Infectious disease associations in advanced stage, indolent lymphoma (follicular and nonfollicular): developing a lymphoma prevention strategy

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Background: Eradication of Helicobacter pylori in gastric mucosa-associated lymphoid tumor can result in lymphoma remission. We prospectively identified/treated infections in nonbulky, advanced stage indolent lymphoma (follicular; nonfollicular lymphoma) eligible for observation.

Materials and methods: Stool H. pylori, hepatitis C and Borrelia serologies, Borrelia and Chlamydia fixed tissue PCR, Chlamydia peripheral blood mononuclear cell PCR and hydrogen breath test for small bowel bacterial overgrowth (SBBO) were obtained.

Results: Fifty-six patients were enrolled. Positive infections: H. pylori (13); hepatitis C (3); SBBO (11). Negative: Borrelia (13); Chlamydia psittaci (12, except one PCR). Lymphoma responses to antimicrobial therapy: H. pylori [one complete response (CR), 24+ months; one transient near CR]; hepatitis C [two CRs, 18+ and 30+ months; one partial response (PR) but hepatitis C virus persistent]; SBBO (one PR, 30+ months). Patients with associated infections, but without lymphoma CR, have required lymphoma treatment sooner than those without initial infections (treatment-free survival at 23.4 months median follow-up, 40.5% versus 74.7%, P = 0.01), indicating a different biology.

Conclusion: Infections are common in advanced stage indolent lymphoma (37.5% in our series). Anecdotal lymphoma responses have been seen and three have been durable CRs (18 to 30+ months) with infection eradication alone. The identification and treatment of associated infections may be a first step towards developing a lymphoma prevention strategy.

Key words: follicular lymphoma, indolent lymphoma, infections, prevention

Introduction

Advanced stage, indolent non-Hodgkin’s lymphomas are characterized by long survival, but are incurable with standard therapies. The histological subtypes include follicular lymphoma (FL) grades I, II and IIIA, and nonfollicular lymphoma (nFL) histologies [small lymphocytic lymphoma, lymphoplasmacytoid lymphoma and marginal zone lymphoma (MZL), both nodal and extranodal, i.e. mucosa-associated lymphoid tissue (MALT), types] [1].

The antigen drive association of gastric MALT lymphoma with Helicobacter pylori is well recognized. Successful antibiotic therapy, to eradicate the H. pylori antigen, can result in lymphoma remission, and with it, lymphoma prevention [2]. We have sought to prospectively identify possible associated infections (candidates for antigen drive) in patients with nonbulky, advanced stage indolent lymphoma as the first step to such a lymphoma treatment/prevention strategy. These patients are often given a recommendation of monitoring (‘watch and wait’) and it is during this period that a window of opportunity may exist to identify and treat antigen drive-related infections.

The primary objective of this study was to evaluate the possible association of infectious diseases in previously untreated patients with advanced stage, indolent lymphoma, and to assess the incidence of infections identified. The secondary objective was to evaluate possible lymphoma response in patients treated for positive infectious disease.

Materials and methods

Patient characteristics are listed in Table 1. Eligibility included: FL (grades I, II or IIIA) or nFL, without evidence of transformation, confirmed at Memorial Hospital; previously untreated, advanced stage (intra-abdominal stage II, III or IV) disease not requiring initial treatment according to Groupe d’Etude des Lymphomes Folliculaires criteria [20]; baseline computed tomography scan of the torso, bone marrow biopsies not
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Median 56 years, range 21–77</td>
</tr>
<tr>
<td>Gender</td>
<td>Male: 23</td>
</tr>
<tr>
<td></td>
<td>Female: 33</td>
</tr>
<tr>
<td>Histology</td>
<td>FL 31</td>
</tr>
<tr>
<td></td>
<td>nFL 25</td>
</tr>
<tr>
<td>FL</td>
<td>Grade I: 21</td>
</tr>
<tr>
<td></td>
<td>Grade II: 10</td>
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<tr>
<td></td>
<td>Grade III: 0</td>
</tr>
<tr>
<td>nFL</td>
<td>SLL = 9</td>
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<tr>
<td></td>
<td>LPL = 0</td>
</tr>
<tr>
<td></td>
<td>MZL nodal = 14</td>
</tr>
<tr>
<td></td>
<td>MALT = 2</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>II 4</td>
</tr>
<tr>
<td></td>
<td>III 22</td>
</tr>
<tr>
<td></td>
<td>IV 30</td>
</tr>
</tbody>
</table>

