Endoscopic Surveillance of Patients With Hereditary Diffuse Gastric Cancer

Biopsy Recommendations After Topographic Distribution of Cancer Foci in a Series of 10 CDH1-mutated Gastrectomies

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Abstract: The management of hereditary diffuse-type gastric cancer revolves around surveillance biopsies and the timing of prophylactic gastrectomy. In the absence of a validated surveillance biopsy protocol, we modeled bioptic diagnostic yield on the basis of the topographic distribution of cancer foci in a series of 10 gastrectomies in *CDH1*-mutation carriers. Complete histologic examination was performed in all cases, and 1817 slides were evaluated for the presence of in situ, intramucosal, or submucosal diffuse-type carcinoma. Detailed maps determined the density of cancer foci. On the basis of the number of sampled glands per biopsy in routine surveillance preoperative endoscopy, we estimated the theoretical number of biopsies necessary for a 90% rate of detection of neoplastic foci, and we evaluated this number, taking into account the regional distribution of these foci. A total of 96 m of gastric mucosa with ~1,193,453 gastric glands yielded 302 cancer foci [in situ (n = 89), intramucosal (n = 209), and submucosal (n = 4)] spanning the width of a total of 1820 glands (8 to 1205 per case; average 182 ± 115). On the basis of the number of glands per stomach and the average number of glands sampled during surveillance biopsy (28.7 ± 1.7; range, 0 to 79; n = 112), the theoretical number of biopsies necessary to capture at least 1 cancer focus was estimated to be 1768 (range, 50 to 5832) to assure a 90% detection rate. Mapping of cancer foci showed the highest density in the anterior proximal fundus (37%) and cardia/proximal fundus (27%). Our results argue for the incorporation of cancer focus distribution into any biopsy protocol, although detection is likely to remain extremely low, and they call into question the validity of endoscopic surveillance.

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Gastric cancer is one of the leading causes of cancer-related deaths worldwide. Although the majority of cases are sporadic, about 10% of gastric cancers are qualified as familial and related to several syndromes and genes.[1–7] Among these, approximately 25% to 40% of families with an autosomal dominant pattern of hereditary diffuse gastric cancer (HDGC) have germline mutations in the *CDH1* gene that encodes the epithelial cell adhesion protein E-cadherin.[8] In this cancer susceptibility syndrome with a penetrance between 70% to 80%, the cumulative lifetime risk of developing gastric cancer is over 80% in both men and women by the age of 80,[9] and affected patients typically develop signet ring cell gastric cancer before the age of 40.[9–12]

HDGC has been defined by the international gastric cancer linkage consortium, and criteria for selection of families for *CDH1* mutational analysis have been established[13] (Table 1). Management currently revolves around surveillance (with early detection and treatment of gastric cancer) or prophylactic gastrectomy in young, asymptomatic carriers of germline truncating *CDH1* mutations who belong to families with highly penetrant hereditary gastric cancer.[14] However, the optimal management of individuals at risk is controversial; there is no proven value in surveillance regimens, and prophylactic gastrectomies are not devoid of potential morbidity and mortality. Nonetheless, given that prophylactic gastrectomy is the only known preventive treatment, strategies to optimize timing of the operation are needed.

The preoperative diagnosis of early diffuse-type gastric cancer is difficult because the tumor cells begin infiltrating the mucosa while preserving a normal surface epithelium, and rarely are any visible lesions spotted endoscopically. Despite improvements in endoscopic techniques,[15–19] biopsy remains the gold standard for cancer detection. Considerable interest in the detection of

TABLE 1. Criteria for Testing and Diagnosis of HDGC Syndrome

Two or more cases of diffuse gastric cancer in first-degree or second-degree relatives, with at least 1 diagnosed under 50 y of age
Three or more cases of diffuse-type gastric cancer in a first-degree or second-degree relative, independent of age at diagnosis
Diagnosis of diffuse-type gastric cancer before 40 y of age without family history
Families with diagnosis of both diffuse gastric cancer and lobular breast cancer with at least 1 case detected before 50 y of age

precursor lesions in HGDC has led to histologic identification of signet ring cell carcinoma in situ, as well as foci of invasive signet ring cell carcinoma confined to the superficial lamina propria typically without nodal metastases (pT1a).[11,20] Notably, there is wide variation in the number and location of these foci both within families and between HDGC kindreds, with average values of over 100 to <20 foci (http://www.genetests.org).[9,21–24] Recently, some authors have recommended that at least 24, and more recently 30, random endoscopic biopsies be taken,[12,25] but we are not aware of any systematic evaluation of these recommendations.

Herein, we provide data generated from mapping and quantifying cancer foci in 10 consecutive gastrectomies within a single institutional HDGC cohort. On the basis of regional variation, we modeled a topographically weighed biopsy protocol and determined theoretical detection rates. Our findings indicate that although surveillance endoscopy diagnosis will remain challenging, incorporation of the cancer focus distribution may be a helpful step in the development of an endoscopic surveillance protocol for individuals with *CDH1* mutations.

MATERIALS AND METHODS

Regulatory Approval

The study was designed as a retrospective analysis of patients who underwent prophylactic total gastrectomy; the institutional review board approved the study protocol.

Patient Population

Patients were first seen between 2006 and 2009 at the High-risk Gastrointestinal Genetics Clinic of the Massachusetts General Hospital, and established screening guidelines were applied (http://www.genetests.org). Each patient underwent at least 1 genetic counseling session, including detailed assessment of medical and family histories and full-length *CDH1* gene sequencing. Here we focus on histopathologic aspects of the cohort; the surgical aspects and clinical management of this cohort are presented elsewhere.[23]

Endoscopy and Gastrectomy

All patients underwent at least 1 upper endoscopy, with (n = 1) or without (n = 9) chromoendoscopy, before prophylactic total gastrectomy with Roux-en-Y esophagojejunostomy.

