Subcutaneous ‘lipoma-like’ B-cell lymphoma associated with HCV infection: a new presentation of primary extranodal marginal zone B-cell lymphoma of MALT

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Background: Hepatitis C virus (HCV) infection has been linked to lymphoproliferative disorders. Marginal zone B-cell lymphoma (MZL) represents one of the most frequent lymphoma subtypes associated with HCV infection. We describe an unusual subset of HCV-associated MZL characterized by subcutaneous presentation.

Materials and methods: A series of 12 HCV-positive patients presenting with subcutaneous nodules that revealed lymphoma infiltration at biopsy. Molecular analysis of immunoglobulin heavy chain (IGH) gene rearrangement and FISH investigations for t(11;18)(q21;q21) and t(14;18)(q32;q21) were carried out in nine patients.

Results: The 12 patients (median age 69.5 years), all with positive HCV serology, presented with single or multiple subcutaneous nodules resembling lipomas. Histologically the lesions showed lymphoid infiltrates, consistent with extranodal MZL of mucosa-associated lymphoid tissue (MALT). Functional IGH gene rearrangements were identified in nine tested patients, with somatic mutations in 82%, indicating a histogenesis from germinal center-experienced B cells. The t(11;18) was found in two of nine cases. Staging did not show any other lymphoma localization. In two patients, a response was achieved with antiviral treatment. Extracutaneous spread to MALT sites occurred in a case.

Conclusions: Our observations expand the spectrum of HCV-associated lymphomas to include a subset of extranodal MZL characterized by a novel primary ‘lipoma-like’ subcutaneous presentation and indolent clinical course.

Key words: hepatitis C virus, IGH rearrangement, MALT, marginal zone lymphoma, subcutaneous tissue

Introduction

Chronic antigenic stimulation resulting from infectious agents is pathogenetically related to various lymphoma subtypes which often primarily arise at extranodal sites. The best documented role of infections in lymphomagenesis is represented by Helicobacter pylori in gastric lymphoma [1] and Epstein-Barr virus (EBV) in Burkitt’s lymphoma and in sinusoidal NK/T-cell lymphoma [2]. The spectrum of infectious agents has been subsequently expanded to include the association of Borrelia Burgdoferi with cutaneous B-cell lymphoma [3], Chlamydia psittaci with ocular adnexal lymphoma of mucosa-associated lymphoid tissue (MALT) [4], and hepatitis C virus (HCV) with lymphoma of liver, salivary glands, and spleen [5–9].

It is now well known that, in addition to hepatic manifestations, HCV infection is linked to a spectrum of cryoglobulinemic and non-cryoglobulinemic B-cell lymphoproliferative disorders. A meta-analysis showed that the prevalence of HCV infection in B-cell non-Hodgkin’s lymphoma (NHL) patients is 15% in comparison with 1.5% in the general population [10]; this association is more evident in geographic areas with high HCV seroprevalence. The pathogenetic link between HCV and some lymphoproliferative disorders has been confirmed by their responsiveness to antiviral therapy [11, 12].

HCV-associated B-cell NHL has distinctive clinicopathological features including a predilection for extranodal localizations and an overrepresentation of the diffuse large B-cell and marginal zone histological subtypes [13, 14]. Recently, the presence of HCV infection has been also detected in cases of primary cutaneous B-cell lymphomas, irrespectively of clinicopathological subtype [15]. However, the subcutaneous tissue has never been reported among the extranodal sites of presentation of lymphomas arising in HCV-positive patients.

Herein we detail the clinicopathological and molecular features of 12 HCV-positive patients, who presented with solitary or multiple nodular subcutaneous lesions clinically...
resembling lipomas. In all cases, histological lesional examination documented a marginal zone B-cell lymphoma (MZL) infiltration, which was strictly confined to subcutaneous tissue. Despite the high rate of local relapses, the lymphoma pursued an indolent clinical course; after a median follow-up of 50 months, 11 patients are alive and one died of lymphoma (11 years after the first appearance of the subcutaneous nodule and 7 years after the histological diagnosis). In two cases, antiviral therapy resulted in lymphoma regression.

materials and methods

patients
Among 101 nongastric primary extranodal MZL of MALT type diagnosed from 1998 to 2006 at the Pathology Section, Department of Human Pathology, Fondazione IRCCS Policlinico San Matteo, University of Pavia [6], we identified 12 patients with primary subcutaneous lymphoma (Table 1).

