Hepatosplenic γδ T-cell Lymphoma is a Rare Clinicopathologic Entity with Poor Outcome: Report on a Series of 21 Cases.

Short title: HEPATOSPLENIC γδ T-CELL LYMPHOMA


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Clinical Observations, Interventions, and Therapeutic Trials

ABSTRACT

We report on the characteristics of 21 cases of Hepatosplenic \( \gamma\delta \) T-cell lymphoma (HS\( \gamma\delta \)TCL), an entity recognized since 1994 by the REAL classification. Median age was 34 years. Patients presented with splenomegaly (n=21), hepatomegaly (n=15) and thrombocytopenia (n=20). Histopathologic findings were homogeneous with the presence of medium-sized lymphoma cells within the sinusoids of splenic red pulp, liver and bone marrow. Marrow involvement was usually mild but could be demonstrated by phenotyping in all cases. Cells were CD3+, CD5-, expressed the \( \gamma\delta \) T-cell receptor and had a non-activated cytotoxic cell phenotype (TIA-1+, granzyme B-). Most cases were CD4-/CD8- (16/18), CD56+ (15/18), expressed the V\( \delta \)1epitope (Vd1+/Vd2-/Vd3-, 9/12) and were negative for EBV (18/20). An isochromosome 7q was found in nine of 13 documented cases. Eight patients had a previous history of kidney transplant, systemic lupus, Hodgkin disease or malaria. Prognosis was very poor with a median survival time of 16 months and all patients but two ultimately dying despite consolidative or salvage high-dose therapy. In conclusion, HS\( \gamma\delta \)TCL is a disease with distinctive clinical, histopathologic and phenotypic characteristics. Bone marrow biopsy with combined phenotyping is sufficient for diagnosis and splenectomy is therefore unwarranted. Current treatment modalities appear to be ineffective in most cases.
Introduction

Human \( \gamma \delta \) T lymphocytes represent a normal subset of post-thymic T cells with cytotoxic functions and preferential homing in some epithelial-rich tissues and within sinusoidal areas of the splenic red pulp.\(^{1,2}\) In 1990, we proposed hepatosplenic \( \gamma \delta \) T-cell lymphoma (HS\( \gamma \delta \)TCL) as a distinct entity among peripheral T cell lymphomas which was recognized on the basis of clinical presentation, pattern of histologic involvement resulting from the sinusal/sinusoidal tropism of neoplastic cells and expression of the \( \gamma \delta \) T-cell receptor (TCR) by tumor cells.\(^ {3,4}\)

Since our initial reports, several cases of hepatosplenic lymphoma expressing a \( \gamma \delta \) phenotype have been reported.\(^ {5-25}\) Among these, Cooke et al.\(^ {11}\) published a series of seven cases with a proven \( \gamma \delta \) phenotype in five of them, providing further evidence that HS\( \gamma \delta \)TCL is a distinct clinicopathologic entity. The identification of this lymphoma subtype, recognized as a provisional entity in the REAL classification,\(^ {26}\) has been further supported by its cytotoxic phenotype\(^ {11,27}\) and its strong association with isochromosome 7q cytogenetic abnormality.\(^ {12,14,25,28-32}\)

More recently, a few cases of HSTCL with sinusoidal infiltration and an \( \alpha \beta \) T-cell receptor phenotype have been reported,\(^ {33-35}\) now considered an immunophenotypic variant of the same disease entity in the WHO classification.\(^ {36}\)

Due to the rarity of the disease and the difficulty to assess the \( \gamma \delta \) T-cell origin which relies on frozen tissue immunophenotyping, most previous reports of HS\( \gamma \delta \)TCL refer to sporadic cases or very limited series with short follow-up and heterogeneous therapies, as recently reviewed by Weidman.\(^ {37}\) This prompted us to analyze the clinicopathologic characteristics and outcome of 21 patients with hepatosplenic T-cell lymphoma displaying a \( \gamma \delta \) TCR phenotype as demonstrated on frozen material that we collected over a 20-year
period. Our results confirm that HSγδTCL is a clinicopathologic entity of cytotoxic T-cell origin. We also show that bone marrow involvement is a constant finding with diagnostic value and that the disease, which may occur in the setting of immunosuppression, has a very poor prognosis.

