original paper

haematologica 2001; 86:609-617
http://www.haematologica.it/2001_06/0609.htm

Treatment of low-grade gastric mucosa-associated lymphoid tissue lymphoma in stage I with *Helicobacter pylori* eradication. Long-term results after sequential histologic and molecular follow-up

CARLOS MONTALBAN,* ALMUDENA SANTON,° DANIEL BOIXEDA,# CLARA REDONDO,° ISABEL ALVAREZ,° JOSE LUIS CALLEJA,* CARLOS MARTIN DE ARGILA,# CARMEN BELLAS°
Departments of Internal Medicine,* Pathology° and Gastroenterology,# Hospital Ramón y Cajal, Madrid, Spain

Correspondence: Carlos Montalban, M.D., Department of Internal Medicine, Hospital Ramón y Cajal, Carretera de Colmenar km 9, 28034 Madrid, Spain. Phone: international +34-91-3368279 – Fax: international +34-91-3369016 – E-mail: cmontalban@hrc.insalud.es

Background and Objectives. Most cases of gastric low-grade mucosa-associated lymphoid tissue (MALT) lymphoma are associated with *H. pylori*. In localized disease (stage I), eradication of *H. pylori* can result in histologic regression of the lymphoma in 50% to 100% of the patients. Moreover, in half of the apparently cured patients a monoclonal rearrangement of the IgH gene can be demonstrated. However, data on the long-term outcome of the patients are scarce. We report the evolution of a series of patients followed-up since 1994 in order to evaluate the long-term outcome of the apparently cured lymphoma.

Design and Methods. From January 1994 to July 2000, 19 consecutive patients with stage I gastric low grade MALT lymphoma were sequentially studied in our hospital. They had all been diagnosed by endoscopy and had had a complete staging (including CT-scan, contrast X-ray of the small bowel, bone marrow biopsies, immunophenotyping of bone marrow and peripheral blood and, in the later years, endoscopic ultrasonography). Diagnosis required established histologic criteria for low grade MALT lymphoma in the samples obtained by endoscopy. The investigation of *H. pylori* status included histologic search, serology and breath test urea-^{13}C. Only patients in stage I disease associated with *H. pylori* were included in the study. Patients received standard triple therapy for eradication of *H.pylori* and after treatment were sequentially followed-up with endoscopies performed every 2-3 months in the first year, every 6 months in the second year and then yearly. Post-treatment biopsies were obtained by endoscopy for histologic studies, *H. pylori* cultures and molecular studies. The criteria of Wotherspoon *et al.* were used for the histological evaluation. Molecular studies were performed with a polymerase chain reaction analysis of the IgH gene using semi-nested procedures with consensus primers for the V_H (Fr3A/Fr2A) and J_H (LJH and VLJH) regions.

Results. After the eradication treatment, 18 of the 19 patients (94.7%) achieved histologic regression of the MALT lymphoma that occurred after a mean of 4.6 months (range 2-19). In 11 of the 18 histologically cured patients (61%) a monoclonal rearrangement of the IgH gene was demonstrated. In 2 patients the monoclonality disappeared completely, but 9 of the 11 patients (82%) had either persistent (3 patients) or intermittently persistent (5 patients) monoclonality for as long as 64 months. None of the patients who achieved a histologic remission (either with or without monoclonality) relapsed after a mean follow-up of 37 months (range 2-78). Two patients were lost to follow-up and another patient died of a gastric carcinoma; the remaining 15 patients are still in histologic remission after a mean period of 43 months (range 5-78). Ten patients studied between 1994 and the end of 1996 are in remission after a mean of 59 months (range 33-78).

Interpretations and Conclusions. In most cases of gastric low-grade MALT lymphoma in stage I eradication of *H. pylori* can produce histologic regression of the lymphoma and this regression can be maintained for years. However, IgH gene monoclonality can be detected and persists in most cases. Although this persistent monoclonality seems to indicate the presence of a latent lymphoma population, over a period of 6 years it has not so far influenced the outcome. These findings indicate that in cases of localized gastric low-grade MALT lymphoma associated with *H. pylori*, the first step of treatment should be eradication of the *H. pylori*; however, a close and long follow-up is essential to determine the ultimate outcome of these patients and the possible significance of the persistent monoclonality.

