Intestinal metaplasia (IM) in the gastric mucosa is characterized by the transformation of normal gastric epithelium and gastric glands into intestinal epithelium and intestinal glands, and it is considered an important precursor to gastric cancer. Patients with histologically confirmed gastric intestinal metaplasia have up to a sixfold increased risk for developing gastric cancer, compared with the population at large. \(^1\) Gastric cancer represents the fifth most common cancer type worldwide, and is the third leading cause of cancer-related mortality. \(^2\) Although its incidence has declined over the past decades, it remains a prominent disease in China; and its early detection represents an important goal for health care.

According to the widely accepted multistep model of gastric carcinogenesis, progression to cancerous disease involves the sequential development of inflammation and atrophy, followed by metaplasia and dysplasia, ultimately leading to carcinoma. Thus, the presence of IM is an important risk factor for gastric cancer development. Therefore, improving the early diagnosis of IM is the cornerstone of strategies aimed at improving the selection of patients that require vigilant surveillance.
Anatomical predilection of intestinal metaplasia

Endoscopic biopsy has developed into the most important avenue for identifying IM. Diagnosis requires histological assessment by a pathologist, which in turn requires representative sampling of the gastric mucosa by taking biopsies. To this end, a standardized protocol is usually followed, which involves taking biopsies at five predetermined sites (two samples from the antrum, two samples from the corpus, and one sample from the angulus). However, whether this standardized protocol maximizes the chance for the adequate diagnosis of premalignant gastric lesions remains controversial. In the study of de Vries et al., involving a Western non-Asian cohort nontargeted biopsies from the lesser curvature had a significantly higher yield as compared with those conventionally obtained of the greater curvature of the corpus in diagnosing atrophic gastritis and IM ($P = 0.05$ and $P = 0.03$). Although these data still require validation in an Asian cohort the study of de Vries et al. strongly suggests that further assessment as to which anatomical parts of the stomach are most likely to develop IM are needed. This consideration prompted us to comprehensively characterize the anatomical predilection for developing IM in the stomach in a large well-characterized Chinese cohort. The results indicate that the standard protocol for biopsy sampling in patients with potential IM may require anatomical adjustment in order to obtain more effective monitoring of gastric cancer risk.

MATERIALS AND METHODS

Ethics statement
This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

Study population
We identified and obtained data from 109,840 patients who underwent gastroendoscopy with sampling of gastric biopsies between January 2008 and December 2013 at the Digestive Endoscopy Center of The First Affiliated Hospital of Nanjing Medical University. All upper gastrointestinal (GI) endoscopic results were retrieved from the Endoscopy Information System (EIS, Angelwin, Beijing, China). Inclusion criteria for this study were endoscopic diagnosis of chronic gastritis (CG), duodenal ulcer (DU), gastric ulcer (GU), reflux esophagitis (RE), duodenogastric bile reflux, and so on. Exclusion criteria were diagnosis of active gastrointestinal hemorrhage, esophageal and gastric varices, esophageal cancer, duodenal neoplasms, pyloric obstruction, cases without definite endoscopic or pathological diagnosis, and rare diseases such as gastric lymphoma or eosinophilic gastroenteritis. In cases where multiple endoscopies were performed for the same patient, only the first report was included in the analysis. Finally, applying these criteria, 78,335 cases were included in this study. All available demographic data (age, gender, address, and others), endoscopic images, endoscopic and pathological findings, and rapid urease test results were retrieved from the EIS. An algorithm written using the Perl programming language was used for data processing (The algorithm is available at the scientific community upon request.).

Endoscopy with biopsy
All 78,335 cases underwent conventional upper GI endoscopy using a standard forward-viewing video gastroscope (Olympus GIF-Q160, Olympus Optical Co., Tokyo, Japan). For subanalysis, the 7019 cases that fulfilled the inclusion criteria (sufficient number of biopsies, no overt cancer, and so on) were analyzed separately in more detail; and among these selected cases, 2012 were *Helicobacter pylori* (*H. pylori*)-positive patients. All procedures were performed by experienced senior endoscopists, who have more than three years experience in endoscopic procedures. For all included cases, biopsies were taken from the following sites according to standard protocol: The lesser and greater curvature of the antrum and the body, the posterior and anterior wall of the antrum, the angulus, the cardia, and the fundus. Generally, three or four biopsies were taken from each person. IM status was evaluated through histology data by expert gastrointestinal pathologists of the Department of Pathology. We considered the diagnosis of IM as confirmed if at least one biopsy displayed IM, irrespective of the anatomical site where it was obtained.