Dissection and Mapping Protocol

A protocol adapted for pathologic examination of gastrectomy specimens was followed.[26] Briefly, unfixed specimens were opened along the greater curvature, pinned down, and fixed in 4% neutral-buffered formalin. Gross photographs were taken and annotated to generate maps. Each stomach was entirely submitted for histologic examination using 3- to 5-mm-thick sections that were routinely processed, paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin. Orientation and microscopic-macroscopic correlation was achieved through annotation on gross photographs (Fig. 1).

Histopathology

Microscopic evaluation was performed by 2 of the authors (H.F., G.Y.L.), and glandular changes were classified into 4 categories: normal; signet cell carcinoma in situ, intramucosal carcinoma, and submucosal diffuse-type carcinoma. Normal was defined as absence of abnormal neck cells, that is, absence of signet ring cells. Signet cell carcinoma in situ was defined as individual neoplastic cells of signet ring type within the confines of the basement membrane (Figs. 2A, B, 3A, B). These appeared as rounded cells with distended cytoplasm with misplaced and crescent-shaped hyperchromatic nuclei. Signet cell carcinoma in situ also included configurations

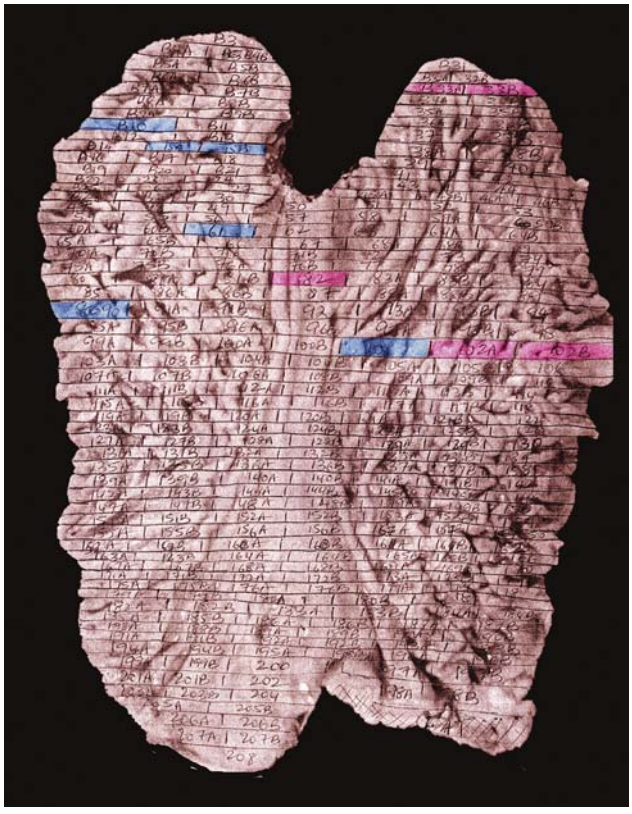

FIGURE 1. Example of evaluation of gastrectomy. The entire specimen is sampled using a mapping technique as illustrated. The color codes indicate sections in which foci of signet ring cell carcinoma in situ and intramucosal carcinoma were noted.

