Reverse Transcription-PCR for t(11;18)(q21;q21) Staging and Monitoring in Mucosa-Associated Lymphoid Tissue Lymphoma

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Abstract

Purpose: Subclinical dissemination as well as persistence after therapy may be difficult to assess on clinical and histologic examinations in patients with mucosa-associated lymphoid tissue (MALT) lymphoma. We have analyzed the use of reverse transcription-PCR (RT-PCR) for the detection of t(11;18)(q21;q21) in histologically infiltrated and normal biopsies at diagnosis and during follow-up to determine its clinical and prognostic effect.

Experimental Design: Twenty-one patients with t(11;18)(q21;q21)+ MALT lymphoma were included in this retrospective study. Presence of t(11;18)(q21;q21) was determined by RT-PCR done on 316 biopsies of various tissues obtained during staging and follow-up.

Results: Infiltration with lymphoma was histologically detected in 67 of 316 biopsies, whereas molecular infiltration was established in 104 of 316 biopsies. All histologically positive specimens were also positive in RT-PCR. There was a good concordance (P = 0.0001) between histology and RT-PCR at the time of disease presentation with only one further infiltration site identified by RT-PCR. In 8 of 12 patients with persistent lymphoma, RT-PCR revealed tumor infiltration in histologically unsuspected sites. Eight of nine treated patients with histologic and clinical complete remission (CR) remained RT-PCR positive. CR on RT-PCR was achieved later than histologic CR (between 13–59 months) without any further therapy in five of these eight patients; only one patient with persistent t(11;18)(q21;q21) relapsed histologically.

Conclusions: This study shows the potential of RT-PCR for t(11;18)(q21;q21) done on routine paraffin-embedded specimens to identify disseminated disease in tissues otherwise not diagnostic of MALT lymphoma involvement. T(11;18)(q21;q21) persistence in patients with clinical and histologic CR does not necessarily require therapeutic intervention.

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma is the third most common lymphoma entity (1). Following the initial description by Isaacson and Wright in 1983 (2), it has subsequently become a focus of interest for both clinicians and pathologists due to its unique clinical and pathologic features. The large majority of cases are diagnosed in the stomach, but MALT lymphoma may affect virtually every organ in the human body (3, 4). Chronic antigenic mucosal stimulation provided by infection, such as Helicobacter pylori, or various autoimmune conditions induces the accumulation of MALT in organs usually devoid of lymphoid tissue. These mucosal lymphocytes become genetically unstable with the acquisition of genetic aberrations leading to transformation into MALT lymphoma. The most frequent translocation in MALT lymphoma is the t(11;18)(q21;q21), resulting in the fusion of API2 and MALT1 genes (5–8). T(11;18)(q21;q21) is restricted to MALT lymphomas and has not been detected in other non-Hodgkin’s lymphomas or reactive lymphoid tissue (9, 10).

MALT lymphoma irrespective of origin is thought to behave as an indolent disease with a prolonged clinical course. Similar to activated normal mucosal lymphocytes, MALT lymphoma cells preferentially recirculate to various mucosal sites (“homing”) due to exclusive interaction between lymphoma cells and mucosal adhesion molecules (3, 11–14). Because dissemination within MALT may occur without generating overt clinical manifestations, thorough staging of patients seems mandatory to assess multifocal involvement before initiation of therapy or in case of relapse (15, 16).

