Linfomas extraganglionares

Coordinador: C. Montalbán. Hospital Ramón y Cajal, Madrid.

Resumen del simposio

Hasta hace pocos años los linfomas extraganglionares se han considerado como una rareza y se les ha prestado poca atención. Este panorama ha cambiado radicalmente por múltiples motivos, el primero de ellos la evolución del propio concepto de linfoma extraganglionar. Aunque cualquier estructura no ganglionar se puede afectar en la diseminación de cualquier linfoma nodal, no cabe duda que en estas áreas aparecen linfomas de manera primaria. En muchas ocasiones no se han reconocido como linfomas, utilizando diagnósticos de seudolinfoma o síndromes linfoproliferativos no bien definidos. Hoy día hay instrumentos que permiten delimitar muchas de estas situaciones como verdaderos linfomas primarios. Ha sido determinante la identificación del tejido linfoide asociado a las mucosa (MALT) y de los linfomas que aparecen en este sistema. Por otra parte el concepto de linfoma primario extraganglionar se aceptaba cuando el linfoma se encontraba exclusivamente en un órgano extraganglionar, pero esto es sólo una parte de la historia, ya que estos linfomas aunque tienden a estar durante largo tiempo localizados y a manifestarse como enfermedad local, a lo largo de su evolución se diseminan y se comportan como los linfomas ganglionares. Los linfomas del MALT, considerados conceptualmente como enfermedad localizada, se ha demostrado que en una primera fase se diseminan a otras áreas del MALT, y posteriormente a áreas linfáticas y médula ósea. En algunos de ellos la leucemización y la afectación medular pueden ya detectarse en el momento del diagnóstico. El reconocimiento de la historia natural de estos linfomas y el estudio de su incidencia en comunidades en las que existen registros fiables, ha permitido reconocer la verdadera frecuencia de los linfomas extraganglionares, que constituye alrededor de un tercio de todos los linfomas. Otro aspecto fundamental ha sido el conocimiento de algunos de los mecanismos etiopatogénicos que ocurren en estos linfomas. El concepto del MALT y de los linfomas derivados del MALT ha permitido saber que estímulos locales específicos en algunas áreas extraganglionares pueden poner en marcha una cadena de acontecimientos que condicionan expansión policlonal de linfocitos B, selección clonal y cambios moleculares que conducen al linfoma y a su diseminación y transformación. Se han reconocido varios estímulos inductores de la aparición de linfomas MALT extraganglionares, bacterias, parásitos, virus y alteraciones inmunológicas primarias. El caso mejor conocido es el del Helicobacter pylori en la aparición del linfoma MALT del estómago, pero también los de Campylobacter jejuni en los linfomas MALT cutáneos y Chlamydia psittaci en los linfomas MALT de la conjuntiva y anexos oculares. Menos clara es la relación de parásitos, como Anisakis y Fasciol, con el linfoma intravascular. Los virus también tienen un papel, ya que en algunas áreas geográficas algunos linfomas gástricos pueden estar inducidos por virus de Epstein-Barr (VEB) o virus de la hepatitis C (VHC). El VEB está presente en los linfomas T nasales, en los linfomas extraganglionares postrasplante y en los cerebrales de los inmunodeprimidos y está relacionado, junto a HHV8, con los linfomas primarios de cavidades. El VHC también está relacionado con algunos linfomas de la zona marginal esplénica, y con linfomas linfoplasmocitarios en otras áreas (ligado) o sistémicos. Situaciones autoinmunes como tiroiditis de Hashimoto y Sjögren inducen la aparición de linfomas MALT tiroideos y de glándulas salivales y la enteropatía por gluten la de linfomas T intestinales. El reconocimiento del linfoma extraganglionar como enfermedad local ha permitido curar a una parte de esos pacientes en las fases iniciales (localizadas) de la enfermedad utilizando tratamientos locales (cirugía o radioterapia) y sobre todo demostrar que en algunas situaciones la eliminación del desencadenante puede interrumpir la secuencia de acontecimientos y conseguir la desaparición del linfoma e incluso su curación, como en el caso del H. pylori y el linfoma gástrico. Si esto sucede en unas áreas extraganglionares es razonable que también ocurra en otras, aunque por el momento no se conozcan los antígenos desencadenantes. También sería razonable que estímulos locales o sistémicos (virales, bacterianos o inmunológicos) y mecanismos similares tengan lugar en los linfomas ganglionares. Los ejemplos...
The incidence rate of NHL, especially extranodal disease, has been increasing steadily for the past two decades. Whether this represents a real increase or results from improved diagnosis and more efficient case registration is as yet unclear. This increased rate was evaluated at between 3-5 % per annum in the gastro-intestinal lymphomas (GIL). From cancer registry data from 14 countries, the calculated incidence rate was 0.21, 0.16, and 0.08 per 100,000 for gastric, small intestinal and colonic NHL, respectively\(^1\).

Primary GIL comprise a group of distinct clinicopathological entities of B-and T-cell types [3]. In occidental countries, B-cell lymphomas are more frequent (over 80 %) while in Southern Japan T-cell lymphomas predominate (> 75 %, intestinal cases). In the Western world B-cell lymphomas of MALT-type (mucosae associated lymphoid tissue-type) are the more common. Most of these lymphomas arise in the stomach\(^2,4\). They may present as typical low-grade (small B-cell) lymphomas or with partial or complete transformation into high-grade (large B-cell) tumours\(^3\). In the Middle Eastern and Mediterranean populations there was a relatively high incidence of small bowel NHL referred as immunoproliferative small intestinal disease (IPSID) comprising alpha-chain disease (α-CD), a special form of MALT intestinal lymphoma, they are not considered in this chapter. Other B-cell lymphomas of the GI tract include mantle-cell lymphoma, which presents as immunophenotypically distinct features and represents 10 % of the GIL.

Gastric lymphomas (GL) of MALT-type

The group of lymphomas called as low-grade MALT-type lymphomas include a number of extranodal lymphomas composed mostly of small cells, that share similar clinical, pathological and molecular features\(^5\). The term MALT-type lymphoma was first used by Isaacson because their histological characteristics recapitulating features of MALT as exemplified by the Peyer’s patches. These tumours initially consist of mucosal small cleaved cell or centrocytic-like cell proliferation colonising the follicular germinal centers with a tendency to invade and destroy glandular epithelium, forming the characteristic lymphoepithelial lesions. With respect to their normal counterpart cells, these lymphomas are defined as extranodal marginal zone B-cell lymphomas of follicular type in the last world Health Organization (WHO) Classification of Neoplastic Diseases of the Hematopoietic and lymphoid tissue\(^6\). These extranodal lymphomas may arise

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**PRIMARY GASTRIC AND INTESTINAL LYMPHOMA MALT-DERIVED AND OTHER LYMPHOMA TYPES**

A. Rouskone-Fourmestraux

Groupe d’Etude des Lymphomes Digestifs (GELD).


Introduction

The incidence rate of NHL, especially extranodal disease, has been increasing steadily for the past two decades. Whether this represents a real increase or results from improved diagnosis and more efficient case registration is as yet unclear. This increased rate was evaluated at between 3-5 % per annum in the gastro-intestinal lymphomas (GIL). From cancer registry data from 14 countries, the calculated incidence rate was 0.21, 0.16, and 0.08 per 100,000 for gastric, small intestinal and colonic NHL, respectively\(^1\).

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from normal native MALT physiologically present in the gut or acquired MALT developed in sites of chronic inflammation in response to either infection or autoimmune phenomena. In the stomach, acquired MALT provides the background for lymphoma development. *Helicobacter pylori* (*H. pylori*) is the only well-established chronic antigenic stimulus that causes the development of gastric MALT. The link between *H. pylori* gastritis and low-grade GL of MALT-type has been shown by data in case control and epidemiological studies and by the regression of the majority of low-grade GL by eradication of *H. pylori*. These prolonged lymphoid reactive proliferations favour the emergence of a pathological clone, which can progressively replace the normal lymphoid population giving rise to a MALT-type lymphoma. As in nodal NHL, low-grade MALT-lymphoma may progress to high-grade lymphoma. The stomach is the more frequently involved site of these low-grade GL.

