Early-Stage Gastric MALT Lymphoma: Is It a Truly Localized Disease?

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Key Words. MALT lymphoma • Monoclonality • Occult disease • VH genes

Disclosure

Marina P. Siakantaris: None; Gerassimos A. Pangalis: None; Evangelia Dimitriadou: None; Flora N. Kontopidou: None; Theodoros P. Vassilakopoulos: None; Christina Kalpadakis: None; Sotirios Sachanas: None; Xanthi Yiakounis: None; Penelope Korkolopoulos: None; Marie-Christine Kyrtsonis: None; Panayia Bobotsis: None; Athina Androulaki: None; Eustratios Patsouris: None; Panayiotis Panayiotidis: None; Maria K. Angelopoulou: None

Section editor George P. Canellos has disclosed no financial relationships relevant to the content of this article.

The content of this article has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from commercial bias.

Target audience: Physicians who wish to advance their current knowledge of clinical cancer medicine in lymphoma oncology.

LEARNING OBJECTIVES

After completing this course, the reader should be able to:

1. In your patients with gastric MALT lymphoma (GML), determine accurate staging and formulate appropriate treatment strategies.
2. Assess early stage GML patients who should be closely monitored for early intervention and manage treatment plans.
3. Design further studies with different modality treatments to explore the impact of occult blood disease on patients’ outcomes.

This article is available for continuing medical education credit at CME.TheOncologist.com.

ABSTRACT

Early-stage gastric mucosa-associated lymphoid tissue lymphoma (GML) is considered a localized disease with an indolent course. Circulating malignant cells have been detected in other early-stage indolent lymphomas by molecular methods. We investigated the incidence of occult blood disease in early-stage GML patients, its impact on clinical outcome, and the similarity between blood and gastric lymphocytic clones. Sixty-two patients with local-
ized GML were included in the study; 51 of them had *Helicobacter pylori* infection. Monoclonality was investigated by leader polymerase chain reaction. Sequencing was performed for the immunoglobulin variable gene (VH) analysis. Blood involvement was absent in all patients by conventional staging methods. In the whole group of 62 patients, the incidence of blood IgH rearrangement was 45%, and this did not correlate with baseline patient characteristics. The monoclonal blood and gastric products of five patients were sequenced and compared with each other. Clonal identity was evident in four of five patients. The VH3 gene was the most frequently used, both in the blood and in the stomach. Early-stage GML is not a truly localized disease because half the patients had a circulating clone, probably identical to the gastric one. The clinical significance of occult blood disease and the potential appropriate intervention need to be further investigated. The Oncologist 2009;14:148–154

![Image](https://www.theoncologist.com)

**INTRODUCTION**

Mucosa-associated lymphoid tissue (MALT) lymphomas represent 7%–8% of B-cell lymphomas. Gastric MALT lymphoma (GML) comprises almost 50% of all MALT lymphomas. GML remains localized for a long period of time and is considered an indolent disease, although transformation to large-cell lymphoma may occur [1, 2]. *Helicobacter pylori* (Hp) infection is associated with GML, as demonstrated by numerous studies [3–5]. First-line treatment for early-stage GML is the eradication of Hp with antibiotics, producing complete lymphoma remission in a large proportion of patients [6, 7]. However, varying percentages (30%–65%) of patients fail to respond to antibiotics, especially those with t(11,18)(q21;q21) translocation [8–10]. For patients who are Hp− and those who fail anti-Hp antibiotics, there is no standard treatment.

As already pointed out, the majority of GML patients present with early-stage disease. However, it has been suggested that early-stage GML is not a truly localized disease, as is the case with localized follicular lymphoma, for which occult blood involvement has been documented by molecular methods [11–13]. The aim of the present study was to investigate whether a clonal lymphocytic population circulates in the blood of early-stage GML patients, to compare it with the gastric malignant clone, and to explore whether it has an impact on patient clinical status and outcome.