In biopsies obtained from staging procedures, diagnosis of MALT lymphoma can readily be made based on its well-described histologic and immunophenotypic features. Lymphomatous lesions as small as “microlymphomas” may be diagnostic (17). However, histologic identification of tumor cells from less confluent lymphomatous lesions is problematic.
Moreover, abundant reactive lymphocytic infiltrates may hamper a clear-cut diagnosis. About t(11;18)+ MALT lymphomas, successful application of reverse transcription-PCR (RT-PCR) on paraffin-embedded specimens should potentially be able to identify even small numbers of tumor cells in biopsies devoid of manifest lymphoma infiltration as judged by conventional methods, including immunohistochemistry. To date, little is known about the value of RT-PCR for the detection of tumor cells harboring t(11;18)(q21;q21) with respect to molecular staging of tissues not suggestive of overt MALT lymphoma infiltration. Moreover, the rate of persistence of t(11;18)(q21;q21) positivity after chemotherapy is unclear. For these reasons, we have retrospectively studied all biopsies available from patients with t(11;18)+ MALT lymphomas by RT-PCR to determine the extent of dissemination and the pattern of t(11;18)(q21;q21) after treatment.

**Materials and Methods**

**Cases.** A retrospective analysis of 21 patients with t(11;18)/API2-MALT1+ MALT lymphoma diagnosed, treated, and followed at our institution was done. The study group comprised 10 men and 11 women with a median age of 63 years (range, 31-82 years). Patients underwent extensive staging according to our standard protocol (15), including endosonography, gastroduodenoscopy with multiple biopsies, colonoscopy, computed tomography scan of thorax and abdomen, magnetic resonance imaging of the salivary glands, otolaryngologic examinations, and bone marrow biopsy. Histologic diagnosis of MALT lymphoma was done according to the criteria outlined in the WHO classification (18), and histologic (re-)assessment of all biopsies was done by a reference hematopathologist (A.C.). Paraffin sections were immunologically phenotyped for demonstration of light-chain restriction and the phenotype CD20+/CD5−/CD10−/CD23−/CD38− CD43−/BCL6−/CLL1−/CD19−/CD103−/CD26−/CD56−/TdT−/CD10−/CD5−, which, in context with the microscopic appearance, is consistent with MALT lymphoma. In all patients referred to our institution for relapsing MALT lymphoma, the original biopsies were also reasseessed, and patients were only included if these, along with information on initial treatment, staging, and response, were available.

**T(11;18)(q21;q21) analysis.** A total of 316 biopsies from various tissues (including stomach, duodenum, colon, and bone marrow) were investigated for the presence of t(11;18)(q21;q21). Biopsies were selected for sufficient amount of tissue and quality of RNA. RT-PCR for t(11;18)(q21;q21) involving API2 and MALT1 was done according to Inagaki et al. (19), with one modification: first round RT-PCR products were amplified in a second round separately and not as multiplex nested PCRs to discriminate the various fusion signals. Where indicated, PCR products were sequenced using dRhodamine dye terminators on an ABI Prism 310 (Perkin-Elmer Applied Biosystems, Foster City, CA). ACTB was amplified in parallel as an internal positive control. To assess the sensitivity of the RT-PCR assay, we did serial dilutions of 10 t(11;18)+ and t(11;18)− MALT lymphomas. In all cases, 4% or more of t(11;18)+ tumor cells were sufficient to detect an API2-MALT1 fusion transcript by nested RT-PCR.

Furthermore, the sensitivity of nested RT-PCR and quantitative RT-PCR was compared. Quantitative RT-PCR analysis was done on the ABI Prism 7000 Sequence Detection System (Applied Biosystems). The following assays were used: assay 1, GAAGAGGAGAGAGGACCTG (forward primer), GCCTTTCACCTCGGTTTCACT (reverse primer), and AGGAAAAGAATCAAGAAATCCAGA (reporter); assay 2, AAGCTCAATACGGGAGAGGAGCA (forward primer), AGCCCTCACTGGTCATCCTTTATCA (reverse primer), and AGGAAAAAGAATCAAATGAAT (reporter); and assay 3, GGAAGAGGAGAGAGGAA (forward primer), AAGGCTGGTCAGTTGTTATCAGA (reverse primer), and CTGAGAAAGGAAAGGAAAGAATCAAGAATAA (reporter). Glyceraldehyde-3-phosphate dehydrogenase served as internal control. Hundred biopsies were compared. These 100 biopsies comprised 44 RT-PCR-positive biopsies and 56 RT-PCR-negative biopsies. Quantitative RT-PCR was negative in all 56 RT-PCR-negative biopsies and negative in 10/44 RT-PCR-positive biopsies. Therefore, we concluded that sensitivity of nested RT-PCR was superior to quantitative RT-PCR in our paraffin-embedded biopsies and conducted our study using nested RT-PCR.