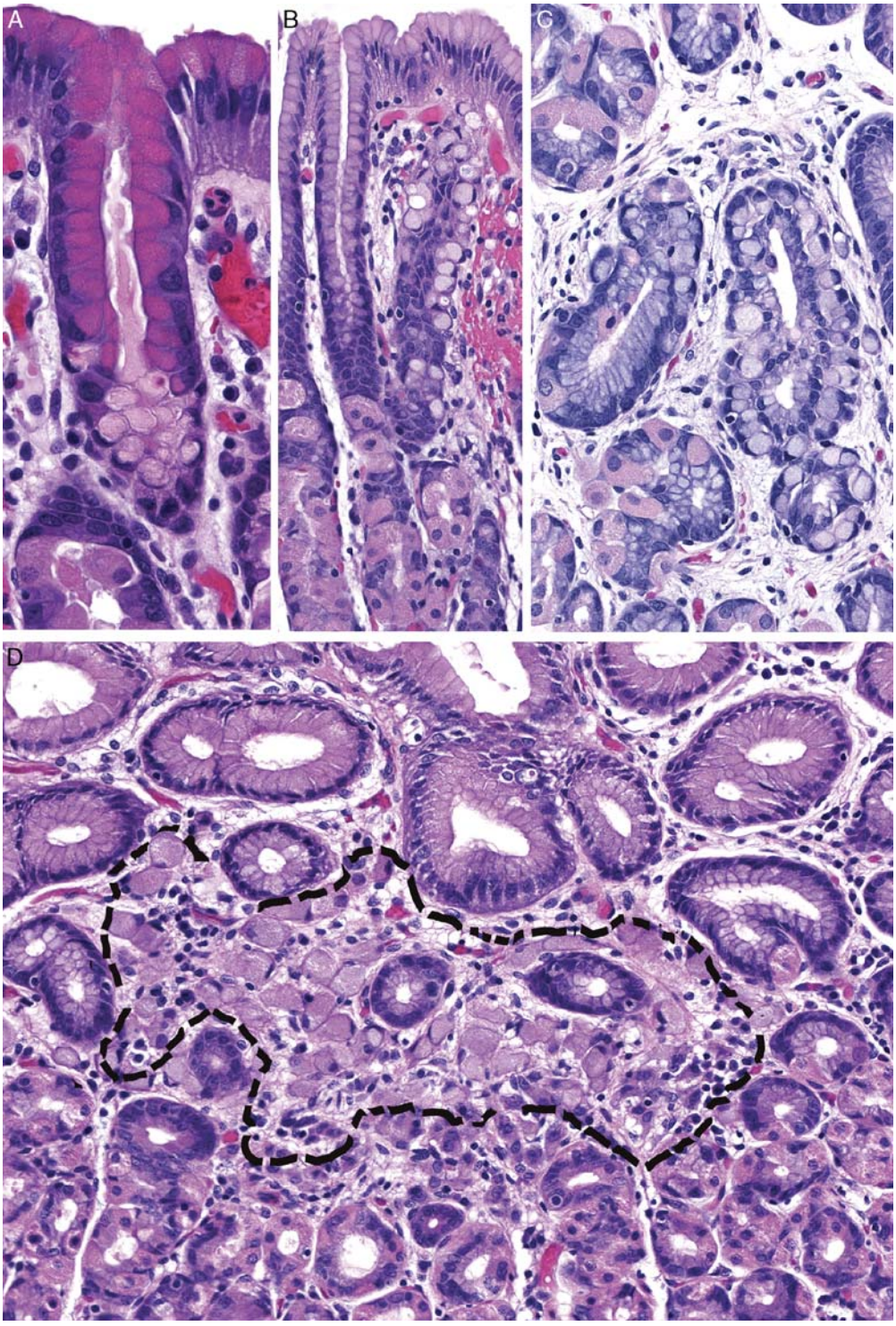

FIGURE 2. Histomorphology of preinvasive (A–C) and invasive (D) hereditary diffuse-type gastric cancer. A, Signet cell carcinoma in situ (pTis) is characterized by large atypical neck cells with intracytoplasmic mucin accumulation that displaces the crescent-shaped, hyperchromatic nucleus to the periphery, resulting in so-called signet-ring cell appearance. B, Another example of signet ring cell carcinoma in situ. The neoplastic cells are confined to the outline of the gastric unit. C, Pagetoid spread of signet ring cell carcinoma in situ. D, Intramucosal carcinoma (pT1a) invading lamina propria around ~4 gastric units (outlined by dotted line).

in which signet ring cells substituted for normal epithelial cells or there was spreading of signet ring cells below normal epithelium (pagetoid spread) (Fig. 2C). Intramucosal carcinoma was diagnosed when infiltrating neoplastic cells were detected outside the confines of a glandular unit, and invasive signet ring cells were present within the lamina propria (Fig. 2D). Submucosal diffuse-type carcinoma was diagnosed when cancer cells extended through the muscularis mucosa into the submucosa. The type of gastric mucosa (antral vs. fundic/oxyntic) was noted, and for each lesion, the total number of affected gastric units was recorded and their position mapped (see below).

Morphometric Evaluation

The mucosal length per block and size of each cancer focus were measured microscopically, and lesions present in adjacent blocks were assumed to be at least 3 mm in diameter. The area of antral-type or fundic-type mucosa was determined and mapped on scanned photographs. The total number of gastric units in the resection specimens was estimated on the basis of the average density of at least 10 randomly chosen gastric gland units in the antral-type and fundic-type mucosa and the assumption that cross-sections of superficial aspects of gastric units (pits) are vaguely circular. The percentage of sampled gastric units was calculated using the average glandular density and total sampled length per block. The subtypes of neoplastic foci were tallied, and the total number of affected glands was used to calculate the percentage of affected gastric units per patient. Review of preoperatively obtained biopsy samples was used to determine the average number of glands sampled per biopsy. The biopsies were obtained using large particle biopsy forceps (so-called "jumbo forceps").

Using the range of affected units, the number of glands per stomach, and the average number of sampled gastric units, we estimated the theoretical number of biopsies necessary to capture at least 1 cancer focus, assuming a clinically suboptimal (50%) and more acceptable detection rate (90%). Identification of site predilection of cancer foci involved the merging of individual density maps into a combined normalized map. The size-proportionate cancer foci were also merged through form fitting into a 2-dimensional coordinate system erected along the minor gastric curvature (x axis) and the maximum sagittal circumference (width) of the opened specimen (y axis). Distribution of cancer foci was also studied in 8 anatomic regions (cardia; anterior and posterior proximal fundus; anterior and posterior distal fundus; lesser curvature; greater curvature and antrum); and the fundus was divided into proximal and distal by halving the lesser curvature (greater and lesser curvatures were defined as 2-cm-wide bands, not split into proximal and distal).

Imaging

Gross and microscopic images were acquired using a Canon EOS 50d 15.1-megapixel 17 to 85 mm zoom lens (Canon, Lake Success, NY) and an Olympus DP70 camera attached to a BX51 light microscope (Olympus America, Center Valley, PA), respectively.