Thirteen cases with primary cutaneous MZL, one case with multiple concurrent MALT sites including subcutis, and one HCV-positive case of ocular adnexal MALT lymphoma with a subcutaneous relapse 4 years after diagnosis were excluded. Four of 13 patients affected by primary cutaneous MZL were HCV positive.

Clinical data were available for all patients, including biochemistry, HCV status, and staging procedures, such as computed tomography scan, gastric endoscopy, and bone marrow biopsy. All patients were treated and/or followed up at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo, University of Pavia.

Approval was obtained from the local Institutional Ethical Committee for this retrospective study, which was based on archival data. Data management and analysis were carried out in accordance to the Helsinki Declaration of 1975, revised in 1983 and 2000.

histopathology, immunohistochemical analysis, and EBV in situ hybridization
Lesional tissue samples were obtained from subcutaneous nodules of all patients at diagnosis, before initiating specific therapies. Three patients had diagnostic biopsies at two distinct anatomical sites. Additional biopsies were carried out at subcutaneous relapse, in five patients. Altogether, 20 formalin fixed and routinely processed lesional samples from 12 patients were available for morphological and immunohistochemical investigations. Additional frozen material was available for seven samples from five patients. In all patients, bone marrow biopsy was carried out at diagnosis [16].

Histological diagnosis of extranodal MZL of MALT was made by three of us (two expert hematopathologists and one dermatopathologist) according to the criteria of 2008 updated World Health Organization lymphoma classification [17].

Automated paraffin section immunostainings (DakoCytomation Autostainer, Glostrup, Denmark) were carried out in all samples, employing the streptavidin–biotin peroxidase revelation system. Antibodies against CD20, CD79a, CD10, CD5, CD21, CD23, CD3, CD38, cyclin D1, bcl-2, bcl-6, bcl-10, MUM1, Mib1/Ki67, immunoglobulin (Ig) light chains (k and l), IgM, IgG, and IgD were used. The EBV status was tested by in situ hybridization (ISH) analysis using a fluorescein isothiocyanate-labeled peptic nuclear acid probe, complementary to the EBV-encoded RNAs (DakoCytomation).

analysis of immunoglobulin heavy chain variable region (IGHV) gene rearrangements
IGHV gene status was investigated in 17 lesional specimens obtained at diagnosis and/or at relapse from 9 of 12 patients; genomic DNA was obtained from paraffin (n = 10) or frozen (n = 7) samples. IGHV rearrangements were amplified with family-specific primers that hybridize to sequences in the IGHV leader, framework region (FR) 1, or FR2 in conjunction with the corresponding IGHV outer primers, in separate reactions for each IGHV family. An alternative set of IGHV primers (J-outer primers) was also used when no amplicon was obtained using the IGHV outer primers. The sequences of IGHV leader, FR1, FR2, and J-outer primers have been previously reported [18].

PCR products were separated by agarose gel electrophoresis, purified using the PrepTact Gel Cleanup Kit (Eppendorf, Hamburg, Germany), and directly sequenced with the ABI PRISM BigDye Terminator v1.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Milan, Italy). Automated sample sequencing was carried out on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Sequences were aligned to the VBASE sequence directory (MRC Centre for Protein Engineering, Cambridge, UK) using MacVector 6.0.1 software (Oxford Molecular Group, Oxford, UK). IGHV gene sequences were considered mutated if the deviation from the corresponding germline gene was ≥2%.

analysis of BCL-1 and BCL-2 rearrangements
BCL-1 [19] and BCL-2 [20] rearrangements were investigated by means of PCR. In particular, for the BCL-1/IGH, a nested PCR was carried out with two successive amplifications using primers homologous to the 5′ sequence of the major translocation cluster of the BCL-1 locus (P2, P3) and to the consensus sequences of the six J-regions of the immunoglobulin heavy chain (IGH) gene (LJH, VLIJH). For the molecular detection of IGH gene fusion with the BCL-2 major breakpoint region (MBR), a standard PCR protocol was employed. The amplification products were electrophoresed in 2% agarose gels and visualized by ethidium bromide staining and UV illumination.