**Results**

**Patient characteristics and molecular staging at initial diagnosis.** Among this series of 21 patients, the stomach was the initial site of disease in 13 (62%) patients. The remaining eight patients had primary extragastric MALT lymphoma, occurring in the lung (n = 5), colon (n = 1), parotid gland (n = 1), and thyroid gland (n = 1). Staging work-up revealed involvement of a further anatomic site in 3 of the 21 (14.3%) patients: one patient (patient 18, see Table 1) with primary gastric MALT lymphoma had involvement of the colon; another patient (patient 1, see Table 1) presenting with MALT lymphoma of the colon had synchronous gastric disease; and in a third patient (patient 5, see Table 1) with pulmonary MALT lymphoma staging revealed involvement of the colon.

At the time of disease presentation, 54 biopsies derived from 18 patients were available for molecular staging (Table 1). Comparison of RT-PCR and histologic data showed identical results in 51 of 54 (94%) biopsies: 24 biopsies were positive and 27 biopsies were negative by both techniques. The remaining 3 of 54 (6%) biopsies were histologically negative but t(11;18)+: patients 16 and 17 with MALT lymphoma of the stomach showed an API2-MALT1 fusion transcript in two additional gastric biopsies; patient 17 was t(11;18)+ in the bone marrow in spite of normal histologic findings. In summary, at presentation, histologic and molecular data showed excellent concordance (P = 0.0001, χ² test), with identification of a further site of disease in only one patient.

**Patient characteristics and molecular staging after therapy.** Among the 21 patients with t(11;18)+ MALT lymphoma, one patient (patient 1) refused any treatment and remained in stable disease for 13 months, whereas the remaining 20 patients received diverse therapies (Table 1). Four cases underwent surgery followed by radiation in one case and treatment with cladribine (2CdA) in two cases. One patient received radiation followed by treatment with 2CdA. Eight patients underwent H. pylori eradication therapy as initial treatment; two of these eight patients are in complete remission (CR) after H. pylori eradication, whereas five of the remaining six patients underwent further therapies due to unresponsiveness to H. pylori eradication. Patients were considered unresponsive to antibiotic treatment if no change of the lymphoma occurred within at a time span of at least 12 months following successful eradication. Seven patients were administered systemic treatment due to disseminated disease; treatment consisted of rituximab (n = 2), 2CdA (n = 3), oral cyclophosphamide (n = 1), doxorubicin, cyclophosphamide, vincristine, and prednisone (n = 1).

After initiation of therapy, 262 biopsies obtained from 18 patients were available for investigation of t(11;18)(q21;q21).
Comparison of molecular and histologic data showed identical results in 228 of 262 (87%) biopsies: 43 biopsies were positive and 185 biopsies were negative by both techniques. The remaining 34 of 262 (13%) biopsies were histologically negative but positive by RT-PCR for t(11;18)(q21;q21). These 34 biopsies represented divergent results in 12 of the 18 (67%) patients. These mismatch (RT-PCR positive and histology negative) biopsies were grouped in (A) cases with apparent histologic MALT infiltration in other biopsies obtained at the same time (interpreted as underestimated dissemination in documented persistent MALT lymphoma) and (B) molecular evidence of subclinical persistence of lymphoma in patients with clinical/histologic CR.

Group A (underestimated dissemination in persistent MALT lymphoma) comprised 13 mismatched biopsies obtained from eight patients (Table 2). In seven of these eight patients, RT-PCR revealed wide dissemination to the same organ (stomach, n = 5; duodenum, n = 1; and coecum, n = 1). In addition, RT-PCR showed tumor dissemination to further, histologic unsuspected sites in four of eight patients (for details see Table 2).