**PATIENTS AND METHODS**

**Patient Characteristics**

Of the 80 patients with GML referred to our department during the last decade, 62 had localized GML (stage I or II1) and were included in the present study. Fifty-nine patients had stage I (95%) and three patients had stage II1 disease according to the modified Blackledge staging system [14, 15]. There were 36 men and 26 women, with a median age of 57 years (range, 33–86). Epigastric pain was the most frequent symptom (46%), followed by bleeding (19%) and belching (12.7%). GML was found during routine investigation in three (4.8%) patients. Hp was found to be positive in 51 of 62 patients (82%).

**Diagnosis and Staging**

The diagnosis was documented by histological and immunohistochemical studies after gastrointestinal endoscopy in all but eight patients, who were diagnosed after surgery. The histological diagnosis was based on Wotherspoon’s histological index [7]. For the documentation of Hp infection, modified Giemsa staining on histological sections was used.

Staging procedures included history and physical examination, CBC with differential, biochemical profile, computed tomography scans of the thorax and abdomen, as well as bone marrow aspiration plus biopsy. For the exclusion of lymphomatous blood involvement, immunphenotypic analysis by flow cytometry was performed, using the following monoclonal antibodies (Becton-Dickinson, San Jose, CA): anti-CD20, anti-CD19, anti-CD5, anti-HLA-DR, anti-CD38, anti-CD23, anti-CD4, anti-CD8, anti-CD45, and anti-CD14 [16]. Staging was evaluated using the modified Blackledge system [14]. In brief, stage I includes cases with a single lesion or multiple noncontiguous lesions in the stomach without serosal penetration, whereas stage II1 refers to cases with gastric involvement and local lymph node enlargement.

**Polymerase Chain Reaction Methodology**

To examine monoclonal B-cell populations in blood and gastric biopsies, analysis of IgH gene rearrangement by leader polymerase chain reaction (PCR) was performed [17–19]. DNA was extracted from blood mononuclear cells according to the phenol-chloroform protocol and from paraffin-embedded, formalin-fixed gastric biopsies after deparaffinization with xylene, ethanol precipitation, and proteinase K digestion, followed by the phenol-chloroform method. DNA was amplified using the MJR System with 20 pM of each of the VH family leader primers corresponding to the VH family leader primers corresponding to the VH family sequences and two consensus downstream JHA and JHB primers corresponding to consensus sequences of the J region (Table 1). The PCR contained 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0), 1.5 mM MgCl2, 200 mM of each deoxynucleotide triphosphate, and 2.5U Taq platinum (Invitrogen, Carlsbad, CA) at a final volume of 50 µl. Thirty-five cycles of amplification...
were performed under the following conditions: denaturation at 94°C for 45 seconds, annealing at 65°C for 45 seconds, and extension at 72°C for 45 seconds. The reaction was completed with a final extension at 72°C for 10 minutes. The PCR products were analyzed on a 3% agarose gel and visualized under UV light after staining with ethidium bromide.

**Cloning and Sequencing**

The PCR products of the expected size were purified by affinity columns (NucleoSpin Extract, Macherey-Nagel, Germany). The recovered DNA was ligated into the PCR 4-Topo vector according to the manufacturer’s instructions (Topo TA Cloning Kit, Invitrogen, Carlsbad, CA). Ten colonies for each sample were randomly picked and grown overnight in 3 ml LB medium. Recombinant plasmids were purified by Qiagen miniprep columns (Qiagen, Germany) and selected by restriction analysis using EcoR1 (Roche Diagnostics GmbH, Mannheim, Germany). The plasmid DNA was sequenced in an automated sequencer (ABI capillary sequencer, MWG Biotech, Ebersberg, Germany) [20]. Sequences obtained from each blood and gastric clone were aligned with germline sequences in the IMGT/V-QUEST directory (http://imgt.cines.fr). The similarity of the VH genes to the closest germline sequences was defined as a percentage in the range of 98%–100% for the unmutated genes and <98% for the mutated ones [21]. Replacement mutations (leading to amino acid change) and silent mutations (no amino acid change) were calculated in framework regions and in complementarity determining regions (CDRs) in all mutated immunoglobulin sequences.