Key words: lymphoma, MALT, gastric lymphoma

The association of *H. pylori* with gastric lymphoma arising in the mucosa-associated lymphoid tissue (MALT) has been well established. *H. pylori* infection causes the appearance of MALT[1,2] as follicular gastritis. It is present in most cases of gastric low-grade MALT lymphoma[1] and the growth of the lymphoma is dependent on the antigenic drive of *H. pylori*.[3] Moreover, eradication of *H. pylori* can make the lymphoma regress.[4]

Since the pioneering paper by Wotherspoon *et al*.[4] reporting the histologic regression of low-grade MALT lymphoma limited to the stomach after *H. pylori* eradication, other series have confirmed this initial response in 50% to 100% of patients.[5-11] However, as the follow-up of the lymphomas that respond to *H. pylori* eradication was not long, it is not clear for how long patients can maintain the remission, what their long-term outcome is and what factors could influence the possible relapse of the lymphoma.

In 1997 and 1998 we reported the preliminary results of a series of patients with stage I low-grade gastric MALT lymphoma treated by eradication of *H. pylori*. These patients had been studied since 1994 and sequentially followed-up with endoscopies to study the histologic and molecular evolution of the lymphoma.[9,10] The initial results showed that up to 90% of the patients achieved total regression of the lymphoma after the eradication treatment and that, once achieved, all patients maintained the remission after a mean time of 18 months. Our results also showed that there was a monoclonal rearrangement of the IgH gene in 60% of the histologically cured patients, as had also been observed by others[4,6,7,8,11,12] and that in most cases the monoclonality seemed to disappear during the follow-up.[7-11]

In the present paper we report the follow-up of 19 patients consecutively studied since 1994. We report the histologic and molecular response to treatment and, although patients were accrued along these years, we can also report the long-term outcome of the patients who were enrolled earliest and have now been followed-up for seven years.

Design and Methods

Nineteen consecutive patients with low-grade stage I gastric MALT lymphoma newly diagnosed at the Hospital Ramon y Cajal (Madrid, Spain) from December 1993 to July 2000 were included in a long-term prospective study to evaluate the response to *H.pylori* eradication as initial and only therapy of their lymphoma. The patients were sequentially followed-up in the Outpatient Clinic of the Department of Internal Medicine. The same group of experienced endoscopists performed all the endoscopies in the Department of Gastroenterology. The same pathologists studied all the histologic samples applying the same criteria, and all the molecular studies were performed in the Laboratory of Molecular Pathology of the Department of Pathology. The initial results from 1994 to 1997 have been previously reported elsewhere.[9,10] This article reports the follow-up of the patients up to December 2000. One of the patients included in the previous reports[9,10] has not been included in the present study, as her diagnosis was made in another hospital.

Initial histologic diagnosis was established by means of endoscopic biopsies obtained from the fundus, body of the stomach, antrum, and duodenum and from any macroscopic tumor or suspicious area. Endoscopic patterns were considered as infiltration, ulceration or tumor pattern, as described elsewhere.[13,14] The infiltration pattern described an enlargement and thickening of the gastric folds, with or without superficial erosions; an ulceration pattern described the presence of isolated ulcers. In addition, a chronic gastritis pattern described the presence of irregular patchy erythematous lesions. Other minor endoscopic changes were considered normal. Biopsy samples were routinely processed for histologic study and stained with hematoxylin and eosin, and Giemsa. Low grade MALT lymphoma was diagnosed when unequivocal histologic features, infiltration by centrocyte-like cells and lymphoepithelial lesions were identified.[4,15] Breath test urea-^{13}C and immunoglobulin G (IgG) antibody measurement assay using a quantitative enzyme-linked immunosorbent assay (ELISA) commercial kit (Helico-G, Porton, Cambridge, UK) were performed as part of the initial study. Staging procedures included physical examination, standard blood and biochemical parameters, chest and abdominal CT-scan, contrast X-ray of the small bowel, bone marrow biopsy and immunophenotyping of peripheral blood and bone marrow lymphocytes. From January 1999 (patients #15 to 19), endoscopic ultrasonography was routinely included as part of the initial study of the patients. Only patients with demonstrated association of *H. pylori* infection and localized gastric disease, stage I of the Lugano[16] and Musshoff systems, were eligible for the treatment protocol. All patients accepted their inclusion in the study. All received eradication therapy, which in patients #1 to 9 consisted of amoxicillin 750 mg plus metronidazole 500 mg tid and omeprazole 40 mg bid daily for 14 days; in patients #10 to #19, the eradication protocol consisted of clarithromycin 500 mg, amoxicillin 1,000 mg and omeprazole 40 mg bid daily for 14 days.