For clarity, results of group B (unknown tumor persistence) are included in the next section.

**Molecular staging in patients with clinical CR.** After initiation of therapy, biopsies were available for molecular monitoring in nine patients with CR (Table 3). The median clinical follow-up was 54 months, with the median molecular follow-up being 28 months. Among these nine patients, one patient with gastric MALT lymphoma had a local relapse 49 months after CR following initial therapy with 2CdA. The other eight patients remained in CR during the follow-up period.

Persistence of t(11;18)(q21;q21) was detected by RT-PCR in the majority of patients after treatment with ongoing
clinical and histologic CR. RT-PCR exhibited histologically undetectable molecular residual disease in eight of nine cases (including the patient who relapsed 49 months after initial CR). Six cases with gastric MALT lymphoma revealed t(11;18)(q21;q21) in gastric biopsies. Two cases with MALT lymphoma in the parotid were t(11;18)(q21;q21)+ at other sites (esophagus, colon, and lymph node). However, five of these eight cases with persistence of t(11;18)(q21;q21) but clinical/histologic CR also became negative for t(11;18)(q21;q21) at a later time without further treatment. The time to RT-PCR negativity was 13 to 59 months, whereas the remaining three patients remained t(11;18)(q21;q21)+ for 12, 14, and 28 months in the absence of histologic relapse. One patient (patient 19) with gastric MALT lymphoma responding to H. pylori eradication was in continuous clinical, histologic, and molecular CR for the whole 22 months of available follow-up.

### Table 2. Histologic and RT-PCR results in patients with persistent lymphoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Histologically positive biopsies</th>
<th>RT-PCR-positive biopsies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colon, stomach, duodenum</td>
<td>Colon, stomach, duodenum</td>
</tr>
<tr>
<td>4</td>
<td>Lung, coecum</td>
<td>Lung, coecum</td>
</tr>
<tr>
<td>5</td>
<td>Lung, colon</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Lung</td>
<td>Lung</td>
</tr>
<tr>
<td>8</td>
<td>Stomach</td>
<td>Stomach</td>
</tr>
<tr>
<td>9</td>
<td>Stomach</td>
<td>Stomach, duodenum</td>
</tr>
<tr>
<td>10</td>
<td>Stomach</td>
<td>Stomach, bone marrow</td>
</tr>
<tr>
<td>12</td>
<td>Stomach</td>
<td>Stomach</td>
</tr>
<tr>
<td>16</td>
<td>Stomach</td>
<td>Stomach</td>
</tr>
<tr>
<td>17</td>
<td>Stomach</td>
<td>Stomach, duodenum, bone marrow</td>
</tr>
<tr>
<td>18</td>
<td>Stomach, colon</td>
<td>Stomach, colon</td>
</tr>
<tr>
<td>20</td>
<td>Stomach</td>
<td>Stomach, duodenum</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*Two hundred sixty-two biopsies were available for molecular staging after initiation of therapy. Thirty-four of 262 (13%) biopsies were histologically negative and RT-PCR positive.

### Discussion

Molecular tests complement histologic diagnosis in a variety of B-cell lymphomas. The assessment of monoclonal IGH rearrangement by PCR, presumably reflecting the proliferating tumor clone, does not require the knowledge of underlying genetic alterations of the tumor cells. In MALT lymphoma, monoclonalpopularity is widely used to evaluate dissemination at diagnosis as well as discrete tumor persistence in follow-up monitoring (16, 20–27). A drawback of monoclonality studies is the fact that monoclonal B cells may not represent the tumor but normal B cells. This may partly explain the unclear role of persistent monoclonality in treated MALT lymphoma patients with CR as well as the demonstration of monoclonality in H. pylori-related gastritis (26, 28–30). By contrast, t(11;18)(q21;q21) occurs only in MALT lymphoma cells and can be regarded as an exclusive tumor-specific marker (9, 10).