Patients and methods

Patient selection. Patients with proven HSγδTCL diagnosed between 1981 and 2001 were selected from the files of the Department of Pathology (Hôpital Henri Mondor, Créteil, France). Selection criteria for this study were: (1) a diagnosis of HSγδTCL established on histopathology and immunophenotyping on frozen and/or fresh material and (2) clinical information and follow-up data available. Twenty-one patients fulfilled these criteria. In thirteen cases, the biopsies had been referred for consultation. Parts of immunomorphologic, genotypic and/or cytogenetic features of some patients of the present series have been reported previously.4,14,32,38,39

Clinical staging. The extent of disease was measured by physical examination, bone marrow biopsy and/or aspiration, cerebrospinal fluid examination and CT scan of chest and abdomen. Staging was performed according to the Ann Arbor system. Performance status was based on the Eastern Cooperative Oncology Group scale (0 to 4). Serum lactate dehydrogenase (LDH) level was expressed as the ratio over the maximum normal value. Patients were retrospectively staged according to the age-adjusted international prognostic index.40
**Histopathologic studies.** Biopsy specimens from initial sites of involvement were reviewed, as well as all initial bone marrow biopsies. Biopsies at progression were also studied in 11 patients. Samples were fixed in buffered formalin or Bouin’s fluid. Paraffin-embedded tissue sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) and/or Giemsa. In all but one cases, a portion of tumoral tissue obtained at diagnosis (spleen, 11 cases; liver, 9; bone marrow, 8) was snap-frozen in liquid nitrogen for phenotypic, genotypic and/or cytogenetic studies. In seven patients, including the case without frozen tumoral tissue, fresh blood and/or bone marrow cells were also immunophenotyped by flow cytometry.

**Immunohistochemical staining.** Cryostat sections of spleen, liver and/or bone marrow specimens were evaluated for T-, natural killer (NK)-, and B-cell differentiation antigens using the alkaline phosphatase/anti-alkaline phosphatase (APAAP) method. Mouse monoclonal antibodies were used to detect the following antigens: CD2, CD3, CD4, CD5, CD7, CD8 (Becton Dickinson, Mountain View, CA); CD30, LMP-1 (Dako, Glostrup, Denmark); CD19, CD56 (Coulter, Hialeah, FL). The expression of the TCR chains was analyzed using βF1, δTCR-1, δTCS-1, Vδ2 and Vδ3 monoclonal antibodies (T-cell Diagnosis, Woburn, MA). βF1 recognizes a nonpolymorphic epitope of the β chain of the αβ TCR heterodimer. δTCR-1 recognizes a nonpolymorphic epitope of the δ chain of the γδ TCR heterodimer. δTCS-1 is directed against a conformational epitope of the Vδ1/Jδ1 junction, whereas Vδ2 and Vδ3 antibodies recognize an epitope of the human Vδ2 and Vδ3 regions of the δ chain, respectively.

After appropriate antigen retrieval, deparaffinized tissue sections were also evaluated with a panel of antibodies including CD20, CD3ε (Dako), CD5 and CD56 (Novocasrta, Newcastle, UK) as well as for formalin-resistant epitopes of cytotoxic cell proteins, i.e. T-cell
intracellular antigen-1 (TIA-1, Coulter) and Granzyme B (Monosan, Uden, The Netherlands). Expression of bcl-2 and p53 (DO7) (Dako) proteins was also investigated in several cases.

*In situ hybridization study.* The presence of Epstein-Barr virus (EBV) RNA was analyzed by non isotopic in situ hybridization (ISH) with EBERs 1-2 oligonucleotide probes (Dako) on deparaffinized tissue sections. Details of the procedure have been previously reported.

*Genomic study.* DNA analysis was performed by Southern blot in the first four patients of our series, as previously reported. Subsequently, clonality was assessed by analyzing TCR γ chain gene rearrangements using a GC clamp multiplex PCR/ denaturing gradient gel electrophoresis (DGGE) procedure.

*Cytogenetic studies.* In nine cases, cytogenetic analysis of marrow, spleen, or blood cells was performed according to standard protocol. In addition, the status of chromosome 7 was investigated by interphase fluorescence in situ hybridization (FISH) in one of these nine cases as well as on archival or fresh material of four other patients, as recently reported elsewhere.
Results

Clinical findings at presentation

Patients had been referred because of splenomegaly and cytopenia. Presenting features also included B symptoms in 14 patients and/or abdominal pain in five. Fifteen patients were male, and the median age was 34 years (range, 16 to 58). Median time from first discovery of these symptoms to diagnosis of HSγδTCL was 60 days (15-180). All patients had splenomegaly and 15 of them hepatomegaly. Physical examination was otherwise normal, showing no enlarged peripheral lymph node. CT scan disclosed no mediastinal or retroperitoneal lymphadenopathy.