Patients were followed-up by sequential clinical and endoscopic examinations; the first control was performed 2-3 months after eradication treatment, thereafter, ideally, every 3 months the first year, every 6 months the second year and yearly after the third year. Breath test urea-^{13}C was also performed in all the successive evaluations. In the post-treatment sequential endoscopies, a set of three biopsies was obtained from each of the above mentioned four areas (fundus, body of the stomach, antrum and duodenum) and from any other suspicious area. One biopsy sample from each

site was used for the histologic study, another one for *H. pylori* culture and the remaining sample for the molecular study. Histologic evaluation was performed following the system described by Wotherspoon *et al*.[4]: score 0: normal, 1: chronic active gastritis, 2: chronic active gastritis with follicle formation, 3: suspicious lymphoid infiltrate in lamina propria, probably reactive, 4: suspicious lymphoid infiltrate in lamina propria, probably lymphoma, 5: low grade MALT lymphoma. Complete histologic regression was accepted to have occurred when no definitive lymphoma was demonstrated in any biopsy samples (grade 0 to 3). Fresh samples were cultured for *H. pylori* in a non-selective medium and in a selective one (Agar Columbia with polymyxin, amphotericin, trimethoprim, vancomycin and nalidixic acid in a microaerophilic atmosphere). Molecular studies were performed by means of polymerase chain reaction amplification of the IgH gene.

Polymerase chain reaction analysis

A polymerase chain reaction (PCR) analysis of the IgH gene was performed as previously described elsewhere.[9,17] High molecular weight DNA was prepared from frozen biopsy material using standard phenol/chloroform extraction. Control DNA samples were extracted from the Raji B-cell line (clonal control) and from reactive lymphoid tissue (polyclonal control). Amplification of the IgH gene was performed on all samples using semi-nested procedures with consensus primers for the V_H region (Fr3A or Fr2A) in conjunction with nested primers directed to the J_H region (LJ_H and VLJ_H). Ten microliters of PCR product was electrophoresed on 5% (Fr2A) or 10% (Fr3A) polyacrylamide mini-gels, which were stained with ethidium bromide and viewed under UV light.

Results

Eleven of the 19 patients in this series were male (58%) and 8 female (42%). Their median age was 57 years (range 26 to 76 years). Seventeen of the 19 patients (89%) had gastric symptoms at presentation: 10 (52.6%) (patients #1, 3, 4, 5, 6, 7, 10, 11, 15, 17) complained of epigastric burning pain, 6 (31.5 %) (patients #2, 8, 12, 14, 15, 16) had gastric bleeding and the remaining patient (patient #9) had epigastric pain and 18 kg weight loss. The two asymptomatic patients were studied because of iron deficiency. The initial endoscopy showed an infiltration pattern in 10 patients (52.6%) (patients #1, 2, 3, 6, 9, 10, 11, 16, 17 and 18), and ulceration pattern in 8 (42%) (patients #4, 5, 7, 8, 12, 13, 14 and 15) and one patient (5%) (patient #19) had a macroscopic chronic gastritis pattern. *H. pylori* was eradicated in all 19 patients after the initial antibiotic treatment. The symptoms disappeared in all patients after eradication treatment. Post-treatment endoscopic patterns, histologic and molecular findings are shown in Table 1. Of the 19 patients, 18 (94.7%) achieved histologic disappearance of the gastric MALT lymphoma after *H. pylori* eradication treatment. The regression of the lymphoma occurred a mean of 4.6 months (range 2 to 19) after the eradication treatment; most cases achieved regression of the lymphoma between 2 and 7 months, but in patients #15 and 16 a consistent histologic regression of the lymphoma was achieved only after 19 and 11 months, respectively. The patient with no initial response was followed-up for 5 months and then treated surgically. The gastrectomy specimen confirmed the presence of low grade MALT lymphoma limited to the mucosa in the fundus, without a large cell component; he has not relapsed after 6 years of follow-up.