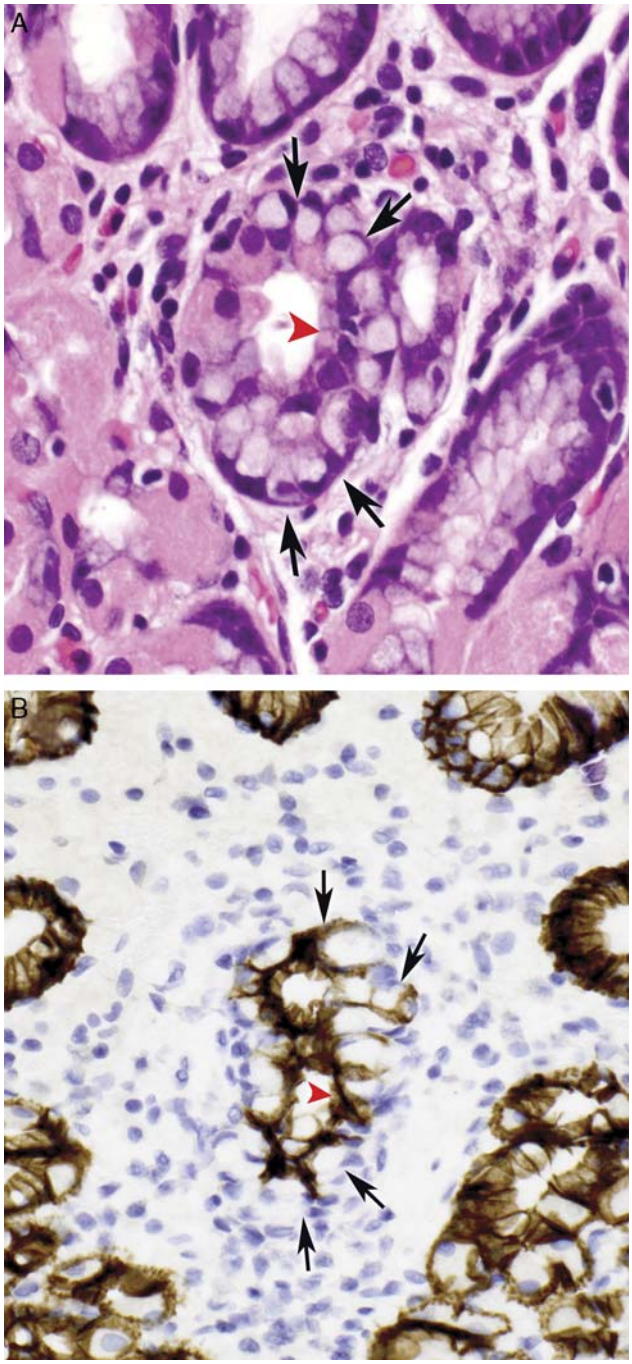

FIGURE 3. Signet ring cell carcinoma in situ (pTis). These microphotographs highlight the residual inner ring of benign cells with preserved E-cadherin immunoreactivity (red arrowheads, A, B), whereas the peripheral crown of neoplastic signet ring cells is not immunoreactive (black arrows, A, B).

Statistical Analysis

Statistical analysis was conducted using the Fisher exact test or Student t test when appropriate. P-values of

TABLE 2. Clinical and Genetic Characteristics in the Cohort

Case	Age	Sex	Mutation Name	Mutation Type	Status (mo)	Reported Family History of Gastric Cancer	Reported Family History of Breast Cancer
1	38	Female	p.R732Q c.2195G > A	MS	AW (48.4)	+	+
2	41	Male	c.1682insA	FS	AW (42.9)	+	–
3	43	Male	c.1901C > T p.A634V	MS	AW (33.8)	+	+ (lobular)
4	42	Male	c.48 + 1G > A	SS	AW (31.2)	+	–
5	38	Female	c.48 + 1G > A	SS	AW (32.2)	+	–
6	42	Male	c.48 + 1G > A	SS	AW (26.7)	+	–
7	50	Female	c.1003C > T p.A335X	NS	AW (19.2)	+	–
8	49	Male	c.1003C > T p.A335X	NS	AW (16.5)	+	–
9	26	Male	c.1003C > T p.A335X	NS	AW (14)	+	–
10	27	Female	c.3G > A	MS	AW (8.2)	+	+

Age indicates the age at the time of surgery.
Patients 4, 5, and 6 are from one family; patients 7, 8, and 9 are from another family.
AW indicates alive and well; F, female; FS, frameshift; M, male; MS, missense; NS, nonsense; SS, splice site.

< 0.05 were regarded as statistically significant. Data were analyzed using Prism 5.0b (GraphPad Software Inc., La Jolla, CA), Microsoft Excel 2008, (Version 12.1.9; Microsoft Corporation, Redmond, WA) or the online statistical toolbox of the Chinese University of Hong Kong by Prof. A. Chang.[27]

RESULTS

The cohort consisted of 10 consecutive patients in 6 different families with a strong history of gastric cancer. All the families fulfilled established criteria for HDGC.[23] In total, there were 6 men and 4 women, with a median age of 41 years at the time of gastrectomy (range, 26 to 50 y). Mutational analysis of the *CDH1* sequence showed 3 missense, 3 nonsense, 3 splice site, and 1 frameshift mutation (Table 2). Median time from genetic testing to surgery was 3 months (range, 1 to 7 mo), and in 9 of the patients, gastrectomy was performed prophylactically, whereas chromoendoscopy and biopsy before surgery demonstrated intramucosal carcinoma in 1 patient (case #3). Median follow-up time was 28.9 months (range, 8.1 to 48.4 mo), and all patients are alive without evidence of malignancy.

The histopathologic analysis of the entire gastric mucosa required up to 490 sections per case (embedded in 120 to 252 blocks per case). The prosection of the gastrectomies yielded a grand total of 1817 slides that were examined (an average of 182 slides per case). On average, 9.6 m of mucosa were examined per case (range, 6.8 to 13.6 m). The average mucosal area per specimen was $227.8 \pm 25.6\,cm^2$ (range, 122.8 to $347.3\,cm^2$). Histologically, the antral-type mucosa corresponds to $\sim 10\%$ (range, 4.7% to 16.4%) of the total gastric surface [fundic: $20576.7 \pm 2401\,mm^2$ (range, 11706 to $31564\,mm^2$) vs. antral: $2206 \pm 261.5\,mm^2$ (range, 578.9 to $3584.6\,mm^2$)]. When related to the lesser curvature (on average 15 cm long), the antrum corresponded to the distal 1.5 cm, which roughly corresponds to the end of the gastric rugal folds.

