009, 1683 cases of gastric MTB have been done at Sir Run Run Shaw Hospital, Zhejiang university, China. With the permission of Chinese Ministry of Health and provincial bureau of health, MTB has been performed in more than 50 hospitals of Beijing, Jiangsu, Jiangxi, Shanxi and Zhejiang as following : Sir Run Run Shaw Hospital affiliated to Zhejiang University, the First Affiliated Hospital of Zhejiang University, the second Affiliated Hospital of Zhejiang University, Zhejiang Province Hospital, the First Affiliated Hospital of Zhejiang Chinese Medical University, Jinhua central hospital, Huzhou central hospital, Lishui city hospital, The third hospital of Wenzhou, Xiyuan Hospital of China Academy of Chinese Medical Sciences, Beijing Friendship Hospital, The third Affiliated Hospital of Beijing University, the second Affiliated Hospital of Tianjing University, Peking Union Medical College Hospital, Xijing Hospital affiliated to Fourth Military Medical University, Dafeng city hospital, the First Affiliated Hospital of Nanchang University, the First Affiliated Hospital of Nanjing medical University

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