In all 18 patients who achieved a complete histologic disappearance of the lymphoma, the remission has been maintained for a mean of 37 months (range 2 to 78 months). No patient relapsed after the initial regression of the lymphoma. Of this group of 18 patients, two were lost to follow-up (patients #5 and 7) after 2 and 8 months. Another patient (patient #11) had a histologic regression of the lymphoma 2 months after eradication treatment, but 2 months later he again complained of gastric pain, asthenia and weight loss. An endoscopy performed then showed diffuse infiltration of the fundus, body and antrum of the stomach with a large ulcer in the junction of the body and antrum. The mapped biopsies obtained showed no lymphoma, but the samples from the body of the stomach demonstrated the presence of a signet-ring cell carcinoma. His disease had an explosive course and he died 2 months later with carcinoma dissemination to abdominal lymph nodes, liver, peritoneum, bones and bone marrow. The 15 remaining patients have been in complete remission for a mean of 43 months (range 5 to 78). The 10 patients included in the study between 1994 and the end of 1996 have been in complete remission for a mean of 59 months (33 to 78 months). *H. pylori* reinfection was demonstrated only in patient #9, 18 months after the initial treatment; he then received another course of eradication treatment which was successful. He has been free from further reinfection for three years.

The molecular studies showed that the patient with persistent active lymphoma had a consistent monoclonal IgH gene rearrangement in all the sequential biopsies. In the 18 remaining patients achieving histologic regression of the lymphoma, polyclonal rearrangement of the IgH gene was found only in 7 patients (39%), whereas a monoclonal rearrangement

Table 1. Clinical, endoscopic, histologic and molecular follow-up.

Patient Age (years)	*Months after Helicobacter pylori eradication*	*Endoscopic Pattern*	*Biopsy scoring*	*PCR IgH gene*	*Outcome after Helicobacter pylori eradication*
1. BMP, 59. Male	1	G	5	M	Failure
	4	G	5	M	Surgery 5 months after eradication.
	5	N	5	M	
2. AMO, 59. Male	2	G	4	P	CR after 76 months
	5	N	2	M	
	10	N	1	M	
	11	N	1	P	
	13	N	1	P	
	19	N	0	P	
	28	G	0	P	
	38	G	2	P	
	49	N	1	M	
	59	N	1	P	
	76	G	1	P	
3. ZRM, 42. Male	2	I	1	M	CR 75 after months
	6	G	1	ND	
	9	G	1	P	
	13	G	1	M	
	20	G	1	P	
	26	N	0	P	
	32	N	0	ND	
	40	I	1	P	
	52	G	0	M	
	64	N	1	M	
	75	N	0	P	
4. RGA, 58. Female	2	G	4	M	CR after
	7	N	1	P	78 months
	12	N	1	P	
	18	N	1	P	
	22	N	1	P	
	30	G	2	P	
	36	N	0	P	
	44	G	1	ND	
	53	N	0	ND	
	59	N	0	P	
	68	N	0	P	
	78	G	0	P	
5. JJF, 60. Male	2	G	2	P	CR after 2 months. Lost to follow-up
6. JBA, 76. Female	2	I	1	P	CR after 69 months
	6	G	1	P	
	10	G	0	P	
	15	G	1	P	
	20	G	1	P	
	25	G	0	P	
	33	G	0	P	
	42	G	1	ND	
	48	G	1	P	
	59	G	1	P	
	69	G	0	P	
7. LGF, 66. Female	3	G	3	P	CR after 8 months. Lost to follow-up
	8	G	3	P	
8. FIS, 71. Female	1	G	4	ND	CR after 75 months
	5	G	2	M	
	12	G	2	P	
	17	G	2	P	
	23	G	1	ND	
	30	N	1	P	
	40	N	0	P	
	48	N	0	ND	
	56	G	1	ND	
	65	N	1	P	
	75	G	0	P	
9. JTV, 26. Male	2	G	4	P	CR after 54 months
	6	N	0	P	
	11	N	0	M	
	13	N	1	M	H. pylori reinfection at
	18	G	1	M	18 months.
	24	N	0	M	Successful eradication
	31	N	0	M	
	37	N	0	ND	
	42	N	0	P	
	54	G	0	P	