### Table 1. Primers for the IgH rearrangement by polymerase chain reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH1 + 7L</td>
<td>5′-ATGGACTGGACCTGGAGG</td>
</tr>
<tr>
<td>VH2L</td>
<td>5′-CAGCTCTCCTGCTGACCTGACCAA</td>
</tr>
<tr>
<td>VH3L</td>
<td>5′-GCTGTTCTTTTCCTGTTGGC</td>
</tr>
<tr>
<td>VH3bL</td>
<td>5′-ATCGAGTTTGGGACCTGAGCTG</td>
</tr>
<tr>
<td>VH4L</td>
<td>5′-GCTCCAGATGGGTCCTG</td>
</tr>
<tr>
<td>VH5L</td>
<td>5′-CTTCTCTGGCTTTCC</td>
</tr>
<tr>
<td>VH6L</td>
<td>5′-CTGCTCTTCTCTCATCTCC</td>
</tr>
<tr>
<td>JHA</td>
<td>5′-GAGGAGACGGTGACCAGG</td>
</tr>
<tr>
<td>JHB</td>
<td>5′-GAGGAGACGGTGACCAGG</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis was performed using the χ² test and Student’s t-test. A probability value of p < 0.05 was considered statistically significant. Progression-free survival (PFS) and overall survival (OS) were calculated according to the method of Kaplan and Meier [22]. The possibility of antigen selection in the mutated sequences was calculated using the multinomial distribution model by Lossos et al. [23].

**Treatment and Follow-Up**

All patients with Hp infection received antibiotics as the sole initial treatment [24, 25]. Patients underwent endoscopy 2 months after the antimicrobial therapy for the evaluation of Hp eradication and lymphoma response. Thereafter, patients were followed by endoscopy with multiple biopsies and imaging studies every 4–6 months for the first 2 years and once a year thereafter up to 5 years. Patients with no response or progressive disease after 4–6 months from antibiotic treatment and patients without Hp infection were treated with other modalities.

**Response Criteria**

For the definition of histological response, the Wotherspoon index [7] and the criteria published by Neubauer et al. [26] were used. Complete response (CR) was defined as the resolution of all disease-related symptoms, the disappearance of endoscopic lesions, and the accomplishment of histological remission according to Neubauer et al. [26], or a score <3 according to the Wotherspoon index.

**RESULTS**

**Molecular Study by PCR**

In all patients, a blood immunophenotypic analysis did not reveal monoclonal B cells. Gastric DNA was available for analysis in 21 of 62 patients, whereas blood DNA was available in all. Monoclonality was documented by PCR in 20 of 21 gastric biopsies (95%). Rearrangement of *IgH* of the same size was detected in the blood of 28 of 62 (45%) patients. The single patient who was molecularly negative in the stomach was negative in the blood as well.

Furthermore, the presence of blood *IgH* monoclonality was correlated with Hp infection and clinical stage. Among the Hp⁺ patients, 26 of 54 (48%) were found with blood *IgH* rearrangement, whereas only two of eight (25%) Hp⁻ patients were *IgH* rearranged in the blood. However this difference was not statistically significant. Twenty-five (42%) patients with stage I had occult blood involvement as assessed by PCR, versus three (100%) of the stage II patients, but again this difference did not reach statistical significance.

**Sequencing Analysis of the VH Genes**

Among the 20 patients with available blood and gastric material, five with positive *IgH* rearrangement in both compartments were randomly picked for sequencing analysis. The VH gene usage as well as the clonal identity to the germline se-
quences in blood and gastric clones for each of the five patients are shown in Table 2. The most commonly represented VH gene both in the blood and stomach was VH3 (in four of five patients) whereas the VH2 family was present in one patient. In four cases (patients 1–4), the monoclonal sequences detected in the stomach were the same as the sequences detected in the blood. In patient #5, two clonal products were evident in the blood by the leader PCR. Sequencing analysis detected only one productive Ig sequence, which was different from the gastric clone. Three patients (patients 2, 3, and 5) had mutated genes and two had unmutated genes. In patients 2 and 3, the gastric clones displayed a higher number of mutations than the corresponding blood clones. The ratio of replacement to silent mutations is shown in Table 2.