As shown in Table 1, thrombocytopenia was present in 20 patients (platelet counts ranging from 25 to 121.10^9/L.), anemia in 15 (hemoglobin levels ranging from 5.7g/dl to 11.3 g/dl) and leucopenia in 12. Only two patients had an elevated white blood cell count with up to 5% detectable atypical lymphoid cells on blood smears. Retrospective examination of blood smears, however, could reveal very few atypical cells in eight other patients, irrespective of whether they were leucopenic or not. A low rate of circulating myeloid precursor cells was observed in eight cases. Monocytosis above 1.10^9/L was found in five cases, one of which was initially misdiagnosed as myelomonocytic leukemia. Liver tests were essentially normal, with slightly elevated levels of serum ASAT, ALAT and/or alkaline phosphatase activities in eight patients. In one patient, a low level of fibrinogen associated with increased triglyceridemia and ferritinemia suggested hemophagocytic syndrome, which was confirmed by bone marrow examination. Serological tests were negative for HTLV1, HIV and HBV in all patients and for HCV in the 12 studied cases.
All patients had initial bone marrow involvement by lymphoma cells as revealed by trephine biopsy in 19 cases and marrow aspirates in the two remaining cases (see below). On this basis, all patients had stage IV disease. According to the presence of an abnormal LDH level and of a performance status >1 (table1), 15 of the 21 patients presented with 2-3 risk factors of the age-adjusted international prognostic index \(^4\) and belonged to its high-risk group.

A significant past history was present in several patients (Table 1). Four patients had received long term immunosuppressive therapy -ie steroids with ciclosporin or azathioprin - for kidney transplantation performed 4, 5, 15 and 27 years prior to diagnosis of HS\(\gamma\)\(\delta\)TCL. A 16 year-old-girl had been followed during five years for systemic lupus erythematosus when she developed lymphoma. Two african patients had a past history of falciparum malaria 4 and 15 years before, respectively. Finally, a 34 year old patient with a recently diagnosed stage III EBV-positive Hodgkin's disease rapidly developed HS\(\gamma\)\(\delta\)TCL after he had completed a third course of MOPP/ABV chemotherapy.
Table 1. Presenting clinical and biological features of patients with HSγδTCL

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<th>Hemoglobin (g/dl)</th>
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<th>PBALC count x10^9/L</th>
<th>Monocyte count &gt; 1x10^9/L</th>
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Abbreviations: WBC, white blood cells; PBALC, peripheral blood atypical lymphoid cells; n= normal value; nd, not done; PS, performance status based on the Eastern Cooperative Oncology Group scale; LDH, serum lactate dehydrogenase.

* biological features of hemophagocytic syndrome
Pathological findings at presentation

Splenectomy was performed in 12 patients either for diagnosis purpose in eight of them or as part of initial therapy in four. In each case, the spleen was enlarged, weighting from 780 g to 6000 g and was free of nodules on cut surface. Histopathology was very similar from case to case. The general architecture was preserved but there was marked hyperplasia of the red pulp and atrophy of the white pulp (Figure 1A). Although at variable extent from case to case, there was an infiltration of both the cords and sinuses of the red pulp by atypical lymphoid cells. In most cases, the neoplastic cells tended to form clusters within the sinuses (Figure 1B). A number of histiocytes were also admixed within lymphoid infiltrates, disclosing features of hemophagocytosis in three cases (Figure 1C). In four cases, infracentrimetric hilar lymph nodes could be analyzed and, although their architecture was preserved, showed a mild sinusal and perisinusal infiltration by neoplastic cells, the detection of which was highlighted by immunohistochemistry.

A liver biopsy was performed in 15 patients, either during splenectomy or as part of initial clinical staging in three cases. In all cases, there was a tumoral lymphoid infiltration in the sinusoids that were variably dilated whereas involvement of the portal tracts was mild or absent (Figure 1D).