(continued on next page)

Patient Age (years)	*Months after Helicobacter pylori eradication*	*Endoscopic Pattern*	*Biopsy scoring*	*PCR IgH gene*	*Outcome after Helicobacter pylori eradication*
10. AEG, 74. Female	2	G	0	P	CR after 51 months
	8	G	2	M	
	11	N	0	M	
	19	G	1	P	
	25	G	3	P	
	32	N	0	P	
	40	G	2	M	
	51	N	0	M	
11. JVL, 72. Male	2	I	0	P	CR after 4 months
	4	U	0/Carcinoma	M	Dead of gastric cancer 6 months after diagnosis of lymphoma
12. TAB, 56. Female	2	N	1	P	CR after 39 months
	5	N	1	ND	
	9	N	1	ND	
	12	N	2	P	
	17	N	0	ND	
	23	N	0	P	
	29	N	0	P	
	39	G	1	P	
13. JMM, 55. Female	3	N	1	M	CR after 39 months
	8	G	2	P	
	11	G	1	ND	
	16	G	1	ND	
	23	G	0	M	
	30	N	1	P/O	
	39	G	0	P	
14. COC, 73. Female	3	G	3	M	CR after 33 months
	7	N	2	M	
	13	N	0	ND	
	20	G	1	ND	
	33	N	0	M	
15. JHM, 59. Male	3	N	5	ND	CR after 21 months
	5	N	2	ND	
	8	N	5	M	
	11	N	4	P	
	15	N	5	P	
	19	N	1	M	
	21	N	1	P	
16. JGH, 50. Male	3	U	5	ND	CR after 19 months
	5	N	0	P	
	8	I	5	M	
	11	I	0	ND	
	14	I	0	M	
	19	G	1	M	
17. FLF, 33. Male	3	G	1	P	CR after 9 months
	5	N	0	P	
	9	N	0	P	
18. BTR, 47. Male	2	G	4	P	CR after 9 months
	5	G	2	P	
	9	G	1	P	
19. MMF, 52. Male	2	G	2	P/O	CR after 5 months
	5	G	1	P	

(a)Endoscopic patterns:[13,14] I:infiltration, U: ulceration, G: chronic gastritis, N: normal; (b) Biopsy scoring: 0: normal, 1: chronic active gastritis, 2: chronic active gastritis with follicle formation; 3: suspicious infiltrate in lamina propria, probably reactive, 4: suspicious infiltrate in lamina propria, probably lymphoma, 5: low grade MALT lymphoma; (c) PCR IgH gene analysis. M: monoclonal, P: polyclonal, O: oligoclonal, ND: not done or PCR failure to amplify IgH gene; (d) CR: complete histologic and clinical remission.