Histological assessment of *H. pylori* status and IM
Biopsy specimens were fixed in 10% PBS-buffered formalin, and embedded in paraffin. The slide sections were stained with hematoxylin and eosin (H and E). A rapid urease test (Pronto Dry, Medical Instruments Corporation, Solothurn, Switzerland) was used to directly examine the status of the *H. pylori* infection. If *H. pylori* infection exists, the breakdown of the urea would produce high concentrations of ammonia. This would then cause the pH to rise and provoke color change from yellow to bright magenta. IM was morphologically recognized by the presence of goblet cells, absorptive cells, and cells resembling colonocytes. The degree of activity of CG was scored by a pathologist based on the number of histologically determined lymphoid follicles, whereas the severity of acute inflammation was scored based on the extent of the neutrophil infiltration. All subjects were assessed according to the updated Sydney classification system.[10] The severity of inflammation, activity, atrophy, and intestinal metaplasia was graded as “no,” “mild,” “moderate,” and “marked,” and was scored on a 0–3 scale, respectively.

Statistical analysis
SAS 9.2 software was used for statistical analysis. Chi-square test was used to compare categorical variables (such as the comparison of IM detection rates among various parts of the
gastric antrum and gastric corpus). The Cochran–Armitage trend test was also carried out for IM when appropriate. Differences in scores were examined using the Kruskal–Wallis test, and all P-values calculated were two-tailed with a significance level of 0.01.

RESULTS

Baseline characteristics

A total of 78,335 patients were included in this study, and data obtained from these patients were included in the analysis. All patients have been previously diagnosed with a digestive system disease [Table 1a]. The mean age of patients in this study was 47.91 ± 14.30 years (range, 5–100 years), and male-to-female ratio was almost 1:1. For the analysis, patients were grouped into seven age groups: <20, 20–29, 30–39, 40–49, 50–59, 60–69, and ≥70. The total incidence of IM in this study cohort was 22.14% [Table 1b], and there was a statistically significant difference in IM incidence among all age groups, compared with this large cohort (P < 0.01). Thus, the existence of IM strongly depends on age. In apparent agreement, after determining IM grade trends by the Cochran–Armitage test in relation to the different age groups, it became apparent that IM incidence increased with age [Figure 1]. The incidence of IM was higher in the male population than in the female population (OR = 1.05, 95% CI: 1.01–1.08, P < 0.01). These characteristics of our cohort are in agreement with those described in previous literature, and we concluded that it was appropriate to analyze the anatomical predilection of gastric IM incidence.

IM incidence at different anatomical sites observed in nontargeted biopsies

When the anatomical location of IM diagnoses based on targeted biopsies was considered, the highest IM incidence appears to be present in the angulus (28.5%, 14,063 of 49,345 nontargeted angulus biopsies; Table 2 and Figure 2). Compared to the greater curvature of the antrum and corpus, the lesser curvature also had a significantly higher incidence of IM diagnoses (P < 0.01). Importantly, significant differences were observed when the five standard proctors for establishing IM diagnosis (the lesser and greater curvature of the antrum, the lesser and greater curvature of corpus, and the angulus) were compared [Table 3], in which the angulus was substantially more prone to display IM compared with the other sites (P < 0.01). Thus, it seems that the standard protocol for sampling the stomach for potential IM does not capture anatomical preferences for the development of this condition.