Table 2. The incidence of associated infection in FL and nFL

<table>
<thead>
<tr>
<th>Infections</th>
<th>Number of positive cases (FL, nFL)</th>
<th>Number of negative cases (FL, nFL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number tested (FL, nFL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>13 (6, 7)</td>
<td>40 (20, 20)</td>
</tr>
<tr>
<td>Hepatitis C = 49</td>
<td>3 (1, 2)</td>
<td>46 (26, 20)</td>
</tr>
<tr>
<td>(27, 22) Borrelia</td>
<td>11 (5, 6)</td>
<td>43 (24, 19)</td>
</tr>
<tr>
<td>Serology = 28 (17, 11)</td>
<td>0 (0, 0)</td>
<td>28 (17, 11)</td>
</tr>
<tr>
<td>Tissue = 13 (8, 5)</td>
<td>0 (0, 0)</td>
<td>13 (8, 5)</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>1 (1, 0)</td>
<td>12 (7, 5)</td>
</tr>
<tr>
<td>Total</td>
<td>21 (10, 11)</td>
<td>35 (21, 14)</td>
</tr>
<tr>
<td>Multiple infections</td>
<td>5 (2, 3)</td>
<td></td>
</tr>
</tbody>
</table>

FL, follicular lymphoma; nFL, nonfollicular lymphoma; SLL, small lymphocytic lymphoma; LPL, lymphoplasmacytic lymphoma; MZL, marginal zone lymphoma; MALT, mucosa-associated lymphoid tissue.

results

Infectious diseases detected are shown in Table 2. Helicobacter pylori stool antigen was positive in 13 of 53 patients (24.5%). Hepatitis C antibody was found in three out of 49 patients tested (6.1%); one of 26 FL [genotype 1b and 1 620 000 hepatitis C virus (HCV) IU/ml] and two of 22 nFL (genotype 1a and >850 000 HCV IU/ml; and genotypes 1b and 873 000 HCV IU/ml). HCV status by PCR was not tested in those who were hepatitis C negative. Small bowel bacterial overgrowth was seen in 11 of 54 patients tested (20.4%). Borrelia burgdorferi fixed tissue PCR was negative in 13 of 13 patients tested. Chlamydia was positive in 1 of 13 (8%). In total, 21 of 56 patients had a documented infection (37.5%), five with multiple infections.

Lymphoma treatment responses:

- One of 13 patients with H. pylori has achieved CR (FL achieving a PET-negative CR for 24+ months since antibiotics). One patient with cutaneous MZL had a transient near CR (for 5 months).
- All three patients with hepatitis C have had objective response [one FL with PR for 24+ months, while HCV improved but persistent; two nFL (one CR with MZL for 30+ months; one CRu with MZL for 18+ months)] and in these two patients, HCV has been eradicated.
- One patient with FL and small bowel bacterial overgrowth (of 11 with positive studies) has had PR for 30+ months after antibiotic therapy.

biostatistics

This was a prospective, hypothesis-generating, exploratory study. Descriptive statistics were utilized to report infection incidence, response to therapy and patient demographics. Time to next lymphoma therapy was measured from enrollment in the study to the date of first lymphoma standard treatment utilizing the method of Kaplan and Meier [4]; univariate analyses were carried out using the log-rank test [5]. Survival analysis was carried out using SPSS 11.0 (SPSS Inc., Chicago, IL).

Infections Number of positive cases (FL, nFL) Number of negative cases (FL, nFL)

- Helicobacter pylori = 13 (6, 7) 40 (20, 20)
- Hepatitis C = 49 (27, 22) 3 (1, 2) 46 (26, 20)
- Borrelia = 11 (5, 6) 43 (24, 19)
- Serology = 28 (17, 11) 0 (0, 0) 28 (17, 11)
- Tissue = 13 (8, 5) 0 (0, 0) 13 (8, 5)
- Chlamydia = 13 (8, 5) 1 (1, 0) 12 (7, 5)
- Total = 21 (10, 11) 35 (21, 14)
- Multiple infections = 5 (2, 3)
Infection (results). (response with antibiotic eradication of associated infection only (see B-cell proliferation, stimulated by which the B-cell neoplasm evolves by a process of T-cell-driven discussion among the infected cohorts (Figure 1).

without lymphoma CR, possibly indicating a different biology not infected; and 40.5% for those with infection eradication but lymphoma CR/CRu to infection eradication; 74.7% for those infected. Stool infection. Associated infections were found to be frequent in both indolent lymphoma cohorts. Stool H. pylori antigen was identified in ~25% of all patients tested, 6% had hepatitis C and 20% of those tested had small bowel bacterial overgrowth. On the other hand, neither Borrelia nor Chlamydia infections were seen, except one patient with C. psittaci (without orbital lymphoma). There were five patients who had more than one infection. All patients were treated for the specific infections identified with a standard course of antibiotics and all have had successful eradication, except for two patients (one with persistent hepatitis C; one with small bowel bacterial overgrowth currently receiving antibiotics).