Initial bone marrow biopsy performed in 19 cases disclosed hypercellular marrow which was shown to contain an infiltration by atypical lymphoid cells (Figure 1E-I). However, the latter infiltration was most often subtle (Figure 1G), better demonstrated by careful histological review together with CD3 immunostaining (Figure 1F-H). To note, this subtle involvement was initially not recognized in six patients, leading to a misdiagnosis of reactive hypercellular marrow in five cases and of chronic myelomonocytic leukemia in
another patient with overt monocytosis at presentation. The pattern of lymphoid infiltration was very peculiar since, in all cases but one, it appeared to be located electively within variably dilated sinuses, resulting into indian files or clusters of neoplastic cells. In only one patient (case 21) was the mild sinusal involvement associated with a more diffuse pattern of infiltration. In two patients, a significant number of histiocytes with features of hemophagocytosis was observed, paralleling the lesions observed in their spleen. In the two patients without initial bone marrow biopsy, involvement was demonstrated by the presence of 10 (case 18) to 40% (case 14) of atypical lymphoid cells on marrow aspirate smears.

Overall, the cytological aspect of lymphoma cells at presentation showed little variation from case to case. Indeed, irrespective of the sites of involvement, neoplastic cells in all but two cases were monomorphic with round or slightly irregular small to medium-sized nuclei containing conspicuous nucleoli and moderately abundant cytoplasm with rare mitotic figures. Only two cases at presentation (Cases 12 and 14) disclosed cell pleomorphism with presence of medium to large cells (Figure 1J). In many cases, a proportion of neoplastic cells in the bone marrow or in the spleen had elongated shapes with dendritic projections, likely resulting from closed contact with endothelial cells and subendothelial matrix. On bone marrow as well as blood smears, abnormal lymphoid cells were usually agranular except in one case disclosing cytoplasmic azurophilic granules in a few cells.

Finally, based on morphologic grounds and phenotypic results described below, the diagnosis was established on spleen specimen in eight patients, on bone marrow biopsy in nine and on liver biopsy in three others. In the remaining patient, diagnosis was based on morphologic and flow cytometric studies of the bone marrow aspirate. Median time from first discovery of cytopenia/splenomegaly to diagnosis of HSγδTCL was 60 days (15-180).
Figure 1.
Immunophenotypic findings

By definition, cases of the present study expressed γδ TCR (δTCR1+) and were negative for αβ TCR (βF1-), as demonstrated on frozen tissue material. As shown in Table 2, the neoplastic cells in all cases expressed the T-cell associated markers CD2 and CD3 but were CD5 negative, whereas CD7 was detected in 9/19 cases. Sixteen cases were CD4-/CD8-, two cases expressed CD8 whereas the three CD8- remaining cases were not interpretable for CD4. CD56 NK-cell antigen was detected in 15/18 studied cases while CD16 was found in only two of the seven cases studied by flow cytometry. Among the 12 cases which could be investigated using the three antibodies reacting with variable epitopes of the δ chain, nine were shown to derive from the Vδ1 subset (δTCS1+), two expressed the Vδ2-encoded epitope (Vδ2+), and the remaining case was negative. CD30 was negative in all cases, as well as CD19 and CD20 B-cell associated antigens.

In addition to studies on frozen sections, immunohistochemistry on routinely fixed paraffin-embedded tissue sections clearly demonstrated the common CD3+/CD5-/CD56+ phenotype of the lymphoma cells and highlighted their sinusal distribution. On routinely fixed material, all investigated cases but one (8/9) were shown to be negative for p53 (<5% tumor cells positive) whereas bcl-2 protein was negative or weakly expressed. All the 20 cases investigated for cytotoxic molecules demonstrated a strong granular cytoplasmic staining for the granule-associated protein TIA-1. By contrast, neoplastic cells were found positive for Granzyme B in only one case (Figure 1K-L).
Table 2: Immunohistochemical, genomic data and EBV status in patients with HSγδTCL

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Abbreviations: nd, not done; ni, not informative; CCs, conventional cytogenetic study; FISH, fluorescence in situ hybridization; R, rearranged; GL, germ-line configuration.

▲ EBV status determined by in situ hybridization with EBERs probes.

* conventional cytogenetic study showed a monosomy of chromosomes 6 and 10

** in this case, conventional cytogenetic study gave a normal result
EBV status

In situ hybridization studies with EBERs probes demonstrated the absence of EBV genome in neoplastic cells, except for the two above described cases with pleomorphic cytological features. Expression of the EBV-encoded latent membrane protein (LMP-1) as studied by immunohistochemistry was negative in all cases (Table 2).