Response to Treatment and Outcome
Among 51 patients with Hp infection, 50 received antibiotics as the sole initial therapy. Eradication of Hp was successful in all patients. Ten of 50 (20%) patients had a complete lymphoma response. Patients who did not respond to antibiotics, as well as those who were Hp− were treated with other modalities, as shown in Table 3. CR to anti-Hp treatment did not correlate with the presence of blood clone. Thus 16% of the blood IgH− patients achieved a CR, versus 21% of the blood IgH+ patients.

During a median follow-up of 67 months (range, 9–179), 15 patients relapsed/progressed—14 in the stomach and one in the liver and small intestine. The 5-year PFS rate for all patients was 75%. The PFS rate for blood IgH+ patients was 70% and the PFS rate was 77% for IgH− patients (p = .27). The 5-year OS rate for all patients was 97%–92% for blood IgH+ and 100% for blood IgH− patients (p = .12). In addition, the presence of blood IgH rearrangement did not correlate with PFS and OS, in either Hp+ or Hp− patients. Furthermore, the presence of blood clonality did not influence patient outcome or the pattern of relapse for patients who received local treatment. In detail, three local relapses were observed among eight patients who were treated with surgery or radiotherapy. Two patients were IgH− and one was IgH+ in the blood.

DISCUSSION
Marginal B-cell lymphomas of the MALT type are considered indolent diseases with a long survival time [27–29]. Half of them involve the stomach and are localized at diagnosis [1, 30]. It is now known that chronic antigenic stimulation resulting from Hp infection plays a central role in the pathogenesis of GML [5, 3, 31]. The accurate staging of a GML is still under investigation [32]. Using the modified Blackledge system, most GMLs are found at early stages, stage I and II1 [33, 15]. It has been proven that occult blood disease may be present using sensitive methods in localized lymphoproliferative diseases such as early-stage follicular lymphoma [12]. In addition, patients with follicular lymphoma in complete remission with conventional staging often have a circulating clone in the blood, demonstrated by PCR [13, 34]. MALT lymphoma is considered a truly localized disease at stages I and II1.

Based on the above observations, we investigated whether a monoclonal population is present in the blood of patients with early-stage GML without any evidence of blood involvement by morphology and immunophenotyping. We studied 62 stage I and II1 patients with histologically proven GML. Leader PCR was found to have an excellent sensitivity (95%) in the detection of the lymphomatous clone in the stomach [17–19]. IgH rearrangement in gastric biopsies was detected in all but one patient with available gastric tissue. Using the same methodology, an IgH rearrangement of the same size was detected in the blood of 28 of 62 (45%) patients. Because the detection of a monoclonal product in the blood per se cannot definitely prove clonal identity between blood and stomach [35], we further compared the sequences of the two compartments. Sequencing was performed in five randomly picked stage I patients with available blood and gastric tissue. Clonal identity between blood and stomach was proven in four of five patients. Although sequencing analysis was not performed in all patients who were IgH+ in the stomach and blood, our findings indicate that a significant proportion of patients with localized GML actually have circulating lymphomatous disease. There is only one other study, by Bertoni et al. [36], describing a similar finding. However, those investigators did not further compare circulating and gastric clones [36]. Other investigators have compared clones in MALT lymphomas between two or more different sites, such as the spleen and stomach, stomach and blood, multiple mucosal sites, and bone marrow, and found the same infiltrating clone [37–40]. However, these cases did not represent localized MALT lymphomas. To our knowledge, this is the first study to specifically address the above question [41, 42]. It is of interest that, in our series, all stage II1 patients (three of three) had a lymphomatous clone in the blood, versus 42% of stage I GML patients, although the difference was not significant. Thus, stage II1 GML cases tended to display occult blood disease more frequently than stage I cases. One could postulate lymphomatous spillover into the blood with growing tumor burden. In accordance with our findings, Thieblemont et al. [11] and Du et al. [11, 43] showed that early-stage GML is already disseminated at diagnosis when a more thorough staging investigation is applied or when biopsies from multiple sites of the intestinal tract are studied by molecular methods. The above studies
have investigated the concomitant occurrence of MALT lymphoma in extranodal sites other than the stomach in GML patients. However, circulating occult blood disease was not addressed in any of those studies. Based on our findings, in which a significant proportion of our patients with localized GML had a circulating clone, one could postulate that the malignant cells can adhere to different extranodal sites and develop into a true lymphoma when the appropriate adhesion molecules and local addressins are encountered [44–48]. The significance of the circulating clone has not been elucidated.