The average density of gastric units in at least 25 randomly chosen sections showed 11 ± 0.36 gastric units (pits)/mm of antral-type mucosa, whereas 12.48 ± 0.35 gastric units/mm were present in fundic-type mucosa. Using the density of gastric units and total gastric area,

TABLE 3. Summary of Cancer Foci Quantification

	Cancer Foci							
Case	SCI	IMC	SMC	Σ	Max. Size (mm)	Bx	LN	Group: Stage (AJCC 2010)
1	0	5	0	5	3.3	Neg.	0/13	IA: pT1a pN0 cM0
2	5	2	0	7	0.6	Neg.	0/13	IA: pT1a pN0 cM0
3	10	122	4*	136	4.0	Pos.	0/16	IA: pT1b pN0 cM0
4	4	7	0	11	1.9	Neg.	0/4	IA: pT1a pN0 cM0
5	8	0	0	8	0.1	Neg.	0/9	0: pTis pN0 cM0
6	4	18	0	22	2.8	Neg.	0/16	IA: pT1a pN0 cM0
7	1	7	0	8	0.3	Neg.	0/13	IA: pT1a pN0 cM0
8	12	7	0	19	0.3	Neg.	0/18	IA: pT1a pN0 cM0
9	8	31	0	39	1.0	Neg.	0/9	IA: pT1a pN0 cM0
10	37	10	0	47	0.5	Neg.	0/10	IA: pT1a pN0 cM0

*The 4 submucosal foci were continuous with intramucosal foci (Fig. 5).
IMC indicates intramucosal adenocarcinoma; LN, lymph node; neg., negative; pos., positive; SCI, signet ring cell carcinoma in situ; SMC, submucosal adenocarcinoma.

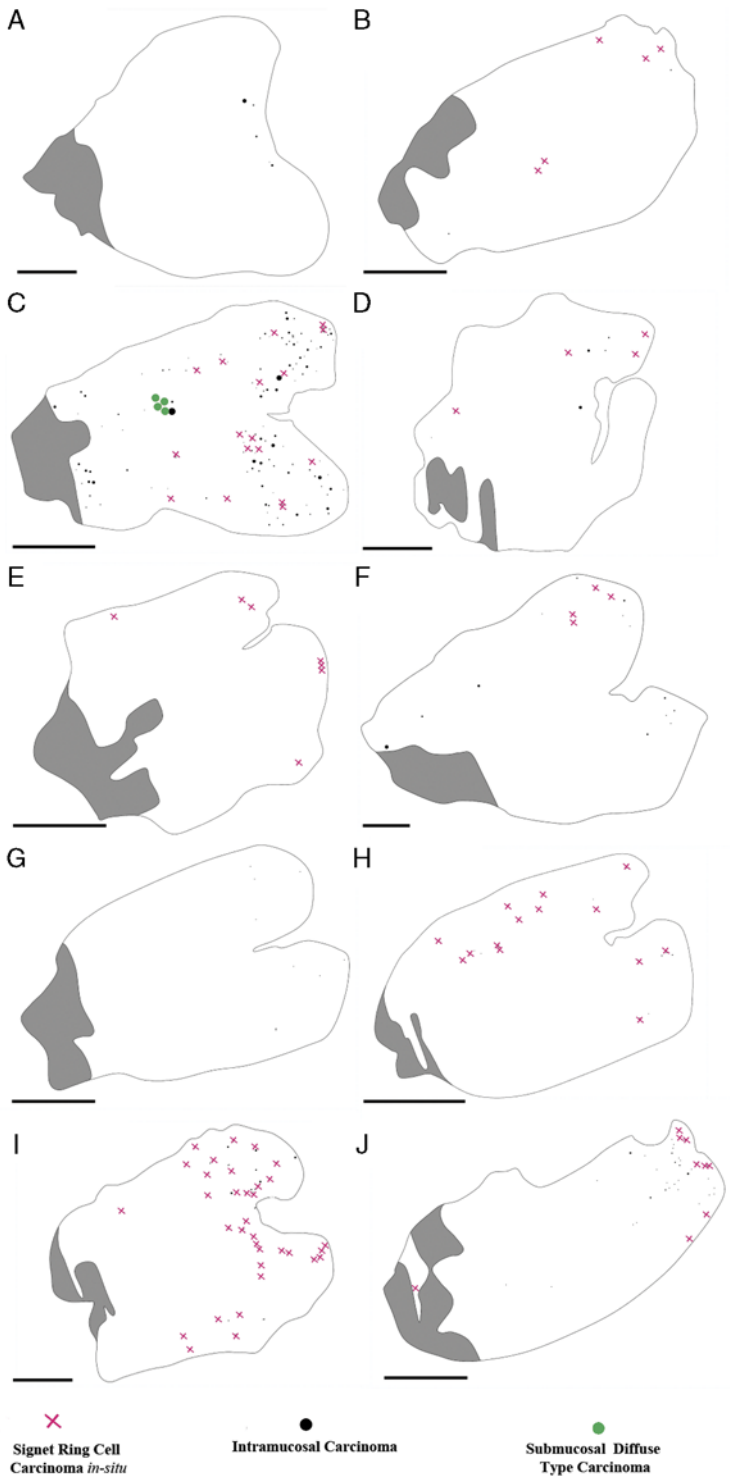

FIGURE 4. Mapping of cancer foci in outlines of all 10 gastrectomy specimens [(A): case #1 to (J): case #10]. Despite the high variability of involvement, the proximal fundus shows the highest density of cancer foci. Note the sparing of antral-type mucosa (gray areas). Scale bars: 5 cm.