Severity of IM at the three main sites for IM development

The tendency of IM to develop into a full-blown cancer strongly depends on the severity of the IM process.

| Table 1a: Demographic characteristics (n=78,335) |
|-----------------------------------------------|
| Age-year median (range) | 47.91 (5-100) |
| Gender no. (%) | Male 40,027 (51.10) |
| | Female 38,308 (48.90) |
| Gastric ulcer no. (%) | Positive 4,763 (6.52) |
| | Negative 68,298 (93.48) |
| Duodenal ulcer no. (%) | Positive 5,624 (7.61) |
| | Negative 68,298 (92.39) |
| Bile reflux no. (%) | Positive 5,564 (7.10) |
| | Negative 72,771 (92.90) |
| Reflux esophagitis no. (%) | Positive 8,591 (10.97) |
| | Negative 69,744 (89.03) |
| Metaplasia no. (%) | Positive 17,340 (22.14) |
| | Negative 60,995 (77.86) |
| Atrophy no. (%) | Positive 2,837 (3.62) |
| | Negative 75,498 (96.38) |
| Dysplasia no. (%) | Positive 4,537 (5.79) |
| | Negative 73,798 (94.21) |

**Table 1b: Correlation among age, gender, and intestinal metaplasia**

| Characteristics | With IM (n=17,340) | OR | 95% CI | P value |
|-----------------|--------------------|----|--------|---------|
| Age (year, mean±SD) | 53.84±12.69 | <0.01 |
| <20 | 55 | 0.32 | |
| 20-29 | 435 | 2.51 | <0.01 |
| 30-39 | 1,926 | 11.11 | <0.01 |
| 40-49 | 3,756 | 21.66 | <0.01 |
| 50-59 | 5,299 | 30.56 | <0.01 |
| 60-69 | 3,824 | 22.05 | <0.01 |
| ≥70 | 2,045 | 11.79 | <0.01 |
| Gender | Male 9,012 | 51.97 | |
| | Female 8,328 | 48.03 | <0.01 |

Anatomical incidence per se is not a sufficient basis with respect to the guidance of endoscopic biopsy sampling; thus, the severity of IM must be taken into account as well. Hence, we analyzed the severity of IM at three anatomical sites that are most likely to develop IM, which are the lesser curvature of the antrum, the lesser curvature of the corpus, and the angulus [Figure 3]. In agreement with the overall incidence, the incidence of mild, moderate, and severe IM in the angulus (19.48%, 10.39%, and 1.20%) and the lesser
curvature of the antrum (12.56%, 3.22%, and 0.25%) were significantly higher, compared with the greater curvature (6.12%, 1.32%, and 0.09%) [Table 4]. These results further support the adjustment of sampling procedures when screening for IM.

Different inflammation scores in the lesser and greater curvature of the antrum

Biopsy samples were simultaneously taken from both the lesser and greater curvatures of the antrum of 7019 cases. This allowed us to compare the relative severity of inflammation in these two anatomical structures. To this end, the incidence of IM in these two sites of the same subjects were compared used a matching Chi-square test. Inflammation was more common in the lesser curvature of the antrum (18.79%, 1319/7019 of all cases) than in the greater curvature of the antrum (4.67%, 328/7019 of all cases; Table 5a). When analyzed according to the updated Sydney classification system, acute and chronic inflammation scores were significantly different in the lesser and greater curvatures of the antrum. For this analysis, patients were stratified according to H. pylori status [Table 5b]. In the H. pylori-positive group, acute-inflammation scores were higher and chronic-inflammation scores were lower in the lesser curvature of the antrum. However, both acute and chronic inflammation scores were higher in the lesser curvature of the antrum than in the greater curvature of the antrum in the H. pylori-negative group (all P < 0.01). This suggests that both IM incidence and inflammation relate to the anatomical location, even if the latter is also strongly influenced by H. pylori status.

DISCUSSION

IM is an important stage in the multistep process that finally leads to full-blown cancer. The progression toward GC is initiated by the development of chronic gastritis, followed by gastric atrophy, intestinal metaplasia and dysplasia, and ultimately resulting in gastric carcinoma. This process typically involves the H. pylori-dependent corruption of gastric morphogen signaling. Gastric cancer is the third leading cause of cancer-related mortality.
monitoring of IM, especially incomplete colonic metaplasia, appears to be a way forward to reducing mortality.\cite{8, 9} Hence, the optimal and early detection and treatment of IM is very important, and is considered to be of utmost importance in this field.\cite{10}

In general, precancerous lesions and early gastric cancer do not have obvious clinical symptoms; and endoscopy, especially endoscopic sampling of gastric biopsies, play a decisive role in the diagnosis. Standard protocols for sampling biopsies have been established, but these have not been linked to the actual incidence of IM at different anatomical locations or to the regional differences of IM severity.\cite{11} This current study aims to fill this void, at least for Asian populations.