Using molecular techniques, it has been demonstrated that, following anti-Hp therapy, there is a persistence of the gastric B-cell monoclonal population in about half of the complete responders [15]. We could hypothesize that the circulating clone might explain the failure of local treatment in a number of patients, or a subsequent relapse in another site after local therapy. However, this hypothesis cannot be supported by our data, because there was no difference in the CR rate with anti-Hp treatment between blood IgH/H11001 and IgH/H11002 patients. Moreover, the presence of occult blood disease did not correlate with the relapse rate or pattern of relapse among patients who received local treatment. In addition, the presence of blood IgH rearrangement did not influence PFS or OS for the whole patient population. Thus, the clinical significance of the circulating clone is not apparent from this study. However, relapses in this indolent disease are rare events, and a higher number of patients is needed to make such correlations.

Another interesting finding was the more frequent usage of the VH3 gene family by blood and gastric clones. A similar finding was recently reported by Sakuma et al. [49].

### Table 2. Results of sequencing study

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>VHGS</th>
<th>Homology to germline sequence</th>
<th>R/S</th>
<th>p-value*</th>
<th>VHGS</th>
<th>Homology to germline sequence</th>
<th>R/S</th>
<th>p-value*</th>
<th>Comparison between blood and gastric sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V2–5*10</td>
<td>100%</td>
<td>–</td>
<td>–</td>
<td>V2–5*10</td>
<td>100%</td>
<td>–</td>
<td>–</td>
<td>Same Igs</td>
</tr>
<tr>
<td></td>
<td>D3–22*01</td>
<td></td>
<td></td>
<td></td>
<td>D3–22*01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>J4*02</td>
<td></td>
<td></td>
<td></td>
<td>J4*02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>V3–74*01</td>
<td>96.1%</td>
<td>FR, 3/2</td>
<td>FR, 0.013</td>
<td>V3–74*01</td>
<td>94.4%</td>
<td>FR, 6/4</td>
<td>FR, 0.028</td>
<td>Same Igs</td>
</tr>
<tr>
<td></td>
<td>D2–2*01</td>
<td></td>
<td>CDR, 5/1</td>
<td>CDR, 0.005</td>
<td>D2–2*01</td>
<td></td>
<td>CDR, 5/1</td>
<td>CDR, 0.034</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J4*02</td>
<td></td>
<td></td>
<td></td>
<td>J4*02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>V3–21*02</td>
<td>96.8%</td>
<td>FR, 2/2</td>
<td>FR, 0.048</td>
<td>V3–21*02</td>
<td>95.49%</td>
<td>FR, 2/2</td>
<td>FR, 0.049</td>
<td>Same Igs</td>
</tr>
<tr>
<td></td>
<td>D5–24*01</td>
<td></td>
<td>CDR, 2/1</td>
<td>CDR, 0.158</td>
<td>D5–24*01</td>
<td></td>
<td>CDR, 2/1</td>
<td>CDR, 0.158</td>
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<td></td>
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<td>J4*02</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>V3–11*03</td>
<td>98.26%</td>
<td>–</td>
<td>–</td>
<td>V3–11*03</td>
<td>98.26%</td>
<td>–</td>
<td>–</td>
<td>Same Igs</td>
</tr>
<tr>
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<td>D3–3*01</td>
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<tr>
<td>5</td>
<td>V3–48*03</td>
<td>95.83%</td>
<td>FR, 5/1</td>
<td>FR, 0.08</td>
<td>V3–48*03</td>
<td>92.71%</td>
<td>FR, 9/7</td>
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<td>D3–22*01</td>
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<td>CDR, 4/2</td>
<td>CDR, 0.04</td>
<td>D3–10*01</td>
<td></td>
<td>CDR, 5/0</td>
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<td></td>
<td>J4*02</td>
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<td>J4*02</td>
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</tbody>
</table>