Genomic study

The results of DNA genotyping performed in the four first patients of these series have been previously reported. These four patients showed a biallelic rearrangement of the δ gene which could be ascribed to Vδ1Jδ1 joining regions in three cases. One of these patients had an unproductive TCR β gene rearrangement. A clonal rearrangement of TCR γ gene was demonstrated by PCR analysis in 10 of the 11 subsequent studied cases (Table 2).

Cytogenetic studies

As shown in Table 2, conventional cytogenetic analysis was performed in nine patients. Karyotype was normal in four patients, showed monosomy of chromosomes 6 and 10 in one whereas an isochromosome 7q was found in four patients, associated with a trisomy 8 in three of them. In addition, the five patients studied by FISH showed an isochromosome 7q.
**Treatment and outcome**

Table 3 summarizes the results of therapy. Patients were given multiagent chemotherapy by CHOP or CHOP-derived (19 cases) or platinum-cytarabine based (two cases) regimens as first-line treatment. Three patients died within two months before completing induction treatment because of disease progression. Four other patients failed to respond to induction and, despite salvage therapy including high dose therapy (HDT) with autologous transplantation in one, died within six to sixteen months from diagnosis.

Overall, 14 patients (67%) responded to induction treatment, nine with complete response and five with good partial response. Five of them, in complete response after completing consolidative sequential chemotherapy, relapsed at various times and died within 10 to 44 months, despite HDT with autotransplantation in one. According to the policy of some institutions, six other patients who responded to induction treatment received up-front consolidative HDT followed by autologous bone marrow (two cases) or peripheral blood stem cells (four cases) transplantation. Two remain in first complete remission at 42 and 52 months from diagnosis, whereas four progressed and died within 13 to 33 months from diagnosis, despite salvage allogeneic transplantation in one. Three other responding patients who underwent up-front consolidative allogeneic bone marrow transplantation died, two from early cerebral toxoplasmosis and the remaining from progression.

Finally, the median survival time of this series is 16 months and only two patients are alive at the time of analysis.
Table 3. First-line treatments and outcome.

<table>
<thead>
<tr>
<th>Case n°</th>
<th>induction phase regimen</th>
<th>induction phase response</th>
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<td>1 <em>spl</em></td>
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<td>CR</td>
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<td>37 mo</td>
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<td>chemotherapy</td>
<td>CR</td>
<td>at mo 16</td>
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<td>auto BMT</td>
<td>CR</td>
<td>at mo 8</td>
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<td>CR</td>
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<td>25 mo</td>
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<tr>
<td>7</td>
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<td>CR</td>
<td>at mo 4**</td>
<td>19 mo</td>
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<td>Failure</td>
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Abbreviations: CR, complete response; PR, good partial response; BMT, bone marrow transplantation; PBSC, peripheral blood stem cells; NE, non evaluable because of cerebral toxoplasmosis; DOD, dead of disease; TRD, transplant related death; *spl*, splenectomy; salvage treatment by auto* or allo** transplantation; ▼, time from end of 1st line treatment; ▲, time from diagnosis.

**Pathological findings at progression**

In all patients, progression remained in the initial sites, resulting into splenomegaly, hepatomegaly, bone marrow and/or blood involvement. To note, recurrent and severe thrombocytopenia was observed in all progressive patients. Prior to death, one patient
developed a leukemic picture due to a high number of atypical lymphocytes (96.10⁹/l) with blastic features. Additional sites of involvement, (i.e. skin, oral mucosa or kidney) were observed in only three patients. Among 11 patients for whom material could be histologically reviewed, eight disclosed cytological features of progression in bone marrow, liver and/or spleen with either blastic (two cases) (figure 1M) or pleomorphic large cell (six cases) appearance. Paralleling these cytological changes, lymphoma cells in the bone marrow tended to extend outside sinuses, blending hematopoietic cells. In the seven studied cases, neoplastic cells retained their original CD3+, TIA1+, Granzyme B- phenotype as demonstrated on paraffin-embedded tissue sections. In two of the four patients with available frozen material, however, it is noteworthy that lymphoma cells had lost detectable TCR γδ expression.