was found in at least one of the samples from the above mentioned areas, in 11 patients (61%) at different moments of the study. Six patients had monoclonality in the initial studies (patients #3, 4, 8, 13, 14 and 15): in two of them a polyclonal rearrangement was achieved after 7 (patient #4) and 12 months (patient #8); another patient (patient #14) showed persisting monoclonality for 33 months and the remaining three patients (patients #3, 13 and 15) had intermittent monoclonality for 64, 23 and 19 months, although the final studies showed polyclonal rearrangements. Five more patients (patients #2, 9, 10, 11 and 16) had polyclonal IgH gene rearrangements in the first endoscopic control, but monoclonality has been intermittently detected throughout the follow-up: two of them had monoclonality for 49 (patient #2) and 31 months (patient #9) with later polyclonal rearrangements; two (patients #10 and

#16) had persistent monoclonality after 51 and 19 months respectively, and in patient #11 monoclonality was detected at the final endoscopy, before he died of gastric carcinoma. As a whole, 9 of the 11 patients (82%) had persistent or intermittently demonstrated monoclonality during the follow-up, which only in two cases evolved to a consistently maintained polyclonal rearrangement. Although the monoclonal PCR fragments in the serial biopsy specimens were not sequenced, in each patient they were the same size implying that the same neoplastic clone seemed to be present. In two patients (patients #13 and 19), the polyclonal IgH gene rearrangement occasionally consisted of three distinct bands, indicating an oligoclonal pattern of rearrangement; in the first one an evolution from monoclonal to oligoclonal and finally to a polyclonal rearrangement was detected and in the second, the oligoclonal rearrangement initially demonstrated evolved to a polyclonal rearrangement.

Discussion

Histologic regression of low grade gastric MALT lymphoma localized in the stomach has been reported in 50% to 100% of the patients who receive *H. pylori* eradication as sole treatment.[4-8,11,12] In our present series 94.7% of the patients achieved a regression of the lymphoma. This is a higher proportion of responses than those obtained in most series. This may be explained in part because only MALT lymphomas associated with *H. pylori* and with limited disease extension, stage I, were included in the study. With this and other published results[4-7,8,11,12] it is now well established that *H. pylori* eradication should be the first step in the treatment of patients with stage I gastric low-grade MALT lymphoma associated with *H. pylori*. However, as all these studies began in 1993-1994, the follow-up of the patients is relatively short and many problems remain to be solved.

The first question is whether or not the lymphoma can in fact be cured after the initial histologic response. The results of the present series indicate that in our environment most patients achieve histologic regression of the lymphoma and that the regression seems to be maintained for years, since our initial patients have been in remission for more that six years without relapse. This has also been the experience of most groups reporting long follow-up periods.[18,19,20] The first series describing the response of gastric low-grade gastric MALT lymphoma to eradication therapy reported by Wotherspoon *et al.*[4] included six patients: all had been in complete remission after six years of follow-up.[18] In the update of the Italian series of Savio *et al.*,[7] comprising 76 patients, 93% achieved a complete remission and only 8% relapsed after a median follow-up of 28 months.[19] In the updating in 2000 of the large German series initially reported by Bayerdörffer *et al.*,[6] including 120 patients, 97 (81%) achieved a complete remission and only 9% relapsed after a median follow-up of 37 months.[20] However, other series have not achieved such good long-term results. The interim analysis in year 2000 of an international prospective group including 189 patients showed that only 55% of the patients achieved a complete regression of the tumor and that 7% relapsed after a median follow-up of 26 months.[21] In the series of Pinotti *et al.*, only 3 of 16 patients (23%) had persistent histologic regression after a mean follow-up of 61 months.[22]

Another question is when to expect the histologic regression of the lymphoma after eradication therapy. In most patients the response is quite rapid and can be seen in the first 2 to 6 months;[5,6] this was also the case in the patients in our series, although two patients achieved apparently sustained responses only after 11 and 19 months. Similarly protracted responses, up to 24 or 27 months, have also been reported.[19,20] It has usually been considered that a prudent expectation for histologic cure might be 12 months;[11,15,23] however, as these two cases show, later responses may also occur and when the patients can be closely followed-up by sequential endoscopies (and ideally with endosonography to early detect eventual extension through the gastric wall), probably a longer period of observation without further therapy might be acceptable.