**Abbreviations:** CDR, complementarity determining region; FR, framework region; R/S, replacement/silent mutation; VHGS, variable heavy gene segment.

* p-value for R/S mutation in FR and CDR according to multinomial distribution analysis.

### Table 3. Treatment and response in Hp⁺ patients who failed antibiotics and Hp⁻ patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hp⁺ patients (n = 41)</th>
<th>Hp⁻ patients (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CR</td>
</tr>
<tr>
<td>CT or CT + RT</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>CT + S</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>S</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>RT</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>

**Abbreviations:** CR, complete response; CT, chemotherapy (chlorambucil or cyclophosphamide, vincristine, and prednisone); Hp, Helicobacter pylori; R, rituximab; RT, radiotherapy; S, surgery.
who analyzed VH gene usage in gastric biopsies of GML patients. They found that the most frequently used genes belonged to the VH3 gene family and specifically VH3–23 and VH3–30. These findings suggest that GML is derived from highly restricted B-cell subsets probably resulting from specific antigenic stimulation, such as with Hp [50–54]. In our study, patients with Hp infection were more frequently monoclonal in the blood than patients without Hp infection, but the difference was not statistically significant; therefore, occult blood lymphomatous disease cannot be attributed to Hp presence. Mutational analysis revealed that the gastric clones of three patients were mutated. In two of these, the sequences in the blood and stomach were identical. Statistical analysis using the multinomial distribution model revealed statistically significant p-values for the CDR in the identical blood and gastric clones of one patient and in the blood sequence of one patient with different clones, meaning that there is probable antigen selection pressure accounting for these mutations [53, 55]. Concerning the patient with different blood and gastric clones, blood PCR resulted in the detection of two different clonal products, and sequencing analysis rendered only one productive sequence different from the gastric clone. We can only hypothesize that the blood clone can be attributed to the oligoclonal expansion also evident in other lymphoma patients, although we were unable to detect a second productive sequence by our methodology [56].

In conclusion, we demonstrated that early-stage GML does not seem to be a truly localized disease, because almost half of our patients had a circulating clone probably identical to the clone in the stomach. However, the clinical significance of the blood clone is not yet clear. In the era of monoclonal antibodies, the early detection of occult blood disease and the appropriate intervention need to be further studied [57, 58].

AUTHOR CONTRIBUTIONS

Conception/design: Gerassimos A. Pangalis, Xanti Yiakoumou, Panayiotis Panayiotidis, Maria K. Angelopoulou

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Data analysis: Marina P. Siakantaris, Theodoros P. Vassilakopoulos

Manuscript writing: Maria P. Siakantaris

Final approval of manuscript: Gerassimos A. Pangalis, Panayiotis Panayiotidis, Maria K. Angelopoulou

Other: Molecular study: Marina P. Siakantaris, Evangelia Dimitriadou; Immunophenotypic analysis: Flora N. Kontopidou; Histologic and immunohistochemical analysis: Penelope Korkolopoulou, Athina Androulaki, Eustratios Patours;Performed endoscopies and collected the gastric material: Panayia Bobotis

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