**Clinicopathological presentation and diagnosis**

Low-grade GL typically occurs in the fifth decade while high-grade GL are diagnosed later: 57.6 compared to 65.3 years in one recent study. This observation suggests that the progression of low-grade to high-grade GL takes about one decade. The presenting symptoms are generally non-specific dyspepsia or gastrointestinal bleeding. The presence of an abdominal mass is a rare phenomenon at presentation. The endoscopic features may range from non-specific macroscopic gastritis or thickened folds to ulcerative or infiltrating lesions in low-grade tumours, whereas high-grade lymphomas generally present as large ulcers or more protruding tumours. Multiple biopsies are generally required for histological diagnosis because of the multifocal nature of the disease and to exclude focal high-grade transformation. Combination with endoscopic ultrasonography allows evaluation of locoregional extension especially in case of parietal tumour infiltration and nodal involvement. Most of early stage low-grade GL are confined to the mucosal and submucosal layer and when invasion to deeper layers are found, high-grade transformation should be suspected. Lymph node involvement is generally locoregional and depends on parietal involvement of the tumour. It is generally believed that low-grade GL remains localised to the site of origin for a prolonged period. However, recent studies have suggested the possibility of dissemination to the small intestine, other MALT organ or even bone marrow. At diagnosis these potential dissemination must be carefully excluded by using a thorough staging procedure including the recording of patients’ physical examination, ileocolonoscopy together with upper gastrointestinal tract endoscopy, small bowel and chest radiography, abdominal computed tomography (CT scan), and Waldeyer's ring examination with endoscopy and biopsies or CT scan, and bone marrow biopsy.

The pathological findings of low-grade GL of the marginal zone of MALT include: typical morphology with small B-cells or centrocytic-like cells, lymphoid-pithelial lesions and clonality defined by using either immunohistochemistry or molecular techniques. Associated *H. pylori* chronic gastritis can render the diagnosis difficult. The presence of scattered clusters of large transformed blastic cells must be considered as a progression to high-grade MALT lymphoma. Coexistence of low-grade MALT and high-grade components in the same tumour is frequent and the observation of identical immunoglobulin gene sequences in both components confirms their clonal identity. A low-grade component is not always detected in high-grade lymphoma especially on small endoscopic biopsies. However, it has been shown that there is no difference in clinical behaviour between high-grade cases with or without low-grade component and should therefore be categorised of MALT-type. *H. pylori* chronic infection induces and sustains an actively proliferating B-cell population at risk of developing genetic abnormalities. A limited number of genetic studies have provided important information of heterogeneity of MALT lymphomas. Allele imbalance of tumour-suppressor genes (DCC and APC) was found in some cases of MALT lymphoma in the transition from chronic gastritis to low-grade and high-grade GL. Thus, genetic instability in proliferating B-cells provides the potential for induced chromosomal abnormalities. Indeed, trisomy 3 was detected in 60 % of low-grade MALT lymphomas arising in various organs. Recent studies are now focusing on additional genetic abnormalities, such as the t(11;18) and t(1;14). Translocation t(11;18) has been reported in 30 % of low-grade MALT GL and one study suggested that it was only present in more advanced low-grade GL, non responding to *H. pylori* eradication and thus appears to be related to *H. pylori* independent survival/growth. Another cytogenetic abnormality, t(1;14), less frequently detected in MALT lymphomas, has also been suspected to confer *H. pylori* independent growth of tumour cells. Finally, further genetic events such as complete inactivation of the tumour suppressor genes P53 and P16, possibly activation of c-myc oncogenes by translocation and other undetermined abnormalities have been suggest to result in high-grade transformation. Routine detection of these translocations could not only lead to much more precise definition of this lymphoma but help the interpretation of therapeutic results and then guide therapeutic strategy.

**Treatment**

As *H. pylori* is now recognised to be the major inducer of low-grade GL, its eradication could be the first step of the treatment.
Currently, the best treatment protocol includes proton pump inhibitor combined with two antibiotics (as clarithromycin or metronidazole and amoxicillin) two weeks, which results in \textit{H. pylori} eradication in more than 90% of infected patients\textsuperscript{13}. As long as the eradication of the bacterium is effective, it has been demonstrated that more than 70% of low-grade MALT GL regress at early stage. Histological complete remission may be achieved between two and 18 months after \textit{H. pylori} eradication. We reported that in \textit{H. pylori} positive status patients with localised GL, in the absence of lymph node involvement carefully assessed by endoscopic ultrasonography, a complete remission rate of lymphoma is expected in 79% of cases\textsuperscript{14}. Moreover, a significant difference in response rate was observed in lymphoma restricted to the mucosa and more deeply seated lesions. Monoclonal expansion remained detected at distance from eradication in some cases been judged histologically in remission\textsuperscript{10,14}. A recent series of six patients followed for 8 years demonstrated that histological and molecular relapses could occur in a transient fashion\textsuperscript{22}. These findings suggest that \textit{H. pylori} eradication removes a growth stimulus from MALT GL without necessarily eliminating the B-cell clone. In the absence of \textit{H. pylori} re-infection the presence of such a clone does not appear to be deleterious and may be self-limiting, however, more prolonged follow-up is mandatory.

Patients after antibiotic failure and persisting tumour and those with \textit{H. pylori} negative status could benefit of regional approach, such as radiotherapy or radical surgical resection with curative intent. Despite the appearance of one dominant lesion, the disease may be multifocal in the stomach, and total gastrectomy was mandatory to cure GL\textsuperscript{23}. In case of larger tumours surgery has the added advantage of documenting a potential high-grade associated proliferation. Nowadays surgery is much less indicated and low-dose (30 grams) radiotherapy seems to give good results and is evaluated in different European protocols\textsuperscript{24-25}. Others have also used chemotherapy and a variety of regimens have been administered, often with results consistent with responses seen in other low-grade NHL\textsuperscript{26,27}. A complete or near complete response is achieved in more than half of the patients followed by relapse in some of them sometimes years after the initial treatment. The wait and see strategy advocated by some authors, could be conceivable only in older patients, owing the risk of high-grade transformation.

In high-grade lymphomas despite anecdotal cases regressing after antibiotics, chemotherapy including anthracycin and now Rituximab, sometimes associated with surgery, is the main treatment for these tumours\textsuperscript{28,29}. Our results including surgery and CHOP led to an overall five-year survival rate of 100% in case of localised tumour\textsuperscript{3}. However, chemotherapy alone is now more often given but in addition it is recommended to eradicate \textit{H. pylori} to eliminate a residual or relapse low-grade component.

B-Primary intestinal B-cell lymphomas

Among gastrointestinal lymphomas, those arising primarily in the intestine have been less extensively reported. We studied the clinicopathological features and outcome of a large series of primary B-cell intestinal lymphomas (IL). They accounted for 99 (28%) of the 361 cases of gastrointestinal lymphomas enrolled in the successive french GELD studies\textsuperscript{30}. Ninety-two of them (93%) were of B-cell phenotype, including 38 diffuse large cell lymphomas, 36 mantle-cell lymphomas (lymphomatous polyposis), 11 follicular lymphomas, 5 low-grade marginal zone lymphomas of MALT-type, and 2 Burkitt’s lymphomas. Clinical presentations differed according to histological subtype: younger age in Burkitt’s lymphomas and older in low-grade MALT-type, obstruction with surgical emergency mainly observed in diffuse large cell lymphomas, endoscopic extensive nodular and polypoid pattern in mantle-cell and follicular subtypes as well as advanced stage, poorer outcome in mantle-cell lymphomas with 44% overall survival at 5 years, prolonged survival in follicular and MALT subtypes despite failure to achieve true complete remission after treatment.