FISH analysis. Interphasic FISH was carried out on routine paraffin sections (3–4 μm). FISH experiments were carried out using the following probes: LSI MALT1 Break Apart strategy for screening of rearrangements, LSI AP2/ MALT1 and LSI IGH/MALT1 Dual Color Dual Fusion for the definition of chromosome rearrangements, and centromeric probes for detection of copy number changes (CEP) of chromosome 3 and 18. All probes, provided by Vysis (Abbott Diagnostics, Wiesbaden-Delkenheim, Germany), were directly labeled with orange and green fluorochromes. The analysis was carried out using direct viewing on a standard fluorescence microscope (Olympus BX51 equipped with a suitable filter set) at ×100 magnification. In each case, >100 nuclei on paraffin-embedded sections were examined from at least five areas

Table 1. Primary MALT lymphoma localizations according to HCV serostatus

<table>
<thead>
<tr>
<th>Localization</th>
<th>Positive</th>
<th>Negative</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>101</td>
<td>45</td>
<td>12</td>
</tr>
<tr>
<td>OAL</td>
<td>20</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Parotid/salivary glands</td>
<td>18</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Waldeyer’s ring</td>
<td>13</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Skin</td>
<td>13</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Colon/small bowel</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Female genitals</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CNS</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The number cases of MALT lymphoma localized to subcutaneous tissue is in bold.

OAL, ocular adnexal lymphomas; CNS, central nervous system.
selected for well-preserved cellular and nuclear morphology to ensure representative samples and to avoid nuclear truncation.

**results**

**clinical features**

The 12 patients (two males and 10 females, median age 69.5 years, range 54–75) presented with single (3 of 12) or multiple (9 of 12) subcutaneous nodular lesions, ranging in diameter from 2 to 5 cm (Figure 1A). Major clinical and virological features are summarized in Table 2. On physical examination, the lesions resembled lipoma (soft and mobile nodules underlying a normal appearing skin). In three patients, due to the benign clinical appearance of the lesions, a diagnostic biopsy was postponed for 2, 3, and 4 years, respectively.

Hematological staging did not detect other lymphoma localizations at MALT sites or nodal disease; no splenomegaly or splenic lesions were found. No history of previous lymphoma was registered. A single patient had bone marrow involvement (case #4) at diagnosis. The Eastern Cooperative Oncology Group score was 0–1 in 11 patients and 2 in one patient. Lactate dehydrogenase was normal in all patients and β2-microglobulin was >3 mg/ml in two patients. Six patients had a small serum IgM monoclonal component (<1 g/dl); one patient had small double-monoclonal component IgMk/IgMk.

All patients were positive for HCV antibodies at enzyme-linked immunosorbent assay test, confirmed by recombinant immunoblot assay. HCV-RNA was detectable in 10 of the 10 patients tested. Seven patients were tested for genotype that resulted as follows: 2a/2c in five patients, 2a in one patient, and 2b in one patient.

Cryoglobulins were found in four patients; patient 2 presented cryoglobulinemic purpura. No patient had autoimmune disorder. One had a history of surgery for renal cell carcinoma 6 months before lymphoma diagnosis.

A complete surgical excision of the subcutaneous tumor was carried out in all cases. Three patients were followed with no additional treatment; nine patients had specific therapy; local radiotherapy in two patients, chlorambucil in two patients, combination chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)-like chemotherapy plus radiotherapy in three patients, rituximab in one patient, and interferon + ribavirine in one patient. A complete disappearance of nodules was obtained in five patients while four patients showed a partial response (more than 50% reduction of lesion size). In particular, the patient 11 treated with rituximab obtained a complete remission lasting 2 years; at first relapse, a second course of rituximab allowed to obtain a second complete remission that lasted for 20 months. The patient 10 was treated with interferon and ribavirin as first-line treatment and had a rapid virological response and a partial response for the lymphoma.