Recently, Salar et al. (31) have compared RT-PCR for t(11;18)(q21;q21) with PCR for IGH to assess residual disease after chemotherapy in three patients with gastric MALT lymphoma. In their experience, the most sensitive method of follow-up was RT-PCR for t(11;18)(q21;q21). These findings show that RT-PCR for API2-MALT1 is a specific and sensitive method for evaluating discrete infiltration with t(11;18)+ MALT lymphoma. Furthermore, several studies have successfully applied RT-PCR for t(11;18)(q21;q21) in gastric MALT lymphoma after eradication therapy (32–34). Therefore, a positive RT-PCR for t(11;18)(q21;q21) in the absence of histologic proof of lymphoma as seen in some of our patients can indeed be regarded as molecular evidence for residual disease.

In the present study, we have analyzed 316 biopsies from 21 patients with t(11;18)+ MALT lymphomas by t(11;18)/API2-MALT1 RT-PCR and correlated the results with the clinical and histologic data. RT-PCR proved to be a reliable test, as all histologically positive cases were also t(11;18)+. Not surprisingly, RT-PCR was more sensitive than histologic examination as reflected by the detection of t(11;18)(q21;q21) in 34 of 249 histologically negative biopsies. However, histologic molecular correlation was good at the time of diagnosis (P = 0.0001), although discrepancies arose/increased after therapy and clinical and histologic CR. At the time of diagnosis, RT-PCR identified only one additional site of infiltration (bone marrow) in one patient. These findings indicate that

### Table 3. Molecular follow-up in patients with clinical and histologic CR

<table>
<thead>
<tr>
<th>Case</th>
<th>Localization</th>
<th>Follow-up (mo)</th>
<th>Relapse</th>
<th>RT-PCR-positive biopsies (mo)</th>
<th>First negative molecular follow-up (mo)</th>
<th>Duration of molecular follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Parotid</td>
<td>63</td>
<td>No</td>
<td>Colon, rectum, lymph node (24*)</td>
<td>41</td>
<td>41 (CR)</td>
</tr>
<tr>
<td>14</td>
<td>Parotid</td>
<td>72</td>
<td>No</td>
<td>Esophagus (28*)</td>
<td>2</td>
<td>28 (RD)</td>
</tr>
<tr>
<td>10</td>
<td>Stomach</td>
<td>54</td>
<td>No</td>
<td>Stomach (32*)</td>
<td>5</td>
<td>13 (CR)</td>
</tr>
<tr>
<td>12</td>
<td>Stomach</td>
<td>38</td>
<td>No</td>
<td>Stomach (14*)</td>
<td>29</td>
<td>29 (CR)</td>
</tr>
<tr>
<td>13</td>
<td>Stomach</td>
<td>80</td>
<td>No</td>
<td>Stomach (24*)</td>
<td>7</td>
<td>35 (CR)</td>
</tr>
<tr>
<td>15</td>
<td>Stomach</td>
<td>66</td>
<td>Yes (stomach 49 mos)</td>
<td>Stomach (32*)</td>
<td>59</td>
<td>59 (CR)</td>
</tr>
<tr>
<td>16</td>
<td>Stomach</td>
<td>29</td>
<td>No</td>
<td>Stomach (4 and 12*)</td>
<td>None</td>
<td>12 (RD)</td>
</tr>
<tr>
<td>19</td>
<td>Stomach</td>
<td>70</td>
<td>No</td>
<td>None</td>
<td>5</td>
<td>22 (CR)</td>
</tr>
<tr>
<td>20</td>
<td>Stomach</td>
<td>51</td>
<td>No</td>
<td>Stomach (14*)</td>
<td>None</td>
<td>14 (RD)</td>
</tr>
</tbody>
</table>

Abbreviations: HP, H. pylori. RD, residual disease as judged by ongoing positivity for t(11;18)(q21;q21).