C-Enteropathy-associated T-cell lymphoma

The term enteropathy-associated T-cell lymphoma (EATL) largely refers to T-cell mainly small intestinal lymphomas associated with the characteristic pathological features of celiac disease (CD) or of non-specific ulcerative jejunoileitis. EATL is a high-grade intestinal T-cell lymphoma of poor prognosis, deriving from an extensive low-grade epithelial T-cell proliferation that in occurs in patients with celiac disease (CD) after prolonged gluten exposure. Patients with overt CD should be convinced to strictly adhere to a gluten free diet, even if their disease is mild. EATL often presents as a surgical emergency with intestinal obstruction or perforation\textsuperscript{31}. The disease may also be revealed in patients with known CD unresponsive to gluten-free diet (GFD), known as refractory sprue. Macroscopically the findings of one or several, ulcerated tumours, mainly jejunal, are most frequently observed, sometimes associated with benign appearing ulcers. Histologically, EATL are largely high-grade lymphomas showing varying degrees of polymorphism. Large tumoral cells very probably derive from a subset of intraepithelial lymphocytes as suggested by their capability to invade crypt epithelium and by their peculiar phenotypes, usually CD3+, CD4+, CD8–, CD103+ or occasionally CD3+, CD4+, CD8+ and CD56+, CD103+ (cells expressing the markers of activated cytotoxic T cells). Cells are arrested in varying stages of activation from one tumour to another. Moreover, clonal T-cell receptors (TCR) \(\gamma\) gene rearrangement is found\textsuperscript{22}.\[XLVI Reunión Nacional de la AEHH y XX Congreso Nacional de la SETH. Simposios\]
Strict adherence to GFD is the single means of prevention the occurrence of EATL. Prognosis is poorer than for B-cell lymphoma. Intensive chemotherapy is the main treatment. Even exclusive total parenteral nutrition is not seen to be effective on reversing mucosa abnormalities, but may be used for nutritional purposes. Surgery is sometimes mandatory for diagnosis or complication.

References


antigens and to deliver co-stimulatory signals to T-cells. In addition, MZ B-cells apparently display the capacity to differentiate into plasma cells and most early antibody-secreting cells are thought to originate from MZ B-cell precursors. Of interest is the fact that physiological MALT usually does not give rise to MALT lymphoma, which usually develops in acquired mucosal lymphoid tissue. According to this concept, it is not surprising that MALT lymphoma of the gut—which contains the largest accumulation of lymphoid tissue in the human body—is relatively rare, while the stomach, which is normally devoid of lymphoid tissue, is the most common site of origin for MALT lymphoma1-3.

This high rate of gastric MALT lymphomas, which comprise about 70% of all MALT lymphoma cases, has lead to the erroneous notion that “MALT lymphoma” may be used as a synonym for gastric lymphoma. However, MALT lymphomas can be found throughout the whole body in organs able to acquire MALT in the course of chronic inflammatory/antigenic stimulation, and they have even been reported in non-mucosal sites such as the dura. To the current knowledge, the most common sites of presentation for MALT lymphoma apart from the stomach are the lung (14%), head and neck area including the salivary glands (14%), ocular adnexa (12%) and skin (11%), whereas none were positive for C. trachomatis and C. pneumoniae. A causative role of the agent was also shown by objective response of the lymphoma in 2/4 patients undergoing antibiotic therapy with doxycycline including one patient with MALT lymphoma. In view of this, further studies are needed to assess the impact of anti-chlamydial therapy in patients with ocular adnexal MALT lymphomas.

In addition to infectious agents, the high risk of patients suffering from various autoimmune conditions for the development of lymphomas has been noted. Especially in case of autoimmune thyroiditis and Sjogren’s syndrome (SS), a high risk for the development of MALT lymphomas in the primarily affected organs has been demonstrated. Accordingly, a 70 fold increased risk for thyroid MALT lymphoma and a 44 fold increased risk for parotid lymphoma is attributed to autoimmune thyroiditis and SS5-7, respectively. This is again in keeping with the MALT lymphoma concept formulated by Isaacsen, that acquired MALT developing in the context of chronic infection or antigenic stimulation may give rise to MALT lymphoma following accumulation of various genetic events. In terms of treatment, however, this association currently does not have an impact on clinical management of the lymphoma, as both conditions are virtually intractable apart from symptomatic management of patients and thus do not allow permanent removal of the immunologic stimuli as opposed to eradication of antibiotic agents. Nevertheless, the high risk of developing MALT lymphomas should be kept in mind in such patients.

Clinical aspects and treatment of non-gastrointestinal MALT lymphoma

In addition to the similar pathological features of gastric MALT lymphoma and MALT lymphomas of non GI-origin, both appear to be characterized by a relatively indolent clinical course. In an analysis of 108 patients, Thieblemont and coworkers found a similar median overall survival for gastric vs non-GI MALT lymphomas, but a significantly shorter time to progression (4.9 years vs 8.9 years) in the latter. Nevertheless, the pattern of dissemination appears to be different, and recent data have suggested that this shorter time to progression reflects the higher rate of dissemination, which might be clinically inapparent upon diagnosis or simply may have been overlooked without vigorous and extensive staging. Data from our own institution have demonstra-
terminado multiorган in involvement in about 50% of non GI-MALT lymphomas as opposed to 30% in gastric MALT lymphoma. Similar figures have been reported by Thieblemont et al10, who have also found dissemination of nongastric MALT lymphoma in 48% of patients. As a consequence, patients should undergo staging including imaging of the salivary and lacrimal glands, ear-nose and throat investigation, gastroscopy/endsosonography with multiple biopsies, CT-scan of thorax and abdomen, colonoscopy and bone marrow biopsy in order to correctly assess the clinical stage before initiation of treatment9.

In non-GI MALT lymphoma, non-surgical management has been more widely applied for initial management as opposed to gastric lymphoma, where surgery has been a mainstay of treatment before application of antibiotic treatment or radiation3. Again, prospective data are lacking, but in a large retrospective series 39% of patients relapsing after a median follow-up of 47 months. In an analysis of primary ocular adnexa lymphomas, 11/19 patients were found to have MALT-type lymphoma12, 8 of whom were treated with radiation only, while two were only resected and one was given chemotherapy. Both surgically treated patients, however, relapsed after 54 and 62 months, respectively, while the other patients remain in complete remission after a follow-up time between 24-140 months.

Taken together, radiotherapy has been the most widely applied form of treatment for patients with localized MALT lymphoma of extragastrointestinal origin. Apparently, this practice results in excellent local control of the disease, as has consistently been shown by Tsang and coworkers13. Data from our own institution, however, have shown a high rate of systemic relapse in patients with MALT lymphoma of the head-and-neck area following local therapy, with 39% of patients relapsing after a median follow-up of 46 months.

In view of the common mucosal immunity and the high rate of relapses (sometimes even after decades), systemic approaches including application of the monoclonal antibody rituximab or chemotherapy are being investigated at the moment. In a recent report, application of fludarabine containing regimens has resulted in complete remission in all 31 patients with nongastrointestinal MALT lymphomas in stage I. While these findings are encouraging, a note of caution should be added due to the relatively short median follow-up time of 3 years. Nevertheless, systemic approaches should further be investigated as front-line management of this disease.

**References**


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...logía. Dado el alto riesgo de extrayéndose células linfomatosas y de realizar un examen del LCR siempre que no existan contraindicaciones, el estudio del LCR puede ser de valor diagnóstico para conocer la extensión local del LNH como para su estudio etiológico con lámpara de hendidura, la TC torácica y abdominal y la biopsia de médula ósea.

Factores pronósticos

La baja incidencia del LCP ha limitado la publicación de series amplias donde se puedan analizar la presencia de factores pronósticos. En un estudio retrospectivo de 378 pacientes con LCP tratados en 23 centros diferentes se identificó como predictores de mortalidad la edad superior a 60 años, un estado funcional superior a 1 de la escala de la ECOG (Eastern Cooperative Oncology Group), niveles altos de lactato deshidrogenasa sérica o de proteínas en el LCR, y afectación de estructuras profundas del cerebro. Con estas variables se diseñó una escala pronóstica en la que el valor 0 significaba el no tener ningún predictor favorable de mortalidad y el valor 5 el tenerlos todos. Al aplicar el modelo al subgrupo de 105 pacientes que recibieron quimioterapia, se obtuvo una supervivencia media actuarial a los 2 años del 80%, aquellos con valores de 2-3 del 48% y los de peor pronóstico (valores de 4-5) del 15%.

Las limitaciones de esta escala pronóstica es la inclusión de valores de LCR que pueden faltar en pacientes en los que la punción lumbar está contraindicada. En otro estudio de 77 pacientes tratados con el mismo protocolo se identificó como predictores de mortalidad la edad superior a 60 años, un estado funcional superior a 1 de la escala de ECOG y la presencia de blastos en el LCR...
sencia de linfoma multifocal o con diseminación me-
ningea. La combinación de estas 3 variables permitió
elaborar una escala de 0 (ningún predictor desfavo-
ral) a 3 (todos los predictores desfavorables) con
predicciones de supervivencia similares a los del estu-
dio previo (Bestell et al., en prensa).