Interestingly, patient 2 relapsed 20 months after CHOP therapy plus radiotherapy and was treated with interferon alone for 6 months and obtained a complete regression of nodules after 1 month of therapy and a virological response. HCV-RNA returned positive 8 months after stopping antiviral treatment and a subcutaneous lymphoma relapse occurred 3 months after virological relapse.

Subcutaneous relapse occurred in six patients (24–48 months from diagnosis). Extracutaneous spread to MALT sites (parotid gland, lung, orbit) occurred in one patient 5 years after diagnosis. After a median follow-up of 50 months (range 12–96 months), 11 patients are alive and a single patient (case #5) died of lymphoma (11 years after the first appearance of a subcutaneous nodule and 7 years after the histological diagnosis). This patient died of cardiac failure with active lymphoma in the lungs; no histological transformation was documented.

**histology**

In all cases, irrespectively from the anatomical area of the primary lesion and/or timing of biopsy (diagnosis or relapse), the cellular infiltrates largely overlapped in both morphological and immunophenotypic features.

**Figure 1.** (A) Lipoma-like nodule in the thoracic wall. (B) Histological examination showed marginal zone B-cell lymphoma (HE ×20) confined to the subcutis, sparing the overlying skin (inset, HE ×2).
Table 2. Clinical features of 12 patients with subcutaneous marginal zone B-cell lymphoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>No. of sites</th>
<th>Sites</th>
<th>Ann Arbor stage</th>
<th>BM</th>
<th>Serum MC</th>
<th>HCV ab</th>
<th>HCV-RNA (UI/ml)</th>
<th>HCV-RNAq</th>
<th>HCV genotype</th>
<th>Crioglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>Female 1</td>
<td>1</td>
<td>Right breast</td>
<td>IA</td>
<td>Negative</td>
<td>IgMk</td>
<td>Positive</td>
<td>Positive</td>
<td>1 600 000</td>
<td>2a/2c</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>Female 1</td>
<td>1</td>
<td>Left breast</td>
<td>IA</td>
<td>Negative</td>
<td>IgMk</td>
<td>Positive</td>
<td>Positive</td>
<td>2 375 740</td>
<td>2a/2c</td>
<td>Positive (5%)</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>Male 2</td>
<td>2</td>
<td>Right paravertebral, left lumbar</td>
<td>IIA</td>
<td>Negative</td>
<td>–</td>
<td>Positive</td>
<td>Positive</td>
<td>3 983 240</td>
<td>2a</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>Female 2</td>
<td>1</td>
<td>Right axilla, abdominal wall</td>
<td>IVA</td>
<td>Positive</td>
<td>IgMk</td>
<td>Positive</td>
<td>Positive</td>
<td>3 496 520</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>Female 2</td>
<td>1</td>
<td>Back</td>
<td>IA</td>
<td>Negative</td>
<td>–</td>
<td>Positive</td>
<td>Positive</td>
<td>17 440 000</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>Female 2</td>
<td>1</td>
<td>Thoracic wall</td>
<td>IIA</td>
<td>Negative</td>
<td>–</td>
<td>Positive</td>
<td>na</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>Female 2</td>
<td>1</td>
<td>Both thighs</td>
<td>IVA</td>
<td>Negative</td>
<td>–</td>
<td>Positive</td>
<td>Positive</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>Female 2</td>
<td>2</td>
<td>Abdominal wall</td>
<td>IVA</td>
<td>Negative</td>
<td>IgMk</td>
<td>Positive</td>
<td>33 000</td>
<td>2a</td>
<td>Positive (4%)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>Female 1</td>
<td>1</td>
<td>Left thigh</td>
<td>IA</td>
<td>Negative</td>
<td>–</td>
<td>Positive</td>
<td>na</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>68</td>
<td>Male 2</td>
<td>1</td>
<td>Back, right thigh</td>
<td>IVA</td>
<td>Negative</td>
<td>IgMk</td>
<td>Positive</td>
<td>2 242 730</td>
<td>2a/2c</td>
<td>Positive (2%)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>72</td>
<td>Female 2</td>
<td>1</td>
<td>Right breast, right gluteus</td>
<td>IVA</td>
<td>Negative</td>
<td>IgMk/IgGk</td>
<td>Positive</td>
<td>104 352</td>
<td>2b</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>74</td>
<td>Female 3</td>
<td>2</td>
<td>Thoracic wall, abdominal wall, right arm</td>
<td>IVA</td>
<td>Negative</td>
<td>IgMk</td>
<td>Positive</td>
<td>317 068</td>
<td>2a/2c</td>
<td>Positive (3%)</td>
<td></td>
</tr>
</tbody>
</table>