we determined the total number of gastric units per specimen. On average, there were 3.2 ± 0.4 million units in the fundus and 267,029 ± 31,644 units in the antrum, resulting in a total of ~3.5 ± 0.4 million units per gastrectomy (reflecting gastric units/pits as the denominator for gastric units). Using the total length of reviewed mucosa, we determined the total number of examined gastric units as ~1.2 million (average, 119,344 ± 8,290 per

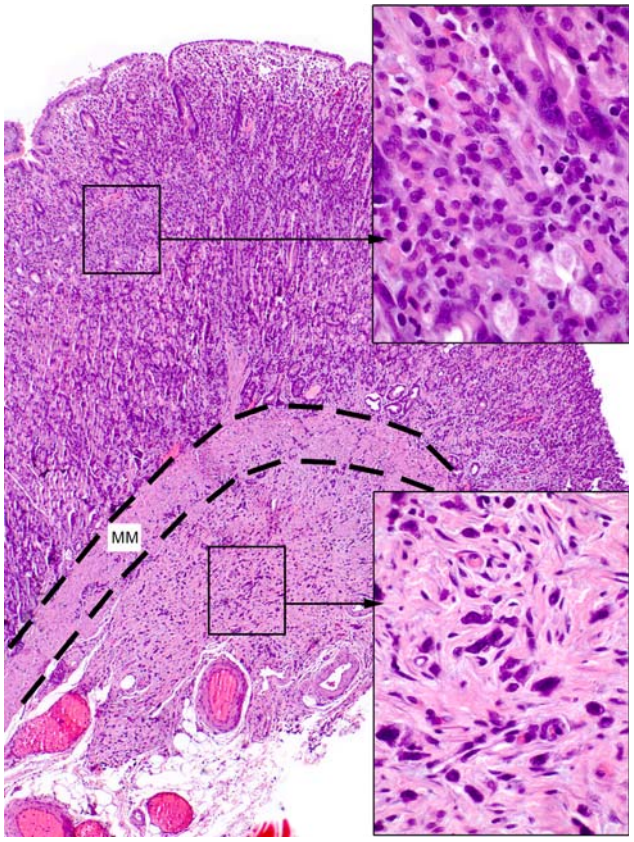

FIGURE 5. Illustration of the sole case with submucosal invasion (case #3). This individual presented with 4 invasive foci (such as the one shown in the lower inset) that were in close association with intramucosal foci (such as the one shown in the upper inset). MM indicates muscularis mucosa.

case; range, 84,440 to 169,035 gastric units). Thus, the histologic review captures only 3.7% ± 0.4% of all gastric units, and we reviewed on average 120,000 of 3.5 million gastric units.

A summary of the examined cancer foci per case is provided in Table 3. Each gastrectomy specimen contained at least 5 separate cancer foci, and we identified a total of 302 cancer foci (range, 5 to 136 foci) (Fig. 4). The largest cancer focus measured 4.0 mm. Eighty-nine foci of signet cell carcinoma in situ were present, and only 1 patient showed superficial submucosal invasion in 4 separate regions (submucosal diffuse-type carcinoma) (Fig. 5). Intramucosal carcinoma was the most prevalent lesion, with a total of 209 separate foci identified in the cohort. The foci of intramucosal carcinoma typically spanned the width of 1 or 2 gastric units (Table 3). In total, 1820 gastric units were affected by cancer (range, 8 to 1205 affected units per patient), and when this was related to the total number of examined gastric units, the average involvement was ~0.12% ± 0.07% (range, 0.008% to 0.71%).

Review of surveillance biopsies available for 6 of the patients showed an average of 28.7 ± 1.7 sampled gastric units (median, 25; range, 0 to 79 in n = 112 samples). On the basis of the number of glands sampled per biopsy (n = 25), the number of gastric units per stomach (~3 million), and the average involvement as determined in the cohort (range, 0.008% to 0.71% of gastric units involved), the theoretical number of biopsies necessary to capture at least 1 neoplastic focus with a detection rate of 50% was estimated to range from 50 to 5832 biopsies (average, 1768). For a detection rate of 90%, the theoretical number of biopsies to observe 1 neoplastic focus ranges from 90 to 11998 (average, 3469) (Table 4).

Mapping of all cancer foci on gastrectomy outlines of each specimen showed absence in the antral-type mucosa and marked variability in fundic-type mucosa. Individual and combined density mapping showed the highest density in the proximal fundus (Figs. 4, 6). Transformation into a coordinate system erected over the lesser gastric curvature allowed optical summation of all cancer foci into a common gastric outline (Fig. 6). Analysis of merged cancer foci demonstrated the following order of cancer prevalence: anterior proximal fundus (37%) > cardia (27%) > anterior distal fundus (12%) >posterior proximal fundus (10%) > lesser curvature (8%) > greater curvature (4%) > posterior distal fundus (2%) > antrum (0%).