Tratamiento
El LCP es un tumor maligno que sin tratamiento
produce la muerte del paciente en pocas semanas.
Los corticoides son colípticos para el LCP y pueden
causar una respuesta parcial o total hasta en el 40 %
de los pacientes no inmunodeprimidos. La respon-
sa sin embargo, es de poca duración y no tiene efecto
curativo. La radioterapia holocraneal era hasta la úl-
tima década el tratamiento estándar de los LCP. La
sobrevida mediana de los pacientes no inmunodepri-
midos tratados con radioterapia es de unos 11 me-
tos con una tasa de sobrevida a los 2 años del 35 %.
Estos resultados son claramente inferiores en los pa-
cientes con LCP y sida, ya que estos suelen morir en-
tes de infecciones oportunistas intercurrentes.

Aunque no ha habido estudios aleatorizados, di-
versos estudios en fase II han combinado tratamien-
tos con quimioterapia y radioterapia con resulta-
dos claramente superiores a los de la radioterapia sola. Estos estudios han permitido llegar a las si-
guientes conclusiones:

1. Los esquemas tradicionales de tratamiento de linfoma sistémica (ciclofosfamida, adriamicina, vin-
cristina, prednisona, CHOP) no son efectivos al no
pasar la barrera hematoencefálica. Aunque el trata-
miento con CHOP produce una respuesta ini-
cial al estar la mayoría del tumor no protegido por la
barrera hematoencefálica, como lo demuestra la in-
tensa captación de contraste, los siguientes ciclos de
CHOP no consiguen erradicar los restos del linfoma.
En un estudio aleatorizado, la adición de ciclos de
CHOP tras la radioterapia no fue superior al trata-
miento aislado con radioterapia.  

2. Los fármacos más activos para el tratamiento del
LCP en pacientes no inmunodeprimidos son el metro-
trexato a dosis mínimas de 1 g/m² y el arabinósido de
citosina (Ara-C) en dosis de 3 g/m². Los regímenes
que incluyen estos fármacos junto con radioterapia
consiguen una tasa de respuestas cercana al 80 %
y una sobrevida media de 3 años. La dosis de metotre-
xato ideal sigue siendo un tema de debate. Es impor-
tante que la dosis consiga unos niveles suficientes de
fármaco en el parénquima cerebral y LCR durante un
tiempo suficiente. Para ello es importante administrar
la dosis en un período no inferior a 4 h. Aunque la do-
sis aconsejable para alcanzar unos niveles de seguri-
dad es de 3 g/m², esquemas con dosis inferiores, pero
siempre superiores a 1 g/m², han demostrado tasas de
respuesta similares. Este dato es importante al consi-
derar que más del 50 % de los pacientes con LCP tie-
nen una edad superior a 60 años y su filtrado glome-
rular puede estar alterado con el consiguiente aumen-
to de toxicidad al usar dosis muy altas de metotrexato.

3. En pacientes mayores de 60 años el riesgo de
capacidad en los pacientes con ventrículos pequeños y no está
recomendado el uso de este fármaco en el LCR; sin
embargo, la colocación del reservorio puede ser difi-
cil en pacientes con ventrículos pequeños y no está
recomendado el uso de este fármaco en el LCR; sin
embargo, la colocación del reservorio puede ser difi-
cil en pacientes con ventrículos pequeños y no está
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PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS: CLINICO-PATHOLOGICAL CHARACTERISTICS AND THERAPEUTIC MANAGEMENT OF RARE ENTITIES

A.J.M. Ferrari1, R. Stefano1, P.A. Scafaro1, S. Dell’Oro1, N. Fringuelli1, L. Politi2, A. Franzin3, M. Foppoli4, M. Ponzoni5, Y. M. Reni6

1 Dept. of Radiotherapy, 2 Neuroradiology, 3 Neurosurgery, 4 Internal Medicine, and 5 Pathology. San Raffaele Scientific Institute. Milan. Italy.

Abstract

Primary central nervous system lymphomas (PCNSL) are aggressive malignancies arising in an anatomical site with certain structural, biological and immunological characteristics and exhibit one of the worst prognoses among non-Hodgkin lymphomas. PCNSL constitute a group of heterogeneous malignancies, and clinical characteristics and therapeutic guidelines reported in the related literature should not be homogeneously applied to all lymphoproliferative disorders primarily arising in the CNS. Differences among some CNS lymphomas could be due to special characteristics of the site where the lymphoma arises or to the particular natural history of some lymphoma entities. This special subgroup of PCNSL is constituted by rare forms of a rare malignancy, which are associated with distinct clinical courses, and, importantly, require different therapeutic approaches. Literature regarding these rare forms of PCNSL is mostly constituted by case reports and small case series, and anecdotal evidence results in a relevant number of management uncertainties. This paper summarizes clinical and therapeutic aspects of the most relevant uncommon forms of PCNSL.

The “common form” of primary CNS lymphoma

Primary central nervous system lymphomas (PCNSL) are rare and aggressive malignancies arising with confinement to the CNS. PCNSL represent 4% of all primary brain tumors, 4-6% of extranodal lymphomas; globally, their incidence has raised in the past 2 decades, both in immunocompromised and immunocompetent individuals, mostly in HIV-negative individuals over 50 years of age. PCNSL are more common among individuals older than 50 years, with a slight prevalence among males; 5-15% of PCNSL patients have a history of a previous cancer. At presentation, PCNSL patients usually display a poor performance status, while systemic symptoms are rare (≤ 2% of cases). Presenting symptoms consist of non-specific motor and/or sensory focal deficit in approximately 50% of cases; cognitive and personality changes, headache and others signs of intracranial hypertension such as nausea, vomiting and papilledema are frequently observed. PCNSL exhibit one of the worst prognoses among non-Hodgkin lymphomas, and the best treatment modality has not been yet identified. In most prospective trials, general criteria for treatment of aggressive lymphoma have been adopted, choosing primary chemotherapy followed by radiotherapy as therapeutic modality. This strategy produced a 5-yr survival of 22-40% in comparison to the 3-26% reported with radiotherapy alone. Systemic high-dose methotrexate is the most effective drug, producing a response rate of 52-100% and a 2-yr OS of 58-72%, while any regimen without this drug-comprehensively performed no better than radiotherapy alone. To date, the addition of other drugs at conventional doses has not consistently improved outcome. In fact, the prognosis of PCNSL remains poor with a great number of local relapses that, after a brief course, inevitably lead to patient death.

Actually, PCNSL constitute a group of heterogeneous malignancies, and the above-summarized clinical characteristics and therapeutic guidelines should not be homogeneously applied to all lymphoproliferative disorders arising in the CNS. In fact, some CNS lymphomas display particular differences with respect to the majority of PCNSL, which could be due to the distinctive characteristics of the site where the lymphoma arises or to the particular natural history of some lymphoma categories. Literature regarding these extremely rare forms of PCNSL is mostly constituted by case reports and small case series. This paper is an effort to review available information on clinical and therapeutic aspects of the most relevant uncommon forms of PCNSL, which requires a separate analysis with respect to the “common form” of PCNSL.


Rare lymphoma localizations in the CNS

Most PCNSL arise in the cerebral parenchyma, presenting as a single lesion, badly delimited, more frequently localized in the frontal lobe and periventricular structures, infiltrating the corpus callosum and the basal ganglia, and with variable perilesional edema. Multiple brain lesions are observed in 30-40% of immunocompetent patients. Lymphomas arising in the eyes, leptomeninges and spinal cord constitute rare forms of PCNSL, which are associated with distinctive clinical behavior and, most importantly, require peculiar therapeutic approaches.

Intraocular lymphoma (IOL) is the most common among the rare forms of PCNSL; comprehensively, ocular involvement is observed in 5-20% of PCNSL. Even if ocular disease can be the sole expression of lymphoma, in 80-90% of cases, ocular involvement is followed, after weeks or months, by the onset of brain lesions. IOL can also occur as an isolated recurrence after parenchymal PCNSL. Lymphomatous cells are able to infiltrate the vitreous humor, retina and choroid; bilateral involvement is observed in 80% of cases. Clinical characteristics of patients with IOL are similar to those of the rest of PCNSL (table 1); however, a more common association with female gender, multifocal disease, systemic symptoms, and CSF dissemination has been reported.