BM, bone marrow; MC, monoclonal component; HCV, hepatitis C virus; NA, not available.

The lymphoma was strictly confined to the subcutaneous tissue, the overlying dermis, epidermis, and adnexa (Figure 1B, inset) being spared; in a single case muscle infiltration was observed. On the contrary, in the 13 cases of primary skin MZL that were excluded from the study, the lymphoma exclusively involved the dermis, showing a perivascular/periadnexal patchy and nodular infiltrate, sparing both epidermis and subcutis.

At scanning power, the infiltrate showed a nodular and diffuse pattern of growth in 7 of 12 cases; the remaining five cases exhibited a diffuse pattern. In all cases, the predominant population cytologically consisted of small- to medium-sized lymphoid cells, centrocyte-like to monocytoid in appearance, consistent with a marginal zone origin (Figure 1B). Scattered, larger centroblast-like cells were intermingled within the infiltrate in all specimens. In most cases (9 of 12), plasmacytic differentiation was found, with the presence of mature plasma cells, mainly distributed at the periphery of the infiltrate.

In 10 of 12 cases, there were remnants of germinal centre-like structures, mostly surrounded and colonized by lymphoma cells. No significant tissue eosinophilia was noticed. Accompanying fibrosis was also observed: it was fine interstitial (5 of 12 cases) or in broad bands (two cases).

In all cases, the antigenic profile of the lymphoma population was consistent with a marginal zone derivation. Tumor cells intensely stained for B-cell markers CD20, CD79a, PAX5, with surface IgM+/IgD− phenotype in 12 of 12 tested cases; in 5 of 12 cases, a minority of lymphoma cells stained for IgG. In the nine cases with features of plasmacytic differentiation, the plasma cells showed clonal cyttoplasmic light-chain restriction (κ in six cases and λ in three cases).

Immunostainings for CD5, CD23, cyclin D1, bcl-10, as well as germinal center markers (CD10 and bcl-6) were uniformly negative. Anti-bcl-2 antibody stained lymphoma cells in 12 of 12 cases but it was negative in the remnants of reactive germinal centers. The proliferation index, evaluated by Mib-1/Ki-67 antibody, ranged in between 5% and 15%. ISH search for EBV infection was negative in all cases.

In a single case (patient 4), bone marrow histology documented a lymphoma infiltration (30%) at diagnosis, with a sinusoidal and interstitial nodular pattern.

**analysis of BCL-1, BCL-2, and IGHV genes rearrangements**

Monoclonal IGHV-D-J rearrangements could be detected in nine of nine tested patients, with the identification of 13 distinct tumor clones. Results of molecular analysis are summarized in Table 3. Of the three patients who underwent biopsy of two distinct nodules at diagnosis, identical clones were identified in two (cases #3 and #8), whereas the two biotop samples were clonally different in one patient (case #4). In two patients (cases #5 and #6), an identical IGHV-D-J rearrangement was identified at diagnosis and at relapse, whereas in three other patients (cases #1, #2, and #7), the clones at diagnosis and at relapse were different. Somatic mutations were detected in 14 of 17 (82%) clonal rearrangements, with a median frequency of mutation of 7.4% (range 3.3%–14%). Among cases with an identical IGHV-D-J rearrangement, patients 5 and 6 shared the most mutational events, whereas in one patient (case #3), only a fraction of mutations was shared by the two clones. In one patient (case #8), an identical, unmutated IGHV-D-J rearrangement was identified both at diagnosis and at relapse. The most frequently used IGHV family was IGHV3 (eight tumor clones), followed by IGHV1 (four tumor clones) and IGHV2 (one tumor clone). In particular, IGHV1-69, IGHV3-07, and IGHV3-23 were the genes most frequently rearranged.