This study has demonstrated that age and gender are independent risk factors for intestinal metaplasia. Consistent with a study carried out by Genta et al.,\cite{11} IM was found in 8.4% of 810,821 unique patients; increased with age, and was found to be more common in male patients than in female patients. With regard to nontargeted biopsy locations, our results have illustrated that IM is prone to occur in the lesser curvature of the stomach; which is consistent with the study of de Vries et al.\cite{10} On this basis, this study further proves that the incidence rate and severity of IM is relatively high in the lesser curvature of the antrum and the angulus. This observation is consistent with the regional differences of H. pylori colonization in the stomach, which occurs more frequently in the lesser curvature.\cite{11} Infection with CagA-positive H. pylori strains is significantly associated with the overexpression of proapoptotic proteins and antiapoptotic proteins in the gastric mucosa, which is mainly in the antral lesser curvature; and this may in turn relate to altered epithelial cell turnover and malignant transformation.\cite{12} A possible reason for the differential H. pylori colonization in the stomach is that the propagation velocity of the antral contraction waves in the greater curvature of the stomach is much faster than in the lesser curvature.\cite{13} Low fluid flow rates of the lesser curvature lead to the accumulation of food, and then the colonization of H. pylori. Our results suggest that the angulus and antrum deserve more attention with respect to biopsy sampling.

In addition to IM per se, we also focused on the inflammation status of the antrum in this study, since gastritis would most likely occur in this site. Our results reveal that the regional severity of gastritis stratifies according to H. pylori status. In the H. pylori-positive group, our results revealed that acute inflammation was more severe and chronic inflammation was milder, compared with the lesser curvature of the antrum. We expect that the reason for this dichotomy lies in differential colonization (the antrum is more prone to be colonized by H. pylori\cite{14}) and patients with H.

### Table 3: Comparison of IM status at five standard biopsy sites

| Biopsy site | IM (n) | \( \chi^2 \) | \( P \) |
|-------------|--------|---------------|-------|
| AL-AG (AL+ AG+), 2,428 (AL− AG+), 1,582 | | | |
| AL-An (AL+ An+), 7,682 (AL− An+), 4,993 | | | |
| AL-CL (AL+ CL+), 4,330 (AL− CL+), 2,830 | | | |
| An-AG (An+ AG+), 4,387 (An− AG+), 7,216 | | | |
| An-CL (An+ CL+), 1,790 (An− CL+), 499 | | | |
| CL-CG (CL+ CG+), 208 (CL− CG−), 2,540 | | | |

AL: Lesser curvature of the antrum, AG: Greater curvature of the antrum, An: Angulus, CL: Lesser curvature of the corpus, CG: Greater curvature of the corpus

**Figure 3:** Severity of IM at the five standard anatomical sites. IM data observed in the stomach of 78,335 cases that underwent gastroduodenoscopy from 2008 to 2013 in Jiangsu and Anhui provinces in China are shown. The percentage of biopsies displaying IM and severity of the extent of IM is shown.

### Table 4: Comparison of IM grades observed in three main gastric locations

| Biopsy site | IM (n, %) | \( \chi^2 \) | \( P \) |
|-------------|-----------|---------------|-------|
| An-GC* (55,841) | 3,415 (6.12), 738 (1.32), 52 (0.09) | | |
| An-LC (54,632) | 6,864 (12.56), 1,759 (3.22), 136 (0.25) | | |
| Angulus (49,345) | 9,612 (19.48), 5,127 (10.39), 592 (1.2) | | |

An-GC: Greater curvature of the antrum, An-LC: Lesser curvature of the antrum, Angulus: Greater curvature of the antrum; *Baseline for comparison; IM was most severe in the angulus, followed by the lesser curvature of the antrum and the greater curvature of the antrum.
**Table 5a: Paired* comparison results in the antrum**

| Biopsy site            | Antrum-lesser curvature | P value |
|------------------------|-------------------------|---------|
|                        | IM (+) | IM (−) |       |
| Antrum-greater curvature |       |         |       |
| IM positive (+)        | 682    | 328    | <0.05  |
| IM negative (−)        | 1,319  | 4,690  | <0.05  |

*Matching Chi-square test was used to compare the IM status of the lesser and greater curvature of the antrum from the same patients.