Most patients seem to achieve a complete and long-lasting histologic regression of the lymphoma, but the reasons why some patients do not respond are not always apparent. It is now clear that gastric low-grade MALT lymphomas not associated with *H. pylori* do not respond to antibiotic treatment.[11,23] In some patients the absence of response or the relapse clearly depend on the presence of a previously present, but undetected, high-grade component.[6] Infiltration of deeper layers of the gastric wall also conditions a worse chance of response[11,23-25] and the detection of significant perigastric lymph node involvement in the endoscopic ultrasonography is a crucial factor for the absence of response.[23] The involvement of the distal stomach may determine a better response.[11] Other factors seem to be the expression of certain specific cytogenetic abnormalities. In fact, in low-grade gastric MALT lymphomas which respond to eradication therapy the fusion transcript API2-MLT of the translocation t(11;18) has not been found,[26,27] whereas it was detected in 75% of patients who failed to respond to eradication treatment.[27] BCL10 gene mutations involved in the translocation t(1;14) were

found in 27% of patients who did not respond to eradication therapy, but in none of the 22 responders, indicating that these mutations may play a role in determining a more aggressive behavior of the gastric MALT lymphomas.[28] Other geographic or genetic factors may also influence the response: there are striking differences in the initial and long-term responses reported in different geographic areas, with a lower proportion of responses and a higher proportion of relapses in a series from U.S.,[11] in one from Italy[22] and in another from Switzerland,[21] although the latter included patients from different countries (United Kingdom, France, Switzerland and Northern Italy). This may be the consequence of specific genetic factors in the patient populations, of environmental factors or of different prevalences of *H. pylori* genotypes, since CagA$^+$ strains might be associated with gastric MALT lymphoma[29] and other distinct genotypes with discrete histologic findings implying different prognoses.[30] However, lymphomas associated with CagA$^+$ *H.pylori* strains do not seem to have a worse response.[23]

At present it is not known whether any specific characteristic of the patient or of the lymphoma may predispose to a late relapse. The only factor that has been associated with a higher risk of relapse has been reinfection with *H.pylori*,[31,32] an occurrence which should, therefore, be specially monitored for during the follow-up.

Another interesting question is the significance of monoclonality in histologically cured patients. In our series, more than half of the patients (61%) had a monoclonal rearrangement of the IgH gene. This has also been found in other series, with monoclonality demonstrated in between 33% and 71% of the patients who achieved a complete histologic response.[7,8,11,18,21,33] In our previous reports,[9,10] and in others,[7,8,11] the monoclonality detected in the early controls seemed to disappear in most patients during the follow-up,[8,9] although its persistence has been occasionally detected for up to 5-6 years.[18,19,33] The disappearance of the monoclonality has not been surprising and, in accordance with an optimistic view, it has been assumed that the regression of the lymphoma is a dynamic ongoing process: the histologic response appears first and the molecular one is evidenced later. However, in the present series, when the patients had a sufficiently long follow-up, it has become clear that this is not the case and that actually in the majority of the cases the monoclonality seems to persist. In our series only two patients who had monoclonality detected in the first controls thereafter showed persistently polyclonal rearrangements. Most of the patients with monoclonality had either persistent monoclonality from the outset (detectable for long periods, up to 51 months) or an intermittently occurring monoclonality (for as long as 64 months). Most probably this latter pattern also represents persisting monoclonality that can only be demonstrated occasionally and the intermittence is probably an artefact due to sampling error or limitations of the technique, considering that monoclonal patterns can be found only in about two thirds of the cases with a confident histologic diagnosis of gastric low-grade MALT lymphoma.[7,34] This continued persistence of monoclonality despite histologic cure in many patients suggests the presence of a latent persistent lymphoma population. The demonstration by Thiede *et al.*[35] that in gastric MALT lymphoma apparently cured after *H.pylori* eradication, the residual clonal lymphocytes have ongoing somatic hypermutation and antigenic selection also supports the concept that a population of the lymphoma cells persists despite histologic regression. This may indicate, as previously considered,[18,22] that the eradication therapy suppresses, but does not completely eradicate the tumor clone. For the time being the final fate of these patients with persistent monoclonality and probably a latent subclinical lymphoma population is not clear. In some series histologic relapses have been found to be more frequent in patients with persisting monoclonality,[8,19,33] but this was not the case in our series. Longer follow-up of the patients and extensive molecular studies may contribute to elucidate the situation. With the present knowledge, these patients should receive no further treatment, but should be closely followed-up.