The search of BCL-1 and BCL-2 rearrangements was negative in 19 of 19 tested specimens. PCR procedures used to analyze BCL-1 and BCL-2 rearrangements can only identify a limited proportion of translocations. PCR sensitivity for detecting BCL-1 gene aberrations is <50% in most studies, whereas PCR sensitivity for BCL-2, using set of primers specific for MBR, reaches 60%–70% [20].

BM, bone marrow; MC, monoclonal component; HCV, hepatitis C virus; NA, not available.
trisomies, tetrasomies) in all tested specimens. (mixed cell populations showing monosomies, disomies, chromosomes 3 and 18, we detected chromosome aneuploidy female patients; we have no explanation for such sex lymphoma diagnosis were postponed for years.

nodules and the absence of symptoms, biopsy and subsequent patients, due to the apparently benign clinical appearance of subcutaneous nodular lesions that mimicked lipomas; in three indolent clinical course. The patients had solitary or multiple unusual primary subcutaneous localization and a relatively extranodal MZL of MALT type, clinically characterized by an features of 12 HCV-positive patients who presented with B-lymphomas including the MZL subtype [15, 23].

HCV has been also documented in primary cutaneous as a paradigmatic example of a HCV-driven with splenic MZL with villous lymphocytes has been proposed in the splenic form [6, 8, 9, 21]. Notably, the association of HCV infection has been reported in MALT lymphoma as well as in lymphomas were more likely to involve the skin, whereas the subcutis was a frequent site of secondary spread and/or relapse; a high rate of secondary subcutaneous spread has been reported also in a series of HCV-associated MALT-type lymphoma of ocular adnexa [27].

Subcutis may be sometimes involved in course of different systemic lymphoma subtypes, whereas it rarely represents the primary site of disease presentation. As a consequence, in this series, morphological differential diagnoses mainly regarded secondary lymphoma localization, reactive lymphoid hyperplasia (so-called pseudolymphoma), and panniculitis-like T-cell lymphoma.

In our patients, a secondary subcutaneous spread was clinically excluded by careful staging procedures; a possible subcutaneous extension by primary cutaneous MZL was excluded by the lack of overlying skin and adnexa involvement [18, 26]. The differential diagnosis between lymphoid hyperplasia and lymphoma may sometimes reveal difficult mainly at extranodal sites. In our cases, the quite monomorphous appearance of lymphoid population, coupled with its massive subcutis infiltration, strongly favored the histological diagnosis of lymphoma. In the cases with a plasmacytic component, immunohistochemical analyses corroborated the lymphoma diagnosis by showing a cytoplasmic Ig light-chain restriction (κ in six cases and λ in preidiction, but other authors as well as our group have already noticed a female prevalence in previous studies on lymphoproliferative disorders associated with HCV infection [6, 24].

Extranodal MZL may affect numerous anatomical sites and organs, but primary soft tissue localizations are exceedingly rare with an approximate incidence of 0.1 per 100 000 [25], that is related to the whole spectrum of primary soft tissue involvement.

At the best of our knowledge, this is the first report of a homogeneous series of primary subcutaneous MZLs associated with HCV infection. Previously, only one case of primary subcutaneous MZL has been reported, by Bailey et al. [26], but within a larger series of primary and secondary MZL mostly involving the skin. In the study by Bailey, primary lymphomas were more likely to involve the skin, whereas the subcutis was a frequent site of secondary spread and/or relapse; a high rate of secondary subcutaneous spread has been reported also in a series of HCV-associated MALT-type lymphoma of ocular adnexa [27].