The most common presenting symptoms are nonspecific visual symptoms such as floaters or campimeter seen in 27% of IOL patients. Comparison with other PCNSL forms

**Table 1. Patients’ characteristics of IOL patients. Comparison with other PCNSL forms**

<table>
<thead>
<tr>
<th></th>
<th>IOL</th>
<th>Other PCNSL</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
<td>58</td>
<td>0.61</td>
</tr>
<tr>
<td>Range</td>
<td>16-74</td>
<td>14-77</td>
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<tr>
<td><strong>Performance status</strong></td>
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<tr>
<td>(ECOG criteria)</td>
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<td></td>
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<tr>
<td>0-1</td>
<td>30%</td>
<td>34%</td>
<td></td>
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<tr>
<td>2-4</td>
<td>70%</td>
<td>66%</td>
<td>0.68</td>
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<tr>
<td><strong>Systemic symptoms</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>9%</td>
<td>5%</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td><strong>Prior cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>5%</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td><strong>Histotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indolent lymphoma</td>
<td>0%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>91%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>9%</td>
<td>22%</td>
<td></td>
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<tr>
<td><strong>T-cell immunophenotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>2%</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Elevated LDH serum level</td>
<td>50%</td>
<td>37%</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Positive CSF cytology</strong></td>
<td>35%</td>
<td>11%</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>High CSF protein concentration</strong></td>
<td>43%</td>
<td>68%</td>
<td>0.07</td>
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<tr>
<td><strong>Multiple lesions</strong></td>
<td></td>
<td></td>
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<tr>
<td>36%</td>
<td>36%</td>
<td>0.99</td>
<td></td>
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<tr>
<td>Involvement of deep structures*</td>
<td>27%</td>
<td>32%</td>
<td>0.62</td>
</tr>
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* Cases of involvement of corpus callosum and/or basal ganglia and/or brain stem and/or cerebellum.

No histopathological differences between IOL and other PCNSL exist, being large B-cell lymphoma the most common histotype. The recognition of lymphoma cells by routine cytology looking at the cellular morphology, with or without immunophenotypic analysis, generates a strong suspicion of the diagnosis. Immunohistochemistry and/or flow cytometry are needed to characterize cellular phenotype and to confirm the diagnosis. Immunoglobulin heavy-chain gene rearrangements analyzed using PCR techniques is detected in nearly all of the cases, and may be useful in confirming the monoclonal nature of the disorder. Intravitreal levels of IL-10 and IL-6 are, probably, adenocysts for B-cell IOL diagnosis. Interestingly, a role for specific infectious agents in the pathogenesis of some cases of IOL has been recently hypothesized; DNA of Human Herpes Virus 8, Epstein-Barr virus and Toxoplasma gondii has been detected in 19, 10 and 13% of cases, respectively.

The best treatment for IOL remains to be defined, and the lack of a routinely assessment and definition of therapeutic response and failure constitutes an important pitfall in the estimation of treatment efficacy. In the past, patients with symptomatic disease were treated with radiotherapy alone, but nearly all of the patients developed early CNS progression and died. A few case reports and small retrospective studies alluded to the efficacy of chemotherapy, and anecdotal promising results using high-dose cytobine, high-dose methotrexate, procarbazine, and nitrogen mustards have been reported. The efficacy of these cytostatics is dependent on intraocular pharmacokinetics, which are not well understood. Preliminary data suggest that micromolar concentrations of methotrexate are achieved in the aqueous and vitreous humors when the drug is given at dose of 8 g/m². Nevertheless, a high rate of persistence of ocular disease have been reported in patients treated with this strategy, which could be explained by the fact that MTX concentration in the vitreous humor, the main site of IOL, is usually lower than those obtained in the anterior chamber of the eyes.

Better disease control combining ocular irradiation to high-dose methotrexate based chemotherapy has been reported. The irradiation of the posterior two thirds of the ocular globes with a dose between 35 and 45 Gy has been recommended, and more recent ex-
Cytology examination in 0-50% of PCNSL4, while NSL2. Meningeal infiltration due to the dissemination of a parenchymal lesion constitutes less than 10% of PCNSL. Leptomeningeal lymphoma becomes a valid alternative in IOL.

Peripheral blood stem-cell transplantation17 and high-dose chemotherapy supported by autologous peripheral blood stem cells have been described, but their actual incidence did not emerge because of the short follow-up of published series. Importantly, the enrolment of patients with IOL in trials assessing the activity or efficacy of chemotherapy as exclusive treatment should be critically discussed9.

Intriguing results with high-dose chemotherapy supported by autologous peripheral blood stem-cell transplantation18 and intravital chemotherapy19 in patients with relapsed or refractory IOL have been reported. Some protocols using intravitreal injections of MTX, with or without thiotepa, are currently ongoing. A weekly intravitreal injection of 400 μg/0.1 ml of methotrexate, for 4 weeks, and once a month thereafter, has been proposed, with encouraging results and reduced morbidity18. This experimental strategy may become a valid alternative in IOL.

Leptomeningeal lymphoma

Leptomeningeal lymphoma without a concomitant parenchymal lesion constitutes less than 10% of PCNSL2. Meningeal infiltration due to the dissemination of lymphomatous cells through the CSF from the subependymal tissues is a more common feature in PCNSL19. In fact, lymphomatous cells are detected by CSF cytology examination in 0-50% of PCNSL1, while autopsic series have demonstrated the presence of meningeal dissemination in 100% of cases. Most leptomeningeal lymphomas are constituted by large B-cells. Only a few cases of indolent lymphomas of leptomeninges have been reported; such cases are mostly represented by marginal zone B-cell lymphomas of MALT-type (see below). A case reported, either by morphology and immunophenotype, by a small B-cell lymphoma has been recently diagnosed at our Institution (Ponzoni M., unpublished data). Presenting symptoms in leptomeningeal lymphomas are variable, depending on the involved site. Increased intracranial pressure, multifocal cranial neuropathies, signs of multilevel root involvement, lumbosacral radiculopathies with radicular pain, increase of intracranial pressure, and confusion have been reported. Neuroradiological features are not pathognomonic and radiological suspicion is sometimes delayed (fig. 1); meningioma is the main differential diagnosis20.

The alterations of CSF, even if not specific, are very useful for diagnostic orientation. In most cases, protein concentration is increased, while glucose concentration is usually normal, being reduced only in the case of massive meningeal infiltration. CSF cytology examination has a fundamental value in the staging for its potential prognostic and therapeutic implications. Unfortunately, it is not possible to identify lymphomatous cells in every case and sometimes not even in the presence of an extended meningeal infiltration. Immunocytochemical analysis and detection of immunoglobulin gene rearrangements by PCR technique have been retained useful in the diagnosis of lymphomatous meningitis when routine cytological examination is inconclusive21. Tumor markers, including lactate dehydrogenase isoenzymes, β-glucuronidase, and β2-microglobulin, may provide indirect evidence of leptomeningeal lymphoma. In some cases with normal features in the CSF, leptomeningeal biopsy is necessary for diagnosis.

With a few exceptions, leptomeningeal lymphoma is reported to be associated with a poor survival (median < 6 months)22,23. Adequate treatment of leptomeningeal lymphoma may be achieved by high-dose systemic chemotherapy or by intrathecal chemotherapy24,25. Some authorities have suggested the concomitant use of both systemic and intrathecal chemotherapy in patients with positive CSF cytology examination. Therapeutic methotrexate concentrations can be achieved in CSF using intravenous dosages ≥3 g/m², and preliminary data suggest that systemic high-dose methotrexate is able to clear CSF from neoplastic cells26,27. On the other hand, drug delivery by using Ommaya’s reservoir permits a better drug distribution in the subarachnoid space. However, the indications and efficacy of intrathecal chemotherapy are debatable considering that its efficacy has not been prospectively assessed and the results of a recent analysis of a large multicentre series suggesting that patients with PCNSL do not benefit.
from intrathecal drug delivery. Moreover, the theoretical advantage of intraventricular drug administration probably does not outweigh the additional risk of infective complications associated with Ommaya reservoir implantation and repeated intraventricular injections and the increased risk of neurotoxicity and chemical meningitis associated with this strategy. Radiation therapy, such as cranio-spinal irradiation, seems to play a palliative role in leptomeningeal lymphomas since it destroys a considerable amount of hemopoietic bone marrow reserve, making troublesome any subsequent chemotherapy.

