[CANCER RESEARCH 50, 4737–4740, August 1, 1990]

Gastric Precancerous Process in a High Risk Population: Cohort Follow-up[1]

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ABSTRACT

In an attempt to characterize the natural history of the gastric precancerous process, 1422 residents of a high risk area of Nariño, Colombia, have been followed from 3–16 years (average 5.1) with repeated gastric biopsies, for a total of 7290 person-years. The original cohort consisted of 1788 individuals yielding a successful completion rate of 79.5%. Comparison of initial and subsequent biopsies revealed a very complex dynamic flow of both progressive and regressive events, suggesting sporadic environmental forces of modulation. One-time measurement of gastric juice, pH, and nitrite failed to predict future events in the gastric mucosa. The net loss of individuals whose gastric mucosa initially showed normal histology or superficial gastritis was 3.3%/year, representing a net gain of 1.7% for chronic atrophic gastritis, 0.9% for intestinal metaplasia, and 0.7% for dysplasia. The incidence rate of gastric cancer in this population was 0.16/100 person-years. The net rates of progression were higher and those of regression lower in older compared to younger individuals. The general pattern detected is that of a slow forward movement in the previously described hierarchical organization of precursor lesions. The presence of progressive as well as regressive changes and the slow pace of change offer special opportunities to inhibit progression through intervention strategies targeting previously identified etiological factors. The difficulties and opportunities offered by the long term follow-up studies as well as the congruency of the findings with current etiological hypotheses are discussed.

INTRODUCTION

Cross-sectional studies of gastric cancer precursors in the high risk population of Nariño, Colombia, have yielded indices of progression and correlations with gastric juice chemistry parameters (1). Such studies cannot provide information on the time required for different steps or on the evolution of a given lesion in different individuals. In order to have a more complete picture of the dynamics of the gastric precancerous process, a cohort of individuals in Nariño have voluntarily agreed to 2 or more endoscopic procedures including multiple gastric biopsies with a minimum interval of 3 years. Since the changes in the standards of living in that community have been minimal, it is expected that the observations reported here adequately represent the natural history of the gastric precancerous process under conditions of high risk.

MATERIALS AND METHODS

The recruitment of the cohort and the methodology for taking and evaluating the gastric biopsies has been described (1). A total of 1788 individuals were recruited between 1973 and 1983. Excluded from the cohort were 14 subjects who were found to have an unsuspected carcinoma at first biopsy. A total of 1422 (79.5%) came back for a second biopsy 3–16 years later, with the following distribution: 3–5 years = 875; 5–7 = 331; 7–9 = 64; 9–16 = 152. They provide a total of 7290 person-years of observation and a mean follow-up interval of 5.1 years. The protocol for the gastroscopy procedure specified the same locations to be sampled. It should be understood, however, that finding the same spot of the previous biopsy in the gastric mucosa is practically impossible. Since the cancer precursor lesions of chronic atrophic gastritis, intestinal metaplasia, and dysplasia appear as multiple foci, a certain degree of random variation in sampling undoubtedly occurs and influences the results. The 358 individuals lost to follow-up are shown in Table 1 which presents the reasons for such loss. Only 116 subjects (6.5%) refused the second biopsy. Twelve subjects developed new gastric cancers. Only 7.6% could not be contacted.

The biopsies were interpreted by the same pathologist (C. C.) following the same protocol and diagnostic criteria agreed upon at the beginning of the study (2). At the end of the study a 10% random sample of all biopsies (328 sets) was selected to be reviewed independently and blindly by 2 different pathologists (P. C. and B. R.). A total of 250 sets of biopsies were reviewed by both pathologists; the remaining 78 were reviewed only by B. R. Table 2 shows interobserver agreement ranging from 76–82.6%. Additionally, all cases with a diagnosis of dysplasia were independently reviewed by the 2 pathologists performing the validation (P. C. and B. R.) and then observed jointly with the original pathologist (C. C.). All cases originally diagnosed as dysplasia were confirmed by the reviewers after 3 such disagreements turned out to be based on different slides which had been unintentionally filed separately and therefore were not in the original sample. It should be pointed out that in a few cases dysplasia was considered "doubtful" or "borderline." Such cases are coded as metaplasia. All diagnoses in the final tabulation are those of the original pathologist.

RESULTS

Table 3 expresses the results in terms of the numbers of cases and the observed/expected ratios for each specific diagnosis in the first and the second biopsy. In many cases biopsies were taken on more than 2 occasions and the results presented express the highest readings (most advanced lesion) in groups of biopsies separated by the longest time interval. The diagnosis of N and SG are combined in one category in view of the presence of inflammatory cells in the great majority of the cases and the difficulties in drawing a line separating normal from mild gastritis.