**Discussion**

We report on the clinical, morphological, phenotypical and genetic characteristics of 21 cases of HSγδTCL, an entity that we initially described in 1990 in two patients.¹ Subsequently, a number of reports have been published, mainly as single cases or very short series.²⁻²⁵ In the present study, we show that this lymphoma has a uniform clinicopathologic presentation, may occur in immunocompromised patients and is characterized by a very poor outcome. In accordance with Cooke et al.,¹¹ we also show a phenotype consistent with its derivation from non activated cytotoxic γδ T cells. Our findings further confirm that, among peripheral T-cell lymphomas, HSγδTCL is a rare and distinct clinicopathologic entity which can be recognized by its characteristic sinusal pattern of infiltration on routine bone marrow biopsies.

In our series, the disease confirmed a predilection for young male adults and presented in all patients with splenomegaly without lymphadenopathy, and hepatomegaly in
most cases. Irrespective of the latter or of abnormal function tests, liver involvement by lymphoma was documented in all biopsies performed at presentation. Notably, at progression HSγδTCL remained preferentially localized within the spleen, liver and bone marrow, without lymph node enlargement. Other sites of involvement were observed at progression in the skin, oral mucosa and kidney of only three of our patients.

Variable degrees of hematological abnormalities were observed in all patients. As in previous reports, thrombocytopenia was the most striking finding being present in all our cases but one, associated with anemia and leucopenia in more than half of the patients. The mechanism of pancytopenia remains unclear. It could result at least in part from the presence of a marked splenomegaly, but as suggested by others \cite{44,45} cytokine secretion by neoplastic γδ T cells, such as interferon gamma, might also contribute to suppression of hematopoiesis. The observation that recurrent thrombocytopenia paralleled disease progression, even in splenectomized patients, favors the second hypothesis. Circulating myeloid precursor cells and/or monocytosis were observed at presentation in several patients of the present series and to our knowledge this has not been reported before. Such abnormalities are most likely unrelated to the degree of marrow infiltration which was mild in all our cases, but might rather reflect some regulatory functions of γδ T cells which are known to promote macrophage activation.\cite{46} The latter might also explain our observation in spleen and bone marrow of numerous histiocytes admixed within the neoplastic γδ T cells, resulting into features of hemophagocytosis in occasional cases, as also reported by others.\cite{17} For clinical purpose, presence of circulating myeloid precursor cells and/or monocytosis in the setting of splenomegaly may be confusing, as illustrated in one of our patients initially misdiagnosed as a chronic myelomonocytic disorder. In agreement with our findings, abnormal lymphocytosis at presentation has been unfrequently reported.\cite{11,47} However in eight patients of our series, careful examination of peripheral blood smears revealed a minor population of abnormal
lymphoid cells identical to those seen in the bone marrow, in accordance with a recent report. A frank leukemic picture, a rarely reported event, was observed in only one progressive patient of the present series.

According to the literature, bone marrow involvement in HSγδTCL is found in approximately two-thirds of the patients at diagnosis. We show here that it can be regarded as a constant feature at presentation and that careful histologic and immunohistologic examination of a trephine biopsy specimen appears to be the adequate and easily feasible procedure which allows the recognition of lymphoma infiltration. The discrepancy between our findings and those reported in the literature is likely due to the peculiar sinusal distribution of tumor cells which, at presentation, is often subtle and therefore difficult to recognize without immunohistochemistry. This infiltration was initially not diagnosed in six patients of the present series but could be easily documented by histological review together with appropriate immunostaining. Thus, in our initial experience, as in several previous reports reviewed by Weidmann, it appears that the underestimation of bone marrow involvement has led to perform splenectomy for a diagnostic purpose. Improvement in recognizing discrete marrow involvement by immunophenotypic studies has now resulted into a change in our diagnostic strategy in HSγδTCL, as shown in the present series in which no splenectomy was performed for diagnosis after 1995.