DISCUSSION

The role of endoscopic surveillance in *CDH1*-mutation carriers is to allow detection of early gastric cancer, preferably when limited to the mucosa (pT1a). Here, we demonstrate that the estimated total number of biopsies necessary to achieve suboptimal (50%) or substantial (90%) diagnostic yield, on the basis of our cohort of 10 patients, is exorbitant and clinically unsustainable; however, in light of serial sampling during ongoing surveillance, these numbers approach realistic levels. Importantly, despite interindividual variability, our mapping demonstrated the highest cancer predilection in the proximal fundic-type mucosa. Thus, our findings argue that incorporation of topographic aspects into biopsy sampling

TABLE 4. Estimated Number of Biopsies

	Entire Stomach			Fundus		
	No. Biopsies			No. Biopsies		
Detection Rate	Average	Low Range	High Range	Average	Low Range	High Range
50%	1768	50	5832	1745	45	5782
90%	3469	90	11998	3141	82	10408

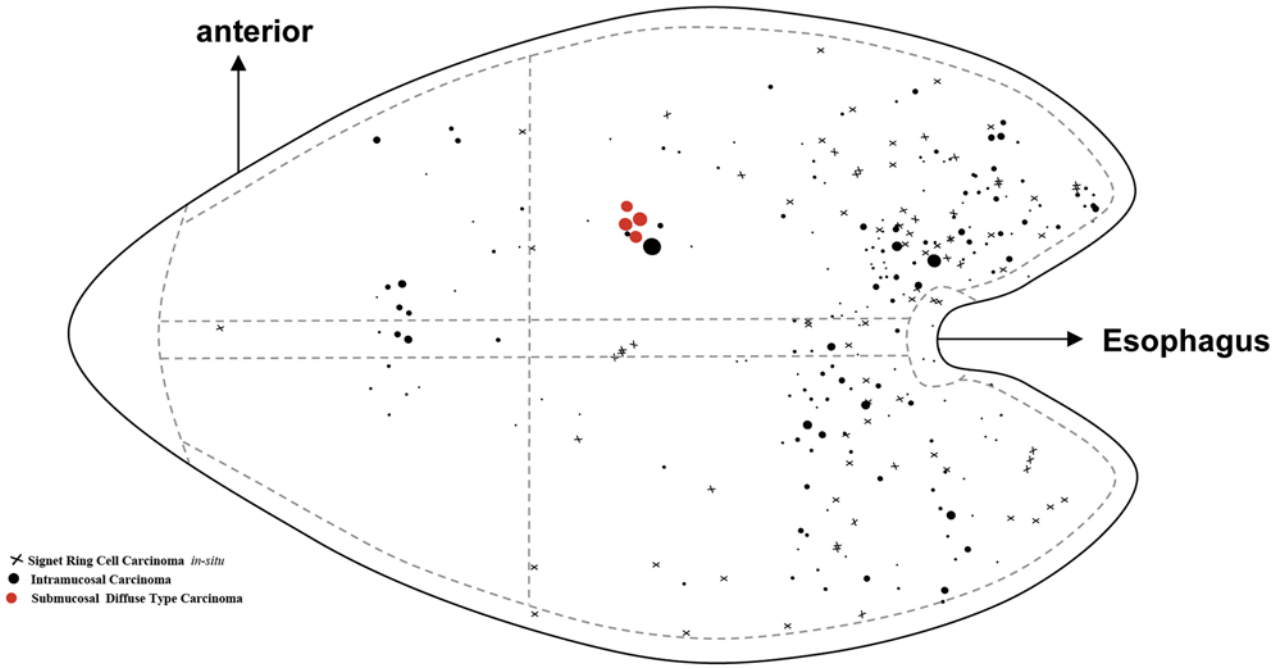

FIGURE 6. Optical summation and mapping of all 302 cancer foci of 10 completely submitted gastrectomy specimens. Transformation into a coordinate system erected over the minor gastric curvature allowed mapping into a common gastric outline (bold) and visualization of merged cancer foci in anatomic regions (hatched lines; see the Materials and Methods section). The highest density of cancer is present in the proximal/anterior and proximal posterior fundus.

strategies could increase diagnostic yield over a prolonged surveillance period.

Total prophylactic gastrectomy has been recommended—even in biopsy-negative patients older than 20 years—whereas annual endoscopic surveillance is recommended for individuals under 20 years.[11,19,20] However, endoscopic screening and random biopsies have limited sensitivity in identifying occult and focal neoplastic lesions. Barber et al[25] noted that only 7 of 28 cases had positive endoscopic biopsies. In our series, cancer foci were detected preoperatively in only 1 of the 10 patients. Shaw et al[19] emphasized the potential advantage of chromoendoscopic surveillance with a reported detection yield of 30%, but also demonstrated that foci <4 mm could not be detected using this technique. In our series, the largest cancer focus measures 4.0 mm, underscoring that most foci were even below the range of improved detection using chromoendoscopy.

A further issue not addressed herein is the inherent risk of missing very small foci of signet ring cell carcinoma in situ on gastric biopsies due to the inherent difficulty of detecting them, even for seasoned pathologists.

When estimating the probability of detecting cancer by using 5 random biopsies, Carneiro et al[26] reported a yield of $<5\%$ in 5 of 9 cases, and $>50\%$ in only 2 cases. In our cohort, the estimated theoretical average number of biopsies to capture at least 1 neoplastic focus was 1768 (range, 50 to 5832) and 3469 (range, 90 to 11998) to assure 50% and 90% detection rates, respectively (Table 4). The magnitude of these figures clearly challenges the practicality of random biopsies for cancer surveillance in *CDH1*-mutation carriers. Nevertheless, these numbers, as well as the previously published prediction model, are likely to underestimate the probability of endoscopic detection, as they are based on random disease distribution. Although previous series have examined the predilection of cancer foci in gastrectomy specimens (http://www.genetests.org),[9,20,22,23] there is no agreement as to whether the proximal (http://www.genetests.org)[9,20] or the transitional zone[22,28] should be preferentially sampled. In our study, we confirm a predilection not only for the proximal stomach but more specifically for oxyntic-type mucosa, and we estimate that 74% of cancer foci are clustered in the cardia and proximal fundus. Nevertheless, even on using only the fundus as the site of detection along with the current recommendation of 30 biopsies per endoscopy,[12] the average number of biopsies necessary for detection remained high in our model: 1745 and 3141 for 50% and 90% detection rates, respectively.