Table 3 shows that the majority of diagnoses did not change in the time interval considered: the diagonal cells account for 873 cases, compared with 451 expected assuming only random changes between the findings of the 2 time periods. Some of the variability in the first *versus* the second biopsy may be due to the sampling of lesions which are multifocal in nature. Extreme differences in the 2 readings were observed infrequently and represent a small fraction of the number expected on the basis of chance variations. The ability to discard an important role for chance variation coupled with good interobserver agreement in blind readings of slides (the latter ruling out systematic observer error as an important source of variation in Table 3) leads us to interpret the data in Table 3 as reflecting information on the progression and regression of precursor stages. A total of 427 lesions progressed and 122 regressed, indicating a ratio of progression to regression of 3.5 progressions for each regression. Overall, 55% of the cases were

Received 11/7/89; revised 3/6/90.

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[1] Work supported by Grant P01-CA28842 from the National Cancer Institute.

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Table 1 *Follow-up status of cohort members by initial diagnosis*

	N+SG	CAG	IM	Dysplasia	Total
Completed	782	261	297	82	1422
Follow-up <3 yr	3	4	1	0	8
Died of gastric cancer	1	2	6	1	10
Died of other diseases	11	7	12	2	33
Emigrated	31	20	12	0	63
Refused:					
Afraid of procedure	34	14	10	1	59
Other illness	9	1	1	1	12
Disapprove of project	10	4	3	0	17
Too busy	5	1	3	0	9
"Do not feel ill"	9	1	0	1	11
Seeing other doctor	4	1	1	0	6
Pregnant	1	1	0	0	2
Wrong person	1	0	0	0	1
Unable to contact	64	41	23	7	135
Total	966	358	369	95	1788

classified as normal or superficial gastritis in the first biopsy and 38% in the second. The ratio of dysplasia to metaplasia was 27.5% for the first biopsy and 36% for the second.

No clear differences in progression or regression were seen between the 2 sexes. A greater proportion of regressions was observed before age 41 years (61 of 177 eligible) than after that age (61 of 200), and this was equally observed for both sexes (data not shown).

The rate of progression and regression of gastric lesions can be approximated by comparing the distribution of the first and second biopsies, taking into account the person-years in each category. The net loss of normals/100 person-years was 3.3. The corresponding data for each diagnostic category in Table 4 show a gain of 1.7% CAG,[3] 0.9% IM, and 0.7% dysplasia. The net gain for carcinoma was 0.03/100 person-years but that number does not include 10 patients who developed gastric cancer diagnosed by local physicians and who died from the disease. The rate calculated on the total number of patients (12) known to have developed gastric cancer in the period of observation would be 0.16/100 person-years.

Information on net gains and losses hides the fact that transitions between categories of gastric pathology may occur in both directions, as shown in Table 5, which indicates the direction of the change based on all diagnoses of higher (progression) or lower (regression) grades. Progression rates for N + SG to atrophy or higher grades and from atrophy to metaplasia or higher grades are greater than the corresponding regressions. The progression of metaplasia to dysplasia is lower than the corresponding regression. The pool of eligibles is greater for metaplasia than for dysplasia, resulting in yearly additions to the dysplasia pool. Rates of dysplasia are affected by sampling errors to a greater extent than other categories. The foci of dysplastic changes tend to be small and may be missed but they tend to be found again in repeat biopsies. Only clearly demonstrable dysplasia was tabulated; borderline or doubtful cases were classified as metaplasia.

Table 6 shows the transition rates by age. There is a tendency for progression rates to be higher and regression rates to be lower in older subjects, but most differences are not statistically significant. No significant difference or specific trend in transition rates by sex was detected.

Gastric juice chemistry parameters in most cases were measured only at first biopsy and on cross-sectional studies showed a strong correlation with histopathology (1, 3). Gastric juice pH and nitrite fail to predict future changes. The progression and regression rates, expressed in terms of person-years, were remarkably stable when comparisons were made between 3–5 and 5–7 years of observation. For longer periods there were not adequate numbers in the different pH and nitrite categories to reach meaningful conclusions. These measures fail to document temporal changes in gastric juice parameters which might have occurred during the period of observation. Similarly, negative results were obtained for several one-time evaluations of dietary items and serum levels of carotene, retinol, tocopherol, and ascorbic acid, apparently indicating that one-time measurements of such parameters are insufficient to monitor changes over time. The only exception to this rule relates to rates of regression from dysplasia to metaplasia in relation to blood retinol levels, which were 2.5/100 person-years for low, 4.6 for moderate, and 8.0 for high retinol levels.