Although this study’s primary aim was to explore possible associated infection and not to assess lymphoma response, we did observe anecdotal responses (see results). Moreover, those patients with associated infections who did not achieve lymphoma CR/CRu to infection treatment appear to have required institution of conventional lymphoma therapy significantly sooner than those without initial associated infections (see Figure 1). The significance of this observation will require future study, as it may simply be an artifact of patients who were monitored more closely following antibiotics.

We are encouraged by these preliminary data indicating that infections are common in indolent lymphoma (37.5% in our series); and that anecdotal lymphoma responses have been seen, even recognizing that spontaneous regressions are often reported. Three CR/CRu (14.3% of those infected) are ongoing for 18–30+ months with infection eradication alone. Like gastric MALT, it is hoped that these data will support a new clinical paradigm, in which potential sources of antigen drive are identified and eradicated, as the first management option in advanced stage indolent lymphoma not otherwise requiring therapy. In so doing, perhaps this prevention strategy will decrease the risk of future lymphoma progression driven by such antigens. To that end, we recommend that all patients with indolent lymphoma be considered for hepatitis C eradication whenever possible, and we have embarked upon a prospective clinical trial testing the role of prolonged clarithromycin antibiotic therapy as first treatment in hepatitis C-negative patients.

discussion

Gastric MALT is a well accepted example of antigen drive, in which the B-cell neoplasm evolves by a process of T-cell-driven B-cell proliferation, stimulated by H. pylori. The lymphoproliferative process occurs over many years, and involves a number of steps before the development of an antigen-independent clonal B-cell malignancy [6]. A similar antigen-driven evolution is also hypothesized for follicular lymphomagenesis; however, the antigens involved are unknown [7].

Many investigators have reported that successful eradication of H. pylori with antibiotic therapy can result in resolution of gastric MALT. H. pylori infection has not been associated routinely with other sites of MZL. However, Campylobacter jejuni in small bowel MALT and C. psittaci in orbital MALT [8, 9] have both been reported.

There is an increased association of indolent lymphoma with chronic hepatitis C infection [10]. Antiviral therapy may eradicate hepatitis C infection in some patients [11], and recent reports document response of both hepatitis C and indolent lymphoma to antiviral therapy alone [12, 13]. Other infectious associations are less well established including cutaneous B-cell lymphoma and B. burgdorferi [14, 15]. There are no direct data at this time to support the hypothesis that small bowel bacterial overgrowth is a source of antigen drive. However, small intestinal disorders may be associated with indolent lymphoma, including immunoproliferative small intestinal disease, celiac sprue and sprue-like syndromes [16]. Given the causal relationship of H. pylori in gastric MALT, it seems reasonable to hypothesize that other infections and/or bacteria may be a source of antigen drive in this site. Given its inaccessibility, we sought to evaluate the conglomerate surrogate marker of small bowel bacterial overgrowth, postulating that any suggestive finding would allow further tailored investigation into the source of a more specific antigen.

The current study analyzed prospectively the incidence of associated infection in FL and nFL (see Table 2). No patients had symptomatic lymphoma or symptomatic infection. Associated infections were found to be frequent in both indolent lymphoma cohorts. Stool H. pylori antigen was identified in ~25% of all patients tested, 6% had hepatitis C and 20% of those tested had small bowel bacterial overgrowth. On the other hand, neither Borrelia nor Chlamydia infections were seen, except one patient with C. psittaci (without orbital lymphoma). There were five patients who had more than one infection. All patients were treated for the specific infections identified with a standard course of antibiotics and all have had successful eradication, except for two patients (one with persistent hepatitis C; one with small bowel bacterial overgrowth currently receiving antibiotics).

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appendix 1

1. **H. pylori stool antigen**: detection by enzyme immunoassay: Focus Technologies, Cypress, CA.

2. **Hepatitis C (serology)**: MSKCC microbiology laboratory. Testing was completed according to hospital standards. If HCV antibody positive, then genotype and quantitative PCR for HCV viral load were obtained.