**Spinal cord lymphoma**

Primary lymphomas of the spinal cord present the greatest diagnostic difficulties among PCNSL. These lymphomas often arise in the upper thoracic or lower cervical regions of the spinal cord. Presenting symptoms are protein, depending on the spinal cord level involved. Patients generally complain radicular symptoms, pain in the legs, a sensory level, and motor or bladder disturbances. Myelography shows a spinal cord widening. MRI usually discloses hyperintense signals on T2-weighted and homogeneous enhancement after administration of gadolinium on T1-weighted images. Increased CSF protein concentration is a common feature. Lymphomas arising in the spinal nerves and ganglia (“neurolymphomatosis”), cauda equina and the sciatic nerve are extremely rare and should be distinguished from the neural infiltration by a systemic lymphoma. Intramedullary lymphoma should be considered in the differential diagnosis of other spinal cord tumors. Timely histological diagnosis and treatment are essential to achieve recovery of neurological function, which is strongly conditioned by pre-treatment neurological status. Prognosis of patients with spinal cord lymphomas is very poor mainly due to delayed diagnosis. Similarly to other PCNSL, corticosteroids and radiotherapy are associated with lymphoma regression and clinical improvement. Only sparse data are available regarding the efficacy of chemotherapy in these lymphomas. However, there is no evidence suggesting that spinal cord lymphomas should be treated in a different way with respect to other PCNSL.

**Rare lymphoma categories in the CNS**

Most of PCNSL are diffuse large B-cell lymphomas. Usually, lymphoma cells grow around the perivascular spaces and invade the surrounding brain parenchyma. Lymphoma cells display mature B-cell phenotype (i.e. CD20 positive) and exhibit a high proliferation rate (50% of MIB1-positive cells). A reactive perivascular T-cell infiltrate (CD3 positive) is evident in one third of cases (Ponzoni M. Personal communication, Barcelona 2004). In contrast to AIDS patients, PCNSL of immunocompetent individuals do not usually carry EBV-genome sequences or proteins. Much rarer lymphoma categories may primarily arise in the CNS: among others, some high-grade categories, such as anaplastic large cell lymphoma, T-cell lymphomas, or intravascular lymphoma, or indolent lymphomas, or plasma cell lymphoma, or MALT-type or immunocytoma, solitary bone plasmacytoma, and Hodgkin’s lymphoma.

**Anaplastic large cell lymphoma**

A few cases of primary CNS anaplastic large-cell lymphoma (ALCL), mostly displaying T-cell immunophenotype, have been reported. In a few cases, primary CNS ALCL carries the characteristic t(2:5) translocation, which is responsible for the production of the fusion protein ALK-1, which may be detected by immunohistochemistry (fig. 2). The parietal lobe is the most common site of disease, although involvement of more than one lobe is the rule. Meningeal involvement and increased CSF protein level seem to be more common in primary CNS ALCL with respect to the rest of PCNSL, while the elevation of lactate dehydrogenase serum levels is a rather unique finding. Similarly to systemic ALCL, a better outcome has been observed in young patients and ALK-1 positive lymphomas. Even if statements about treatment strategy and prognosis cannot be drawn with sufficient confidence, the overall therapeutic approach to primary CNS ALCL does not seem to differ greatly from the current therapy of PCNSL. Importantly, meningeal prophylaxis with intrathecal chemotheraphy or high-dose methotrexate-based chemotherapy appears advisable considering the common involvement of meninges.

**T-cell lymphomas**

One to 4% of PCNSL displays T-cell phenotype. For unknown reasons, the incidence of T-cell PCNSL appears to be higher in Japanese patients (8% of cases). The diagnosis of T-cell PCNSL can be difficult and possibly is overestimated due to the presence of reactive perivascular T-cell in-
filtrate, which could interfere with the interpretation of immunophenotyping. In particular, a diagnosis of T-cell lymphoma should be avoided in the case of pre-biopic administration of corticosteroids. Since these drugs may cause lysis of malignant B-cell, only reactive T-cells are sampled, thus mimicking either an inflammatory process or a T-cell lymphoma. In comparison to B-cell PCNSL, patients with T-cell PCNSL have a younger median age and a higher prevalence among males, but these findings were not definitively established. Half of T-cell PCNSL shows an infratentorial lesion, while leptomeningeal involvement is comparable in B- and T-PCNSL. Differences from therapeutic and prognostic points of view between T-cell and B-cell PCNSL remain matter of debate; some authorities have suggested a more favorable course for T-cell lymphoma. Therapeutic comparisons between these subgroups are difficult because only small and heterogeneously treated, retrospective series are available.

**Intravascular lymphoma**

Intravascular lymphoma (IVL), formerly known as proliferating angioendotheliatomatisis, is an aggressive and disseminated malignancy characterized by widespread proliferating B- or T-cells within the lumen of vessels, with no or minimal involvement of the extravascular parenchyma. IVL affects elderly patients, without gender prevalence, resulting in poor performance status, B-symptoms, anemia, and elevated lactate dehydrogenase serum levels. CNS, other than skin, is the most commonly involved organ in IVL diagnosed in Western Countries; while, in Japanese IVL patients, CNS is usually spared. Exclusive CNS involvement is, however, an extremely rare condition; systemic dissemination or multiorgan infiltration is the rule. Clinical presentation is varied, ranging from isolated symptoms, such as fever, pain or local symptoms, to diverse combinations of B-symptoms and rapidly progressing manifestations of multiorgan failure due to microvascular occlusion and infarcts. Neurological symptoms are present at diagnosis in 34% of IVL cases diagnosed in Western Countries; they are extremely heterogeneous, and neuroimaging confirms CNS involvement only in half of symptomatic patients. Histopathological hallmark is the presence of large lymphoid cells within vessel lumina (fig. 3); concomitant extravascular infiltrates of neoplastic large lymphoid cells within vessel lumina (endothelial cells; & eosin). The growth of neoplastic large cells (open arrows) occurs exclusively within blood vessel lumen (endothelial cells; full arrows). Neoplastic lymphocytes show large nuclei with one or more nucleoli and sparse cytoplasm; 98% of cases display a B-cell immunophenotype.

**MALT lymphoma**

Low-grade marginal zone B-cell lymphoma of MALT-type primarily involving the CNS usually arises in leptomeninges. This is an indolent malignancy affecting females, and usually presenting as a single intracranial lesion (Ann Arbor stage IEA). Its main differential diagnoses are meningioma and, less frequently, acute subdural hematoma. A variety of therapeutic options have been used to treat meningeal MALT lymphomas, including radiotherapy, systemic chemotherapy, and partial surgical excision. It is of high importance to recognize this entity, and to distinguish it from other small B-cell lymphomas considering that, as their counterparts in other organs, MALT lymphomas have favorable clinical outcomes and excellent long-term prognosis with local therapy alone. As reported for other extranodal MALT lymphomas, consolidation radiotherapy seems to be superfluous after complete surgical resection.

**Lymphoplasmacytic lymphoma (immunocytoma)**

Immunocytoma has been retained as the 4-13% of all PCNSL. However, most of CNS immunocytoma cases have been reported before the introduction of the WHO classification, where MALT-lymphoma has been formally included. It is likely that a more stringent definition of immunocytoma (i.e. lymphoplasmacytic lymphoma) may not include all the reported cases of PCNSL with “immunocytoma” morphology, which actually may represent examples of MALT lymphomas. Immunocytoma is characterized by a diffuse
growth of small B-cells that share intermediate characteristics between lymphocytes and plasmacells. The phenotype is characteristic of B-cell, but differs from small B-cell lymphoma for its CD5 and CD23 negativity. Similarly to systemic disease, immunocytoma affects young women. MRI and MR spectroscopy findings indicating the low-grade nature of the malignancy have been reported [45]. In MR spectroscopy, CNS immunocytoma exhibits a lesser degree of metabolite changes and no significant change on follow-up due to its highly differentiated nature [46]. Experience with high-dose methotrexate-based chemotherapy is anecdotal; lymphoma response to this strategy was modest [40]. On the other hand, radiation therapy seems to be associated with an acceptable disease control, while available survival data are insufficient.