**Table 5b: Inflammation scores in the lesser and greater curvature of the antrum**

| Antrum L-G | Number | Average | SD     | Min | Max |
|------------|--------|---------|--------|-----|-----|
| N-biopsy-HpY | 7,019 | 0.0208  | 0.327  | −3.000 | 5.000 |
| C-score-HpY  | 7,019 | 0.01151 | 0.3880 | −2.000 | 2.0000 |
| A-score-HpY  | 7,019 | 0.0403  | 0.5660 | −3.000 | 3.0000 |
| N-biopsy-HpN | 4,666 | 0.0378  | 0.4021 | −3.000 | 5.0000 |
| C-score-HpN  | 4,666 | 0.0199  | 0.3575 | −2.000 | 3.0000 |
| A-score-HpN  | 4,666 | 0.0168  | 0.4369 | −3.000 | 3.0000 |

SD: Standard deviation. Based on Kruskal–Wallis Test, the comparison of the D-value between the lesser and greater curvature of the antrum between the H. pylori-positive and H. pylori-negative groups were analyzed, respectively (all P<0.01). In the H. pylori-positive group, acute-inflammation score was higher and chronic-inflammation score was lower in the lesser curvature. However, both acute and chronic inflammation scores were higher in the H. pylori-negative group. Antrum L-G, the D-value of inflammation scores between the lesser and greater curvature of the antrum in different conditions; N-biopsy, biopsy number; C-score, score of chronic inflammation; A-score, score of acute inflammation; HpY, H. pylori-positive; HpN, H. pylori-negative.

Pylori infection have a significantly higher neutrophil-to-lymphocyte ratio, compared with those without H. pylori. The degree of neutrophil infiltration decreases spatially from the lesser curvature to the greater curvature. In early-stage infection, lesions display acute inflammation characterized by neutrophil infiltration, which changes into chronic inflammation characterized by lymphatic infiltration, and the wide formation of lymphatic follicles at later time points. Hence, differential induction of chronic inflammation may underlie regional differences in IM. Further effects of provoking regional differences in IM incidence in the stomach may relate to mixed infections. It was reported that 23.3% of H. pylori-infected patients had mixed infections, and such infections were associated with distinct histological features in the antrum and corpus. Mixed infection may exist in our specimens, and may show regional differences; explaining the findings of this current study. A final factor may be the non-H. pylori-related gastritis or other digestive tract diseases that are likely to occur at these sites. IM is closely related to acute and chronic inflammation, and inflammation not related to H. pylori might be important. Nonetheless, these exact factors underlying the anatomical distribution of IM in the stomach remain largely speculative and require further research. Biopsies from the lesser and greater curvature of the antrum should be routinely taken for patients infected with H. pylori, whereas biopsies should be more exclusively taken from the lesser curvature in noninfected patients. Our results generally provide anatomical guidance for biopsy and evidence for the notion that patients should be stratified according to H. pylori status. With this recommendation, we would also like to consider the study conducted by Liu et al., which revealed that there were major geographical differences in the overall prevalence of atrophy and intestinal metaplasia. Scores for intestinal metaplasia were generally low except for Xi-an (55%), Japan (44%), and Shanghai (32%). It is possible that our results only fit the Southeast-China population (mainly in Jiangsu and Anhui), in which the prevalence of precancerous lesions differs from others. Thus, we call for studies similar to ours in other cohorts.

**CONCLUSION**

IM is most likely to develop in the anulus and the lesser curvature of the stomach. An adequate biopsy scheme for diagnosing IM should involve generous sampling from the lesser curvature of the antrum, lesser curvature of the corpus, and the anulus; especially in H. pylori-negative patients.