3. **Borrelia (serology and PCR)**: red top tube sent to MSKCC microbiology laboratory. If positive, patient had other confirmatory serological studies as indicated. All patients had tissue blocks of positive lymphoma lymph node or other tissue sites submitted for PCR analysis. The specimens were submitted to IS, Department of Microbiology and Immunology, New York Medical College for analysis.

methodology  
Borrelia burgdorferi DNA present in specimens were detected by nested PCR amplification of the chromosomally encoded flagellin gene (flaB) and the plasmid-encoded outer surface protein A (ospA) gene. The flaB and ospA PCR result in 353 and 147 bp DNA fragments, respectively. The flaB assay were carried out essentially as described by Barbour et al. [17] ospA PCR were carried out using ospA5′-GGGAATAGCTGATTATTTAGCC-3′ and ospA5′-TTTCAACTGTGATCCCTC-3′ as forward and reverse primers, respectively, in the first round of PCR, and ospA5′-CTGCAGCTTGAACTTCAGGCACTTC-3′ and ospA5′ as forward and reverse primers, respectively in the second round. Dried DNA specimens were suspended in 50 µl of water. PCR were carried out in 25 µl reactions containing 5 µl of DNA and 100 mM (each) deoxynucleoside triphosphates, 1.25 U of Taq DNA polymerase and 20 pmol of each primer in a ‘DNA engine’ thermocycler (MJ Research, Watertown, MA). For both target genes, second-round PCR was accomplished by transferring 1 µl of the first-round reaction into 24 µl of the second-round master mix. The thermal cycling profile of both first- and second-round PCR consisted of one 3 min cycle at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. To avoid cross-contamination and sample carryover, pre- and post-PCR sample processing and PCR amplification were carried out in separate rooms and all fluid transfers were carried out with plugged pipet tips to eliminate aerosols. Amplified DNA products were detected by agarose gel electrophoresis in Tris-borate-EDTA buffer. For any positive PCR specimens, the PCR products were sequenced by automated DNA sequencing.

4. **Chlamydia**: cell preparation tubes tube was initially processed at MSKCC and then submitted to Dr CAG of Johns Hopkins University for PCR analysis of peripheral blood mononuclear cells. All patients had tissue blocks of positive lymphoma lymph node or other tissue sites submitted for PCR analysis. The specimens were submitted to CAG of Johns Hopkins University for analysis. The method of PCR detection [18] is outlined below and was carried out by robotics to avoid contamination. Three touchdown enzyme time release (TETR)-PCR assays were used to amplify different DNA sequences in the variable regions of the 16S and 16S–23S spacer ribosomal RNA (rRNA) genes specific for Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci as improved tests for sensitive diagnosis and rapid species differentiation. The TETR-PCR protocol used 60 cycles of amplification, which provided improved analytical sensitivity (0.004–0.063 inclusion-forming unit of Chlamydia species per PCR). The sensitivity of TETR-PCR with primer set CTR70–CTR71 was 96.7% and the specificity was 99.6%, compared with those of the AMPLICOR PCR for the detection of C. trachomatis in vaginal swab samples. TETR-PCR for C. pneumoniae with primer set CPN90–CPN91 was 90% sensitive and 93.3% specific compared with a nested PCR with primer set CP1/2–CPC/D for clinical respiratory samples. TETR-PCR for C. psittaci with primer set CPS100–CPS101 showed substantial agreement with cell culturing for animal tissue samples. Primer sets were then combined into a single multiplex TETR-PCR test. The respective 315-, 195- and 111-bp DNA target products were precisely amplified when DNA from each of the respective Chlamydia species or combinations of them was used. Multiplex Chlamydia TETR-PCR correctly identified one strain of each of the 15 serovars of C. trachomatis, 22 isolates of C. pneumoniae and 20 isolates of C. psittaci. The primer sets were specific for each species. No target products were amplified when DNA from C. pecorum or a variety of other microorganisms was tested for specificity. TETR-PCR with primers selected for specific sequences in the 16S and 16S–23S spacer rRNA genes is a valuable test that could be used either with individual primers or in a multiplex assay for the identification and differentiation of Chlamydia species from culture isolates or for the detection of chlamydial in clinical samples.

5. **Small bowel bacterial overgrowth (H2BT)**: a hydrogen breath test (H2BT) [19] carried out to assess for bacterial overgrowth. H2BT protocol (carried out in the lymphoma clinic): we first collected a baseline basal breath sample (time 0) from the patient. The patient then consumed 50 g of dextrose, >5 min. We then collected additional sequential breath samples every 15 min, for a total of 2 h (times 15, 30, 45, 60, 75, 90, 105 and 120 min). Each breath sample was captured in an individual air collection bag (QuinTron, Milwaukee, WI). Each bag was then labelled with patient identification information, and the timing of the sample. Following the H2BT, all breath samples were shipped to MC, Director of Gastrointestinal Physiology Laboratory, at the University of Michigan Medical Center, for analysis. Each breath sample was analyzed for hydrogen and methane gas by a QuinTron MicroLyzer (QuinTron). An increase of hydrogen or methane, by 12 parts per million or more, over baseline, was defined as a positive indicator for bacterial overgrowth.

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