Finally, it is possible that in some patients, several years of surveillance could eventually provide a sufficient number of biopsies for early cancer detection. However, our data clearly emphasize the importance of and need for novel optical techniques that enhance optical contrast and enable more refined strategies for mucosal evaluation and biopsy targeting.

REFERENCES

1. Chompret A, Brugieres L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br J Cancer*. 2000;82:1932–1937.
2. Iwama T, Mishima Y, Utsunomiya J. The impact of familial adenomatous polyposis on the tumorigenesis and mortality at the several organs. Its rational treatment. *Ann Surg*. 1993;217: 101–108.
3. Koornstra JJ, Mourits MJ, Sijmons RH, et al. Management of extracolonic tumours in patients with Lynch syndrome. *Lancet Oncol*. 2009;10:400–408.
4. La Vecchia C, Negri E, Franceschi S, et al. Family history and the risk of stomach and colorectal cancer. *Cancer*. 1992;70:50–55.
5. Park JG, Park KJ, Ahn YO, et al. Risk of gastric cancer among Korean familial adenomatous polyposis patients. Report of three cases. *Dis Colon Rectum*. 1992;35:996–998.
6. van Lier MG, Wagner A, Mathus-Vliegen EM, et al. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am J Gastroenterol*. 2010;105:1258–1264; author reply 1265.
7. Zanghieri G, Di Gregorio C, Sacchetti C, et al. Familial occurrence of gastric cancer in the 2-year experience of a population-based registry. *Cancer*. 1990;66:2047–2051.
8. Caldas C, Carneiro F, Lynch HT, et al. Familial gastric cancer: overview and guidelines for management. *J Med Genet*. 1999;36: 873–880.
9. Pharoah PD, Guilford P, Caldas C, et al. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology*. 2001;121:1348–1353.
10. Carneiro F, Charlton A, Huntsman DG. Hereditary diffuse gastric cancer. In: Bosman FT, Carneiro F, Hruban RH, et al, eds. *WHO Classification of Tumours of the Digestive System*. 4th ed. Lyon: IARC; 2010:59–63.
11. Fitzgerald RC, Caldas C. Familial gastric cancer - clinical management. *Best Pract Res Clin Gastroenterol*. 2006;20:735–743.
12. Fitzgerald RC, Hardwick R, Huntsman D, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. *J Med Genet*. 2010;47: 436–444.
13. Brooks-Wilson AR, Kaurah P, Suriano G, et al. Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. *J Med Genet*. 2004;41:508–517.
14. Huntsman DG, Carneiro F, Lewis FR, et al. Early gastric cancer in young, asymptomatic carriers of germ-line E-cadherin mutations. *N Engl J Med*. 2001;344:1904–1909.
15. Cisco RM, Ford JM, Norton JA. Hereditary diffuse gastric cancer: implications of genetic testing for screening and prophylactic surgery. *Cancer*. 2008;113:1850–1856.
16. Davila RE. Chromoendoscopy. *Gastrointest Endosc Clin N Am*. 2009;19:193–208.
17. Kato M, Kaise M, Yonezawa J, et al. Trimodal imaging endoscopy may improve diagnostic accuracy of early gastric neoplasia: a feasibility study. *Gastrointest Endosc*. 2009;70:899–906.
18. Lim YC, Debiram I, O'Donovan M, et al. Trimodal imaging to identify microscopic foci of signet ring cells in hereditary diffuse gastric cancer. *Gut*. 2011;60:A192–A193.
19. Shaw D, Blair V, Framp A, et al. Chromoendoscopic surveillance in hereditary diffuse gastric cancer: an alternative to prophylactic gastrectomy? *Gut*. 2005;54:461–468.
20. Kaurah P, MacMillan A, Boyd N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. *JAMA*. 2007;297:2360–2372.
21. Hebbard PC, Macmillan A, Huntsman D, et al. Prophylactic total gastrectomy (PTG) for hereditary diffuse gastric cancer (HDGC): the Newfoundland experience with 23 patients. *Ann Surg Oncol*. 2009;16:1890–1895.
22. Oliveira C, Seruca R, Carneiro F. Hereditary gastric cancer. *Best Pract Res Clin Gastroenterol*. 2009;23:147–157.
23. Pandalai PK, Lauwers GY, Chung DC, et al. Prophylactic total gastrectomy for individuals with germline CDH1 mutation. *Surgery*. 2010;149:347–355.
24. Rogers WM, Dobo E, Norton JA, et al. Risk-reducing total gastrectomy for germline mutations in E-cadherin (CDH1): pathologic findings with clinical implications. *Am J Surg Pathol*. 2008;32:799–809.
25. Barber ME, Save V, Carneiro F, et al. Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic surveillance of individuals at risk. *J Pathol*. 2008;216:286–294.
26. Carneiro F, Huntsman DG, Smyrk TC, et al. Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening. *J Pathol*. 2004;203:681–687.
27. Chang A. StatTools. Available at: http://department.obg.cuhk.edu.hk/researchsupport/statmenu.asp. Accessed April 10, 2010.
28. Charlton A, Blair V, Shaw D, et al. Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone. *Gut*. 2004;53:814–820.