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At the best of our knowledge, this is the first report of a homogeneous series of primary subcutaneous MZLs associated with HCV infection. Previously, only one case of primary subcutaneous MZL has been reported, by Bailey et al. [26], but within a larger series of primary and secondary MZL mostly involving the skin. In the study by Bailey, primary lymphomas were more likely to involve the skin, whereas the subcutis was a frequent site of secondary spread and/or relapse; a high rate of secondary subcutaneous spread has been reported also in a series of HCV-associated MALT-type lymphoma of ocular adnexa [27].

Subcutis may be sometimes involved in course of different systemic lymphoma subtypes, whereas it rarely represents the primary site of disease presentation. As a consequence, in this series, morphological differential diagnoses mainly regarded secondary lymphoma localization, reactive lymphoid hyperplasia (so-called pseudolymphoma), and panniculitis-like T-cell lymphoma.

In our patients, a secondary subcutaneous spread was clinically excluded by careful staging procedures; a possible subcutaneous extension by primary cutaneous MZL was excluded by the lack of overlying skin and adnexa involvement [18, 26]. The differential diagnosis between lymphoid hyperplasia and lymphoma may sometimes reveal difficult mainly at extranodal sites. In our cases, the quite monomorphous appearance of lymphoid population, coupled with its massive subcutis infiltration, strongly favored the histological diagnosis of lymphoma. In the cases with a plasmacytic component, immunohistochemical analyses corroborated the lymphoma diagnosis by showing a cytoplasmic Ig light-chain restriction (κ in six cases and λ in

**Table 3. Molecular features of nine patients with subcutaneous marginal zone B-cell lymphoma**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
<th>Patient 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of biopsy</td>
<td>D</td>
<td>R</td>
<td>D</td>
<td>R</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>EBERs</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>BCL-1</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>BCL-10</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>t(11;18)(q21;q21)</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>t(14;18)(q32q21)</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>J</td>
<td>4b</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>4b</td>
<td>4b</td>
<td>4b</td>
<td>5a</td>
<td>3b</td>
</tr>
<tr>
<td>% Mutation</td>
<td>7.2</td>
<td>13</td>
<td>5.4</td>
<td>0</td>
<td>11</td>
<td>13</td>
<td>5.4</td>
<td>7.0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

EBER, Epstein–Barr virus encoded RNA; D, biopsy at diagnosis; R, biopsy at relapse; Neg, negative; Pos, positive; NA, not assignable.

**FISH analysis.** Nine cases have been analyzed by FISH. A t(11;18)(q21;q21) (API2/MALT1) translocation was observed in two of nine (22%) cases (patients 1 and 5) whereas the search for t(14;18)(q32q21) (IGH/MALT1) translocation was negative in all cases. Using centromeric probes for both chromosomes 3 and 18, we detected chromosome aneuploidy (mixed cell populations showing monosomies, disomies, trisomies, tetrasomies) in all tested specimens.

**discussion**

Reports in the literature dealing with lymphoproliferative disorders associated with HCV infection, indicate variable incidences for the various lymphoma histotypes; this variability may in part reflect different diagnostic classification approaches and/or geographic distribution.

Although all histological lymphoma types can virtually be found in HCV-positive patients, peripheral B-cell-derived malignancies are the most common, including MZL, lymphoplasmacytic lymphoma, and diffuse large B-cell lymphoma.

Among MZL subtypes, a special association with HCV infection has been reported in MALT lymphoma as well as in the splenic form [6, 8, 9, 21]. Notably, the association of HCV with splenic MZL with villous lymphocytes has been proposed as a paradigmatic example of a HCV-driven lymphoproliferative disorder [22]. Recently, the presence of HCV has been also documented in primary cutaneous B-lymphomas including the MZL subtype [15, 23].

Herein we detail the clinicopathological and molecular features of 12 HCV-positive patients who presented with extranodal MZL of MALT type, clinically characterized by an unusual primary subcutaneous localization and a relatively indolent clinical course. The patients had solitary or multiple subcutaneous nodular lesions that mimicked lipomas; in three patients, due to the apparently benign clinical appearance of nodules and the absence of symptoms, biopsy and subsequent lymphoma diagnosis were postponed for years.