*After clinical and histologic response.
histologic examination is a reliable method in the assessment of dissemination at diagnosis of MALT lymphoma. These data, however, are in slight contrast to results obtained by Wotherspoon et al. (17) and Du et al. (20), who detected multiple foci of involvement missed by conventional histology and immunohistochemistry using molecular methods. In contrast to our data, these studies were partly carried out in gastrectomy specimens, whereas we have used forcep biopsy from stomach, duodenum, colon, and bone marrow samples (obtained by puncture of the crista iliaca). Therefore, our data might include a sampling bias as opposed to the possibility to evaluate the whole stomach from a gastrectomy specimen. To our knowledge, our data are nevertheless the first to systematically study dissemination of MALT lymphoma outside the stomach by means of a sensitive molecular method.

Patients with t(11;18)+ gastric MALT lymphoma are thought to be mostly unresponsive to antibacterial treatment, even at early localized stages (32). Correspondingly, six of eight patients with initial eradication did not develop CR. In keeping with the notion that MALT lymphoma is almost always disseminated within the stomach (17, 20), RT-PCR revealed wide dissemination of gastric MALT lymphoma within the stomach with additional subclinical infiltration of duodenum and bone marrow during persistent disease. However, eradication resulted in clinical and histologic CR in two patients, with available follow-up for 12 and 70 months, respectively. The latter patient also achieved a continuous molecular remission. Various local and systemic treatment approaches to MALT lymphoma have been tested, including chemotherapy with alkylating agents, nucleoside analogues, and oxaliplatin and combination regimens (35–38). When evaluating various therapeutic strategies, it has to be kept in mind that relapses of MALT lymphoma can occur very late (median time, 47 months but up to >300 months) and tend to affect distant organs (39). Thus, RT-PCR may help to evaluate therapeutic effects in MALT lymphoma on a molecular basis due to its reliability and sensitivity. We have investigated various tissues from nine patients with clinical and histologic CR and a median follow-up of 56 months. RT-PCR revealed persisting tumor infiltrates not detected by histology in eight of the nine cases affecting both local and distant organs. However, only one t(11;18)+ persistent patient has relapsed thus far, whereas the other patients remained in clinical continuous CR. Interestingly, five patients changed from molecular positive to molecular negative between 13 to 59 months without any further treatment. These data indicate that a prolonged persistence of t(11;18)(q21;q21) is not necessarily associated with relapse and may even result in complete molecular remission without further treatment. One might in fact argue that the three cases with persistent t(11;18)(q21;q21) had a shorter follow-up (13–28 months) than the patients with conversion to a RT-PCR-negative state, which took up to 59 months. Thus, it might be possible that these patients would become t(11;18)+ with prolonged follow-up. One of the most important findings from our study, however, is the fact that various forms of treatment (including chemotherapy and radiation) are able to eradicate minimal residual disease, albeit after a prolonged time compared with conventional histologic assessment. This is in keeping with findings obtained by Alpen et al. (30), who have reported that persistence of monoclonality is a rare event after chemotherapy or radiation in gastric lymphoma, whereas up to 50% may remain positive following sole H. pylori eradication. According to the well-known disappearance of monoclonality following radiation and chemotherapy, we have not done monoclonality analysis, as the large majority of patients included in our series had undergone chemotherapy and radiation.

Based on the experiences at our institution, RT-PCR is a reliable and sensitive test for evaluation of tumor infiltration in fresh and archival biopsies of t(11;18)+ MALT lymphomas. RT-PCR may facilitate histologic diagnosis in biopsies with unclear lymphocytic infiltrates as negative RT-PCR results are very likely to rule out tumor infiltration. A watch and wait strategy may be feasible for patients with clinical and histologic remission and positive RT-PCR results, as conversion to negative RT-PCR takes longer than CR on conventional histology. Therefore, the ongoing presence of t(11;18)(q21;q21) does not necessarily imply a negative prognostic factor in such patients and should not lead to therapeutic interventions in the absence of clinical/histologic relapse.

References


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