**Plasmacytoma**

CNS involvement in multiple myeloma is a rare condition [1]. In half of reported cases, CNS was the sole site of disease. Clinical presentation usually consists of multiple neurologic symptoms, occasionally of space occupying lesion-related symptoms, increased intracranial pressure, optic neuropathy, and optic nerve compression [41]. Determining whether an intracranial plasmacytoma is solitary or not is important from a prognostic point of view. In fact, patients with solitary CNS plasmacytoma have more favorable outcome in comparison to patients with systemic myeloma involving the CNS. Intracranial plasmacytoma is unlikely to develop systemic multiple myeloma in patients with initial negative systemic staging [1]. Thus, involved field irradiation with 50 Gy has been reported as an acceptable strategy in these patients [41], while solitary cases, mostly with large lesions, have obtained clinical benefit from chemotherapy [42]. Cases with brain plasmacytoma associated with multiple bone lesions should be treated like multiple myeloma, but CNS bioavailability of used drugs should be taken into account when therapeutic strategy is planned.

**Hodgkin’s disease**

Primary CNS Hodgkin’s lymphoma constitutes 1.4% of all lymphoproliferative disorders arising in the CNS [3]. Secondary involvement of the CNS by Hodgkin’s disease is more rare (0.2-0.5% of cases). Intracranial Hodgkin’s disease may present as an isolated brain lesion, meningeal dissemination with infiltration of subdural neural tissue and/or brain compression without direct infiltration, or as a calvarium lesion with meningeal and/or cerebral infiltra- tion. Nodular sclerosis is the most frequently reported subtype (∼50% of cases), and it affects equally males and females, with an age ranging between 50 and 85 years [43]. The role of systemic chemotherapy remains to be defined, while it has been suggested the use of intrathecal chemotherapy in patients with positive CSF cytology examination [44].

**Five-year view**

A number of fundamental clinical and biological challenges on PCNSL remain to be addressed [3]. This is particularly relevant in the field of therapeutic management, where several questions remain unanswered. A more extensive and coordinated, multidisciplinary cooperation will become the main strategy to address some of the fundamental clinical and biological research questions for PCNSL. One of the risks of this strategy is, however, to apply conclusions from multicentre trials to every PCNSL case. Actually, rare lymphoproliferative entities primarily arising in the CNS require a special methodological approach and should be investigated separately from the “common”, primary CNS diffuse large B-cell lymphoma. Since these are rare forms of a rare malignancy, a major effort to collect cases from several cancer centers around the world appears advisable. In fact, the creation of a shared historical database that would pool information from an adequate number of cases for every lymphoma category represents a promising clinical research strategy in this field. In addition, it is critical that investigators share archival tumor tissue, which is especially important for frozen tissue since this is a rare resource in PCNSL. With these strategies, the most interesting clinical, molecular, pathologic, and therapeutic issues regarding rare forms of PCNSL will be better investigated. In the next years, international, multidisciplinary cooperation will be the winning strategy in the multifaceted fight against the “common” and “rare” forms of PCNSL.

**References**


47. The Application of Molecular Studies to the Characterization of Cutaneous T Cell Lymphoma


Molecular Pathology Program, Centro Nacional de Investigaciones Oncológicas. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid. "Pathology Department, Hospital Universitario de Getafe. Madrid.

Introduction

Mycosis fungoides (MF) is a peripheral T-cell lymphoma characterized by the infiltration and accumu-
Translation of tumoral T cells in the skin (epidermal and dermal infiltration). It is the most frequent type of cutaneous T cell lymphoma (CTCL), representing more than half of all lymphomas originating in the skin. The knowledge surrounding the aetiology and tumorigenic mechanisms in MF is limited, and the clinical and pathological diagnosis of MF has proved very difficult since it bears many resemblances to common inflammatory dermatoses (ID) such as interface and spongiosic dermatitis, conditions involving infiltration and accumulation of benign T cells in the skin. The difficulties in the study of MF are further compounded by the small number of tumoral cells, which may be present in the skin, with tumoral T-cell infiltrate often accounting for only 5-10% of the total tissue cells.

cDNA microarrays are a powerful technique, which allow the analysis of the expression of thousands of genes simultaneously. These types of studies have revolutionised the study of cancer including lymphoma and leukaemia, allowing finer tumour classification, prediction of treatment response, prediction of overall survival, analysis of drug resistance and the identification of new therapeutic targets. In an effort to improve the characterization of these tumours, molecular analysis using the CNIO OncoChip cDNA microarray has been used for examining the expression profile of a total of 53 MF and 11 ID samples, allowing the identification of an MF tumorigenesis model, construction of a 6-gene MF prediction model and the identification of 2 clusters subclasses of MF, one of which tends to include more aggressive type MF cases including tumoral MF forms.

Results

Data normalization

The first major problem in the study of MF is the low number of tumoural cells present in the skin, with T

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<th>Gene Symbol</th>
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<th>Adjusted p value</th>
<th>Function</th>
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cell infiltrates often accounting for only 1-5% of the total number of cells in the tissue. Therefore during cDNA microarray analysis much of the signal comes from normal cells in the skin, and not from the cells of interest. In preliminary studies of 6 ID and 8 MF cases, using hierarchical clustering techniques, ID and MF cases could not be separated (fig. 1A). We attempted to eliminate the signal coming from normal surrounding tissue in both sample types by normalizing each MF and ID sample against a pool of normal non-photoposed skin samples. This technique allowed cases to be clearly separated into 2 groups, using hierarchical clustering (fig. 1B). Therefore this method of normalization against normal samples is a valid method for enriching the data obtained from microarray data in samples where the number of tumoral cells is low, allowing better clustering and classification of these tumours. For the remainder of the study this type of normalization was used for all cases of MF and ID using a total of 6 normal skin samples for normalization.

**MF signature: Genes differentially expressed between MF and ID cases**

To identify genes implicated in MF tumourgenesis, a total of 29 MF and 11 ID samples were used. Data from microarray studies was normalized against the average signal from 6 normal skin samples. To identify the genes significant in distinguishing between MF and ID cases, we used a t-statistic, and unadjusted p-values were obtained from permutation tests. Adjusted p-values were obtained using Benjamini & Hochberg’s procedure for the control of the False Discovery Rate. Genes with unadjusted p-values < 0.001 and adjusted p-values < 0.1 were deemed to be significant. A total of 27 genes significant in the separation of MF from ID samples were identified (table 1). These genes are involved in a large variety of functions such as cell cycle control, apoptosis and signal transduction. However the most striking finding is the upregulation of many genes implicated in activation of NF-κB and TNF signalling including TRAF1, BIRC3 and TNFRSF5 (CD40).

**MF prediction model construction and validation by blind testing**

In order to validate the 27-gene MF prediction model, gene clustering was carried out using the SOM algorithm, which defined a total of 6 gene clusters. An average for each gene cluster was calculated and this average was used for validation using discriminant analysis in the study group and blind set of 24 low-stage MF patients, yielding a success rate of 100% in both cases (table 2).

In order to identify a smaller subset of class predictor genes, the gene with the lowest p-value was selected from each cluster generated by the SOM algorithm. The final class prediction model of 6 genes (table 3) was validated by discriminant analysis. This class prediction model classifies all cases with 97.3% accuracy in the original sample set and with 97.0% accuracy in the blind set (table 4).

**Identification of MF subgroups**

In order to identify subgroups of MF patients, unsupervised hierarchical clustering analysis was performed of all 53 MF samples, and 2 main clusters of MF were identified (fig 2). At the same time, the heterogeneity of the MF samples was analysed in terms of clinical (treatment responders vs. non-responders), histological (plaque vs. tumour stage, large cell component), or immunophenotypical (common (CD4+/CD8–) vs. aberrant phenotype, high proliferative index vs. low proliferative index and immunostaining for STAT1, STAT3, or CD30 antibodies) characteristics of the patients. The Fisher’s exact test was used to find significant difference between the two clusters. Some characteristics associated with more aggressive phenotypes have a tendency to be found in cluster 2. For example, all tumour-stage disease cases (100%, p: 0.061), along with cases expressing activated STAT3, an oncogene which has been demonstrated to play a role in apoptosis escape in MF cell lines, are over-represented in Cluster 2 (47% compared to 17%, p: 0.069). Other characteristics associated with aggressive disease (large cell component, high proliferative index, and high stage disease) are more predominant in cluster 2 although these differences do not reach significance.