**Financial support and sponsorship**

This work was supported by the National Natural Science Foundation of China (Project No. 81101800), the 12th Six Talents Peak Project of Jiangsu Province (No.2015-WSN-028) and the Research Project of Education of Nanjing Medical University (No.NY2222015030).

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Gomez JM, Wang AY. Gastric intestinal metaplasia and early gastric cancer in the west: A changing paradigm. Gastroenterol Hepatol (N Y) 2014;10:369-78.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
3. de Vries AC, Haringsma J, de Vries RA, van Grieken NC, Meijer GA, et al. Biopsy strategies for endoscopic surveillance of premalignant gastric lesions. Helicobacter 2010;15:259-64.
4. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161-81.
5. den Hoed CM, Holster IL, Capelle LG, de Vries AC, den Hartog B, Ter Borg F, et al. Follow-up of premalignant lesions in patients at risk for progression to gastric cancer. Endoscopy 2013;45:249-56.
6. Ajdarkosh H, Sohrabi M, Moradniani M, Rakhshani N, Sotodeh M, Hemmasi G, et al. Prevalence of gastric precancerous lesions among chronic dyspeptic patients and related common risk factors. Eur J Cancer Prev 2015;24:400-6.

7. Bleuming SA, Kodach LL, Garcia Leon MJ, Richel DJ, Peppelenbosch MP, Reitsma PH, et al. Altered bone morphogenetic protein signalling in the Helicobacter pylori-infected stomach. J Pathol 2006;209:190-7.

8. Rugge M, Capelle LG, Cappellesso R, Nitti D, Kuipers EJ. Precancerous lesions in the stomach: From biology to clinical patient management. Best Pract Res Clin Gastroenterol 2013;27:205-23.

9. Jass JR, Filipe MI. The mucin profiles of normal gastric mucosa, intestinal metaplasia and its variants and gastric carcinoma. Histochem J 1981;13:931-9.

10. den Hollander WJ, Kuipers EJ. Current pharmacotherapy options for gastritis. Expert Opin Pharmacother 2012;13:2625-36.

11. Genta RM, Sonnenberg A. Characteristics of the gastric mucosa in patients with intestinal metaplasia. Am J Surg Pathol 2015;39:700-4.

12. Cabral MM, Mendes CM, Castro LP, Cartelle CT, Guerra J, Queiroz DM, et al. Apoptosis in Helicobacter pylori gastritis is related to cagA status. Helicobacter 2006;11:469-76.

13. Imai Y, Kobayashi I, Ishida S, Ishikawa T, Buist M, Yamaguchi T. Antral recirculation in the stomach during gastric mixing. Am J Physiol Gastrointest Liver Physiol 2013;304:G536-42.

14. Park YH, Kim N. Review of atrophic gastritis and intestinal metaplasia as a premalignant lesion of gastric cancer. J Cancer Prev 2015;20:25-40.

15. Farah R, Khamisy-Farah R. Association of neutrophil to lymphocyte ratio with presence and severity of gastritis due to Helicobacter pylori infection. J Clin Lab Anal 2014;28:219-23.

16. Chi J, Lu M, Fu B, Nakajima S, Hattori T. Induction of gastritis and gastric ulcer in Mongolian gerbils infected with Helicobacter pylori. Chin Med J (Engl) 1999;112:998-1000.

17. Sheu SM, Sheu BS, Lu CC, Yang HB, Wu JJ. Mixed infections of Helicobacter pylori: Tissue tropism and histological significance. Clin Microbiol Infect 2009;15:253-9.

18. Koshiba Y, Koizumi W, Sasabe M, Katoh Y, Okayasu I. Association of Helicobacter pylori-dependent gastritis with gastric carcinomas in young Japanese patients: Histopathological comparison of diffuse and intestinal type cancer cases. Histopathology 2000;37:124-30.

19. Liu Y, Ponsioen CI, Xiao SD, Tytgat GN, Ten Kate FJ. Geographic pathology of Helicobacter pylori gastritis. Helicobacter 2005;10:107-13.