For purposes of special studies, in cases in which the biopsy was considered less than adequate, and in cases of doubtful or definite dysplasia, more than 2 biopsies were taken at variable intervals. Complete tabulations of multiple biopsies showed that dysplastic changes were not detected in every biopsy but were frequently found in repeat biopsies after a negative result. This illustrates well the problem of sampling errors in evaluating the prevalence of dysplasia. Although it is common to have a dysplasia negative biopsy after a dysplasia positive biopsy (55 subjects), repeated biopsies often detect dysplasia again in the same patient (22 cases). A negative diagnosis after a dysplasia diagnosis may represent sampling problems rather than biological regression. Table 7 shows a few selected cases with multiple biopsies, illustrating the rather frequent false negative readings in such patterns. The phenomenon has been previously well documented in other organs such as the cervix uteri and the colon (4, 5).

DISCUSSION

Our study was conducted in a rural underdeveloped community of very low economic resources. In the high risk adult community of rural Nariño in Colombia, where socioeconomic changes impacting on life style have been minimal in recent decades, the net loss of individuals whose gastric mucosa showed normal histology or superficial gastritis was approximately 3.3%/year, representing a net gain of 1.7% for CAG, 0.9% for IM, and 0.7% for dysplasia. The progressive loss of normals was strongly reflected in the cross-sectional analysis at the time of the first biopsy in which the yearly weighted average of loss was 5% (normals at age 18–24 years = 77%; 25–34 years = 63%; 35–44 years = 35%; 55 years or older = 41%). The long term follow-up and the high rate of subject retrieval illustrates the feasibility of such studies in highly motivated communities. Our series is probably the largest which has been followed for the longest time and reported in recent times. Similar large series have been studied in Finland (6) and Yugoslavia (7) and have provided similar cross-sectional results. Progression and regression rates for different histopathological stages, based on prolonged follow-up of large cohorts, are reported here for the first time. Hopefully they will be useful for interpopulation comparisons and for clinical trials utilizing intermediate end points.

Our results illustrate the complexity of the dynamic flow of gastric precancerous lesions as well as the opportunities and problems of trying to monitor them with sequential biopsies. Although sampling problems exist, the general message seems to be that there is a slow forward movement of lesions but that

[3] The abbreviations used are: CAG, chronic atrophic gastritis; IM, intestinal metaplasia; SG, superficial gastritis; N, normal.

Table 2 *Blind readings of gastric biopsies, random sample*

Pathologist C. C.	Pathologist P. C.					Pathologist B. R.				
	N+SG	CAG	IM	Dysplasia	Carcinoma	N+SG	CAG	IM	Dysplasia	Carcinoma
N+SG	97	24	3	0	0	123	30	2	0	0
CAG	23	42	4	0	0	15	73	4	1	0
IM	1	0	49	2	0	1	1	69	0	0
Dysplasia	0	0	1[a]	2	0	0	1[a]	2[a]	4	0
Carcinoma	0	0	0	0	2	0	0	0	0	2

[a] Disagreement due to accidental separate filing of original slides showing dysplasia.

Table 3 *Joint distribution of gastric pathology reported on first and second biopsy*

Second biopsy	First biopsy									
	No. of biopsies						Ratio of observed/expected			
	N+SG	CAG	IM	Dysplasia	Total	%	N+SG	CAG	IM	Dysplasia
N+SG	496	23	18	3	540	38.0	1.67	0.23	0.16	0.10
CAG	174	147	53	9	383	27.0	0.83	2.09	0.66	0.41
IM	94	80	176	16	366	25.7	0.47	1.19	2.30	0.76
Dysplasia	15	12	50	54	131	9.2	0.21	0.50	1.82	7.20
Carcinoma	1	0	1	0	2	0.1	0.91		2.50	
Total	780	262	298	82	1422					
%	54.8	18.4	21.0	5.8		100.0				

Table 4 *Net gain or loss in absolute numbers*

	Initial	Repeat	Net gain or loss	Per 100 person-years
N+SG	780	540	−240	−3.3
CAG	262	383	+121	+1.7
IM	298	366	+68	+0.9
Dysplasia	82	131	+49	+0.7
Carcinoma	0	2	+2	+0.03[a]
Total	1422	1422		

[a] See text.