We also show that immunohistochemistry in routinely-fixed bone marrow biopsy specimens is a satisfactory approach for the identification of the neoplastic lymphocytes, by demonstrating clusters of CD3+, CD5- lymphocytes, usually with a CD8-, TIA1+/Granzyme B- non activated cytotoxic phenotype and highlighting their sinusal distribution. The latter phenotype however also characterizes the recently reported cases of HSTCL which share the same clinicopathologic features but express the αβ TCR and are considered a variant of the disease, now referred as “Hepatosplenic T-cell Lymphoma” in the current WHO
classification. \textsuperscript{36} Therefore, immunophenotyping on frozen tissue biopsy samples is required to determine the expression of \(\gamma \delta\) TCR, as shown in the present study. Flow cytometric study on cell suspensions might be an alternative when frozen tissue samples are not available, although it may be hampered by the low percentage of neoplastic \(\gamma \delta\) cells in marrow aspirates. \textsuperscript{11,47}

Although 16 cases of HSTCL expressing \(\alpha \beta\) chains have been described in the last years, \textsuperscript{33-35} the relative frequency and the prognostic relevance of \(\gamma \delta\) versus \(\alpha \beta\) phenotype in HSTCL remain unknown. In our institution, HSTCL with \(\alpha \beta\) phenotype was observed in only three patients over the period of this study and thus likely represents a very rare condition. It is worth noting however that a recent report indicated that \(\gamma \delta\) phenotype is an adverse prognostic factor among cutaneous T-cell lymphomas. \textsuperscript{49}

In view of their common non activated cytotoxic profile and their similar tissue distribution, it is tempting to speculate that both \(\alpha \beta\) and \(\gamma \delta\) variants of HSTCL could represent proliferation of NK/T cells, which participate with NK cells in the innate immune system. \textsuperscript{50} Indeed, normal NK/T cells comprise subsets of both \(\gamma \delta\) and \(\alpha \beta\) T cells with similar cytolytic properties involving cell-surface receptor molecules referred as killer immunoglobulin-like receptors (KIR), able to recognize MHC class I determinants on target cells. \textsuperscript{51} It would be of interest to further investigate whether both \(\alpha \beta\) and \(\gamma \delta\) variants of HSTL express KIR molecules, as recently reported in one case of HS\(\gamma \delta\)TCL. \textsuperscript{52}

Normal human \(\gamma \delta\) T cells comprise two major subsets according to the use of \(V\delta 1\) or \(V\delta 2\) chains, which have distinct patterns of tissue distribution and bear different functions. \textsuperscript{53} In the present study, we extend our preliminary results \textsuperscript{39} showing that nine out of 12 investigated cases of HS\(\gamma \delta\)TCL use the \(V\delta 1\) gene, a finding in agreement with a recent report. \textsuperscript{54} Interestingly, it has been established that normal \(\gamma \delta\) T cells which reside in spleen
express predominantly the Vδ1 gene. The observed Vδ1 usage in HSγδTCL contrasts with the predominant expression of Vδ2 gene by cells of nonhepatosplenic γδ T-cell lymphoma which develop in skin and mucosal tissue.

It is noteworthy that a remarkable past history was present in eight of our patients. Four patients had received long term immunosuppressive therapy for kidney transplantation, an observation in accordance with previous reports of HSγδTCL occurring as a late-onset posttransplant lymphoproliferative disorder of host origin. Two other patients had experienced falciparum malaria, one had been followed for systemic lupus erythematosus whereas the remaining patient developed HSγδTCL during therapy delivered for an EBV-positive Hodgkin’s disease. Interestingly, all these conditions have been associated with expansion of γδ T-cells, presumably as a result of chronic antigenic stimulation. An increase of γδ T cells has been reported in both peripheral blood and kidney biopsies of renal allograft recipients, possibly as a result of alloreactive response to MHC class II antigens and/or cytomegalovirus infection. In addition, a potential role for γδ T cells in autoimmune disorders has been proposed in view of the accumulation of these cells in rheumatoid arthritis, celiac sprue, polymyositis, multiple sclerosis and systemic lupus erythematosus. The depletion of γδ T cells has proved to dramatically reduce the severity of rheumatoid arthritis in a mouse model. Furthermore, Plasmodium falciparum exoantigens have been shown to stimulate the proliferation of γδ T lymphocytes from postprimary infection patients and accumulation of these cells has been observed in both spleen and peripheral blood of patients with Plasmodium falciparum malaria. Finally, γδ T cells which are known to participate in the immune response to several viruses can respond in vitro to EBV-infected human Burkitt cell lines and on the other hand the CD30 antigen expressed by Reed-Sternberg cells can trigger in vitro the proliferation of a γδ T-cell clone.
It has been shown that the Vδ1 T-cell subset is increased in organ allograft recipients and is induced *in vitro* by EBV-infected Burkitt cell lines, whereas expansion of the Vδ2 subset has been observed in autoimmune diseases such as multiple sclerosis and systemic lupus erythematosus. Interestingly, in accordance with a previous report, the γδ neoplastic cells in the two investigated kidney recipients of the present series had a Vδ1 phenotype, as well as the malignant cells in the patient with EBV-positive Hodgkin lymphoma. Similarly, the Vδ2 subset was used by cells of our HSγδTCL case following systemic lupus erythematosus. Therefore, in the context of antigen-driven stimulation of reactive γδ T cells, it is tempting to speculate that neoplastic transformation could result from a multi-step process involving impairment of the immune system (like in the patients receiving immunosuppressive therapy) and/or additional genetic alterations such as isochromosome 7q. The latter aberration was present in nine out of thirteen documented cases, further confirming the association between HSγδTCL and isochromosome 7q, which was reported as a hallmark not only of HSγδTCL, but also of its unusual αβ immunophenotypic variant. Although the mechanism by which it might contribute to the pathogenesis of HSTCL is unknown, its previously reported accumulation in forms with features of cytologic progression suggests that it benefits the outgrowth of malignant clones.