A final problem in relation to patients with gastric MALT lymphoma is the development of other cancers, especially gastric carcinoma. In this series one patient developed a gastric carcinoma, despite the fact that *H. pylori* had been successfully eradicated and the lymphoma apparently cured. In other series, patients treated only with *H.pylori* eradication also developed other cancers during the follow-up.[19,21] Whether there is an increased risk of second cancers in relation with gastric MALT lymphoma is not yet clear. When statistical studies were used, a significantly increased incidence was not demonstrated,[36,37] but it is a common clinical observation that the association of gastric lymphoma and gastric carcinoma can occur.[36,38] It is also clear that *H. pylori* seems to be a crucial factor for both, and although its eradication can cure the lymphoma, it is not known whether this can have a favorable impact in patients who could develop a gastric carcinoma. The finding of morphologic alterations (atrophy and intestinal metaplasia) of the gastric mucosa adjacent to primary MALT lymphomas[39] suggests that these early changes may contribute to the development of gastric carcinoma, even after *H.*

pylori eradication. All these data indicate that patients with gastric MALT lymphoma should also be followed-up for early detection of the eventual occurrence of gastric carcinoma.

In summary, our results indicate that in our environment most patients with stage I low-grade gastric MALT lymphoma related to *H. pylori* can be histologically cured with eradication therapy. However, most patients have persisting monoclonality of the IgH gene suggesting the persistence of latent lymphoma, and the ultimate outcome of the disease is not yet known. Patients with apparent histologically cured low-grade gastric MALT lymphoma should be closely followed-up for various reasons: to detect *H. pylori* reinfection, to detect eventual relapses of the lymphoma, to evaluate the significance of the persisting monoclonality and to detect early stages of eventually occurring gastric carcinoma. The only adequate way to follow up these patients is the use of sequential endoscopies and of multiple laboratory (microbiological, histologic and molecular) techniques. The patients should be followed-up for life, until more precise knowledge or more accurate techniques permit the identification of special risk groups.

Contributions and Acknowledgments

CM designed and co-ordinated the study, was responsible for the clinical management of the patients, and wrote the manuscript. CB directed the molecular study. The remaining authors contributed equally and take specific responsibilities for different aspects of the study: AS performed the molecular studies; DB and CMA performed the endoscopies; CR and IA were responsible for the histologic studies. IA also contributed to the final form of the manuscript. JLC contributed to the clinical management of the patients. All contributors revised and approved the final version of the manuscript.

We are indebted to Dr. L. Abreu and Dr. JL. Calleja in the Department of Gastroenterology of Clinica Puerta de Hierro, Madrid, for their assistance in performing the endoscopic ultrasonography.

Funding

This work was supported in part by grants FISS 94/483, 97/275 and 99/285 from the Spanish Ministry of Health.

Disclosures

Conflict of interest: none.

Redundant publications: this paper is the result of an ongoing long-term prospective study. Early data from the first patients up to 1994 , 1996 and 1997 were published in 1995 (Lancet,1995:347:789), 1997 (reference #9) and 1998 (reference #10).

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Emanuele Zucca, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Zucca and the Editors. Manuscript received February 23, 2001; accepted May 2, 2001.

Potential implications for clinical practice

Our results show that most patients with stage I low-grade gastric MALT lymphoma can achieve long-lasting histologic regression after *H.pylori* eradication. This should be their first step of treatment.

Despite histologic cure, most patients have persistent IgH gene monoclonality that suggests that a latent lymphoma also persists.

Patients should have long-term endoscopic follow-up for early detection of *H.pylori* reinfection, eventual relapse of the lymphoma or the appearance of a gastric carcinoma and to elucidate the long-term significance of persistent IgH gene monoclonality.

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