Table 5 *Rate of transition in gastric pathology to all lower or higher stages between first and second biopsy/100 person-years*

Transition	At risk	*N*	Rate/100 person-years
N+SG → atrophy	780	284	7.5
Atrophy → N+SG	262	23	1.7
Atrophy → metaplasia	262	92	6.7
Metaplasia → atrophy	298	71	4.4
Metaplasia → dysplasia	298	51	3.2
Dysplasia → metaplasia	82	28	5.7

such movement is not constant and linear in all individuals. The use of the person-years concept is the most efficient option to introduce a time dimension to the analysis of the precancerous process. The concept is ideally suited to study cohorts in which death (an unavoidable event) is the end point. If less certain events are the end points, such as the development of tumors or precancerous lesions, the person-years concept yields an approximate measure of incidence; it does not provide exact measures of the progressive and regressive phenomena but is the best available approximation.

The difficulties posed by sampling multifocal lesions are well illustrated in flow charts of dysplasia-metaplasia changes, which suggest that most dysplastic lesions are persistent but not always found in sequential individual biopsies.

Our cross-sectional studies based on the first gastric biopsy revealed an association between gastric chemistry parameters and histopathological findings. Our follow-up of the cohort, however, failed to detect a predictive value for the initial gastric chemistry parameters on the events leading to progression of lesions. This finding casts some doubts about the a causal association of the two phenomena. It is not clear at this point whether this lack of association reflects some obscure biological process or some unclear shortcoming of our methodology. It thus appears that the precancerous process represents a complex alternation of enhancing forces which may produce indicators of high risk (high pH and nitrite) and retarding forces which produce indicators of lower risk (low pH and nitrite) at different times. Monitoring the gastric microenvironment, therefore, requires measures at different times.

Progression and regression events probably reflect forces of modulation of the precancerous process which act in both directions with an intensity which is variable in time. Such forces are probably mostly environmental as suggested by epidemiological and experimental studies implicating irritants such as excessive salt and mutagen precursors such as nitrite in the progression and inhibitors such as ascorbic acid and β-carotene in regression events (8). Recent findings illustrate the complexity of the gastric microenvironment which probably determines the fate of the precursor lesions of the mucosa. Special functions of the gastric mucosa are being described, such as the ability to concentrate ascorbic acid (9) and β-carotene,[4] 2 potent antioxidants whose role at that anatomic site is not well understood. It could be related with protection against toxic products of oxidation such as free radicals. The atrophic gastric mucosa is associated with low levels of factors which can inhibit amine nitrosation (10). One of these factors has recently been identified as reduced ascorbic acid. A study of patients with atrophic gastritis reported that most of the ascorbic acid found in the gastric juice was in the oxidized state (9). The depletion of reduced ascorbic acid in the gastric juice

[4] S. Alam, personal communication.

Table 6 *Rate of transition to all lower or higher stages in gastric pathology between first and second biopsy/100 person-years*

Transition	≤40 yr		≥41+ yr	
	Rate/ 100 person-years	95% Confidence interval	Rate/ 100 person-years	95% Confidence interval
N+SG → atrophy	7.0	6.0–8.1	8.5	6.9–10.5
Atrophy → N+SG	1.7	0.93–2.9	1.6	0.73–3.0
Atrophy → metaplasia	5.8	4.3–7.7	7.9	5.8–10.6
Metaplasia → atrophy	5.4	3.8–7.4	3.7	2.6–5.1
Metaplasia → dysplasia	2.1	1.1–3.5	4.0	2.8–5.5
Dysplasia → metaplasia	7.9	3.8–14.5	4.9	2.9–7.7

Table 7 *Examples of findings in repeated biopsies from the same patient*

Case	Yr															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
121	D[a]		A			A				M						
312	D				MG			D								
378	D				A			D								
1303	N				D	M										
1305	M				D	M										
2019	D	DM							M							
2171	MA					A					D					
2179	M				A					A					D	
2182	D	D			A					D						
2212	M				M											AD
2388	D					M	D				M					
2634	M				D						D					
2708	D	D					M	D				M				
2774	MD				A		D									
2776	DA		G				D									

[a] A, chronic atrophic gastritis; M, intestinal metaplasia; G, chronic gastritis, not otherwise specified; D, dysplasia.

of these patients by nitrite may lead to a situation in which nitrosamine formation is no longer blocked. This has been demonstrated in gastrectomy patients through the use of the modified *N*-nitrosoproline test (11). In Nariño, patients with intestinal metaplasia or dysplasia had a high level of correlation between urinary nitrate and *N*-nitrosoproline, indicating a selective increase in nitrosation of amines in the gastric microenvironment in patients with such lesions (12) but not in patients with normal mucosa or lesser degrees of chronic gastritis.

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