In the present series, however, five cases were interpreted as negative for the presence of isochromosome 7q by conventional cytogenetics. Since these cases were studied on bone marrow samples with subtle involvement, it cannot be excluded that the karyotype reflects the normal rather than the neoplastic cell population, in agreement with our finding that in one patient isochromosome 7q was detected by FISH whereas karyotype appeared normal by conventional cytogenetic study. Alternatively, isochromosome 7q-negative HSTCL cases have been occasionally reported by FISH, which may indicate that different molecular genetic pathways are involved in the pathogenesis of this lymphoma.
Despite a satisfactory response to induction treatment in two thirds of our patients, the long-term therapeutic results are poor. Relapses occurred early, median survival was 16 months with only two patients surviving at the time of analysis. These results are in accordance with those previously reported.9,11 In our series, irrespective of whether prior splenectomy had been performed or not, patients treated with CHOP or CHOP-like first-line regimens ultimately died, despite consolidative or salvage high-dose therapy in many of them. Thus, therapeutic strategies which have proved to be efficient in other subtypes of aggressive lymphoma, such as diffuse large cell lymphoma, have failed in HSγδTCL and new treatment modalities are needed. In this respect, 2’-deoxycoformycin has been recently shown to display an in vitro selective cytotoxic effect on γδ tumoral T-cells77 and to induce complete response in two patients, but these reports lacked long-term follow-up.78,79 It is noteworthy that the two patients of our series who received a platinum-cytarabine based induction regimen are in continuous complete remission at 42 and 52 months. Such a regimen has previously proved to be effective in aggressive lymphoma in relapse.80-82 Our observation should encourage the use of such a potentially synergistic combination in patients with HSγδTCL.

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LEGENDS TO FIGURES

Figure 1: Histopathology of the spleen, liver and bone marrow in hepatosplenic γδ T cell lymphoma. In the spleen, (A) the general architecture is preserved with a marked hyperplasia of the red pulp; (B) at a high magnification, medium-sized neoplastic cells are located within the cords and sinuses of the red pulp (case 15) and (C) in another case (case 12), histiocytes are admixed, disclosing some features of hemophagocytosis. In the liver (D), the neoplastic lymphoid cells infiltrate variably dilated sinusoids (case 1). In bone marrow biopsy (E-I), the marrow is usually hypercellular (E,) and exhibits an elective sinusal infiltrate composed of medium-sized atypical lymphocytes (E,F) which is more obvious in E (case 7) than in G (case 17). In the latter case, the mild infiltrate is strongly highlighted by immunostaining showing the CD3+, CD5-negative phenotype of the neoplastic cells (H, I). In rare cases, atypical cytology was observed at presentation (J), with presence of pleomorphic medium and large cells within the hepatic sinusoids (case 14). Neoplastic cells display a non-activated cytotoxic profile with strong granular cytoplasmic staining for TIA1 (K) but absence of Granzyme B expression (L) as shown in the spleen (case 2) (the arrow indicates the rare Granzyme B-positive lymphocytes which act as internal positive control). Cytological features of progression with blastic appearance was observed in the heavy infiltrated bone marrow of case 1, at relapse (M); compare the cytology with that observed in the liver at presentation, as seen in figure D.
References


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