Interestingly, in this series, we observed a prevalence of female patients; we have no explanation for such sex predilection, but other authors as well as our group have already noticed a female prevalence in previous studies on lymphoproliferative disorders associated with HCV infection [6, 24].
three cases). A subcutaneous panniculitis-like T-cell lymphoma was easily excluded because its clinical, histological, and immunophenotypical features strikingly diverge from low-grade B-cell MZL [28].

Molecular analysis of IGH gene rearrangements documented in all tested lesional samples (both at diagnosis and at relapse) the presence of a clonal rearrangement. The most frequently used IGHV family was IGHV3, followed by IGHV1 and IGHV2 and in particular, IGHV1-69, IGHV3-07, and IGHV3-23 were the genes most frequently rearranged. Such findings are in keeping with those reported by previous studies analyzing IGH gene rearrangements in HCV infection-associated B-cell lymphomas, irrespectively from the histological subtypes [29–31].

The presence of somatic mutations was found in 14 of 17 (82%) clonal rearrangements, indicating that most B-cell clones giving rise to this subcutaneous lymphoma are histogenetically related to B cells that have experienced germinal center reaction [32].

In some patients, the lesions presented and/or relapsed at different anatomical sites; notably, four patients had different IGHV-D-J rearrangements at diagnosis (case #4) and at relapse (cases #1, #2, and #7), respectively. On such bases, it seems reasonable that these lymphomas may arise from different antigen-driven clonal expansions synchronously or metachronously presenting at different anatomical sites. The fact that different lymphoma clones may arise at different anatomical sites has also been observed in other virus-associated lymphoid malignancies, namely post-transplant lymphoproliferative disorders [33].

Our molecular investigations documented a biased IGH gene usage and the presence of somatic hypermutation. These findings, coupled with the lymphoma regression after antiviral therapy observed in two patients, seem to indicate that HCV may play a causative role also in this peculiar subset of MZL. Actually, a recent study which analyzed the molecular features of Ig gene rearrangements in monoclonal B-cell populations derived from HCV-associated lymphoid proliferations has demonstrated that HCV may act as an exogenous trigger in certain stages of B-cell lymphoproliferation (i.e. type II cryoglobulinemia as well as in some overt B-cell NHL) [34, 35]. It is conceivable that chronic HCV infection, likewise other infectious agents such as H. pylori, represents a constitutive source of antigens that trigger a sustained lymphoid proliferation, giving a selective advantage to lymphoid clones that initially remain dependent upon antigen stimulation. In this setting, additional oncogenic events may occur, leading the lymphoid proliferation to become independent of antigenic stimulation.

The putative pathogenetic role of HCV in driving these monoclonal antigen–experienced B-cell lymphoproliferations confirms the hypothesis that antiviral treatment, if clinically feasible, may be the first choice therapeutic strategy in this peculiar lymphoma subset. Antiviral treatment of HCV-positive lymphoma is indicated if a specific hematological treatment (i.e. chemotherapy) is not mandatory due to the indolence of disease and if the age of patients is not too advanced for interferon with or without ribavirine. Recently, a number of recurrent structural and numerical chromosomal alterations have been reported in MALT lymphoma from different anatomical sites. Four main chromosomal translocations have been associated with the pathogenesis of MZL, MALT type: t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)(p14.1;q32), resulting, respectively, in the production of a chimeric protein (API2/MALT1) or in transcriptional deregulation of BCL-10, MALT1, and FOXP1 because of juxtaposition to the IGH promoter region [36–39]. In our series, the API2/MALT1 translocation was observed in two of nine cases (22%) by FISH technique, whereas MALT1/IGH translocation was uniformly absent. No case showed immunostaining for BCL-10.

These findings are in keeping with the overall incidence of t(11;18)(q21;q21) in MALT lymphomas, which is ~20%.

Clinicians should be aware of this further unusual primary site of lymphoma presentation in HCV patients because the benign ‘lipoma-like’ lesional appearance and indolent clinical behavior may result into diagnostic delay.

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