To identify the biological factors responsible for the clustering observed within the MF cases, a Student’s T test comparing MF clusters 1 and 2, was performed. Genes with unadjusted p-values < 0.001 and adjusted p-value < 0.06 were deemed to be significant. A total of 557 genes whose expression was significantly different between these MF clusters were iden-
tified. To simplify the analysis, all ESTs and hypothetical genes were excluded from further analysis leaving a total of 368 named genes implicated in the differentiation of MF cluster 1 and cluster 2. Cluster 1 cases, which tend to be less aggressive in terms of STAT3 expression and disease type, show increased expression of growth factors and interleukins, some oncogenes and positive cell cycle regulators. Cluster 2, which tends to include the more aggressive cases, shows upregulation of some genes implicated in NF-κB activation in addition to upregulation of a large number of oncogenes, positive cell cycle regulators and anti-apoptotic genes. Furthermore reduced expression of some important tumour suppressor genes is observed compared to Cluster 1 cases.

Discussion

Activation of the NF-κB and TNF anti-apoptotic signalling in the tumorigenesis of MF

A total of 27 genes are differentially expressed between MF and ID cases (table 1), 20 of which are upregulated in the MF cases while the remaining 7 genes are downregulated. Most interesting is the upregulation of many genes directly implicated in the regulation of TNF signalling and NF-κB activation including BIRC3, TRAF1, LYN, HCK, TNFRSF5 and PRKCH in addition to other important genes, which may play an indirect role in TNF signalling and NF-κB activation such as STAT4.

NF-κB is activated primarily through signalling by TNF superfamily receptors and ligands such as CD40 (TNFRSF5), which is overexpressed in MF cases. The TRAF protein family bind to activated TNF family receptors and subsequently induce phosphorylation and degradation of the NF-κB inhibitor, IκB. This allows NF-κB to translocate to the nucleus and activate transcription, leading to cell growth and survival. TRAF7 has been identified here as being upregulated in MF cases.

The inhibitor of apoptosis proteins (IAP) inhibit apoptosis including apoptosis induced through signalling by death receptors such as FAS, TRAIL and TNFR1. These receptors possess an intracellular...
death domain and induce apoptosis by recruitment of TRADD and subsequent activation of caspase 8 through adapter proteins such as FADD. The IAP/BIRC family, including BIRC3, which is upregulated in MF cases, inhibit cell death induced by death receptors thus allowing NF-κB activation to proceed. The role of BIRC3 (cIAP2) has been described in a variety of lymphomas and leukemias and its involvement in treatment resistance has been previously described.

The protein kinase C family plays an important role in some signalling pathways responsible for activation of NF-κB particularly those involving the T and B cell receptor complexes and those involving cytokines such as TNF and IL1 in certain cell types. Protein kinase C, an isoform of which is overexpressed in MF cases, is responsible for the degradation of IκB and activation of NF-κB.

HCK and oncogene LYN are tyrosine kinases upregulated in MF cases. HCK is responsible for production of TNF in murine cells and both HCK and LYN have been implicated in TNF production in human monocytes. In this study, in addition to HCK and LYN upregulation in MF cases, TNF was overexpressed although significance was just outside the stringent range set here. Since apoptotic signalling through death receptors including the TNF receptor 1 (TNFR1) is likely to be abrogated due to overexpression of IAPs such as BIRC3 (cIAP2), overexpression of TNF would lead to NF-κB activation through TNF receptor 2 (TNFR2) and to a lesser extent through TNFR1. Additionally, HCK and LYN and the oncogenic c-MYC are induced by IL2 signalling via JAK2 and STAT4 activation. In this study, STAT4 is overexpressed in MF cases, as is the IL2 receptor (IL2R), but with a lower significance.

In summary, MF tumorigenesis is associated with activation of the NF-κB survival pathway through TNFR2 and other TNF family receptors lacking a death domain such as CD40, in addition to inhibition of the pro-apoptotic pathways of TNFR1 and other death receptors. IL2 may also play a critical role in MF tumorigenesis through its receptor by activating JAK2 and STAT4 and subsequently inducing expression of oncogenes such as c-MYC, LYN and HCK. LYN and HCK participate in an autocrine loop by promoting endogenous TNF production and thereby auto-stimulating TNFR1 and TNFR2 NF-κB activation pathways and creating a feedback loop of TNF survival pathway activation. NF-κB apoptotic pathway silencing may be due to overexpression of IAP family members such as BIRC3 (cIAP2) causing caspase inhibition.

**MF prediction model**

The prediction model using the average signal from the clusters of all 27 genes, can correctly assign class in 100% of both the original MF series and the blind set of 24 MF patients (table 2). Since some genes, although individually significant in separating MF from ID cases, may provide redundant information, the 27 genes in the tumorigenesis model were grouped using the SOM clustering algorithm, which defined 6 clusters. Subsequently the gene with the lowest p-value from each cluster was chosen and this 6-gene prediction model correctly classified 97.3% of the original series and 97.0% of the MF blind set. Further weight to the validation of this prediction model is provided by the exclusive presence in the blind set of cases with early stages of MF, whose histology more closely mimics that of ID cases.

**Clusters of MF cases based on gene expression patterns**

Hierarchical clustering of 53 MF samples revealed 2 main MF clusters. Some characteristics associated with more aggressive phenotypes have a tendency to be found in cluster 2 cases, including tumour-stage disease and cases expressing activated STAT3.

Cluster 1 cases, which tend to be less aggressive show increased expression of a large number of genes including growth factors such as those of the fibroblast growth factor family (FGFR1, FGFR5), epidermal growth factor family (GRB7, EGF3, ERBB3), platelet derived growth factor family (PDGFA), vascular endothelial growth factor family (ANGPT1, ANGPT3) and the transforming growth factor family (BMP5, TAB1, TGFBR3, MADH5P, GDF5). In parallel, higher expression of critical oncogenes such as HRAS (in addition to RAB1, RAB4, RAL2B, TIM) and interleukins (IL5RA, IL7, IL1RAPL1, IL11) is observed accompanied by upregulation of positive cell cycle regulators such as cyclin A1 (CCNA1), cyclin dependent kinase family genes (CDK12, CDKL1) and cell division cycle genes (CDC27, CDC20). Overexpression of a limited number of genes implicated in the NF-κB pathway is also observed in cluster 1 cases, including BIRC4 (XIAP) and receptors and ligands such as TNFSF11A (RANK) and TNFSF4 (OX40L).

Cluster 2, which tends to include the more aggressive cases, shows upregulation of some genes implicated in NF-κB activation including BIRC2 (c-IAP1) and TNFRSF12 (APO3L1, TWEAK receptor). Parallel to upregulation of NF-κB activation genes, upregulation of oncogenes such as MYC, FOS, VAV3, WNT and PRM1 is observed, accompanied by upregulation of positive cell cycle regulators such as cyclin D2 and interleukin receptors such as IL2RB. Other oncogenes such as PYN, STAT3, PIM2 and MYCN (nMYC) are also expressed at a higher level in cluster 2 cases but with lower significance. The overexpression of STAT3 in cluster 2 cases lends support to the observation that activated STAT3 protein is a feature more common in cluster 2 cases. Interestingly oncogenes FOS, MYC and PYN and IL2RB play an important role in NF-κB activation. Furthermore cyclin D2 (CCND2) is a transcriptional target of NF-κB, underlying the potential importance of NF-κB activity in this cluster of cases. Finally cluster 2 cases show lower expression of a num-
ber of genes with tumour suppressing properties as compared to cluster 1 cases. These include HIC1, LGI1, DLEU1, ST14, MSH5 and PTEIN, whose alteration has been described in lymphoid malignancies. Taken together these results suggest that while NF-\( \kappa \)B activation may be a common event in the tumorigenesis of MF, the two MF cases clusters identified show different characteristics in terms both of NF-\( \kappa \)B activation and expression of oncogenes, growth factors, interleukins and cell cycle control genes. Cluster 1 cases tend to be less aggressive and these cases are more dependent on overexpression of growth factors, interleukins and positive cell cycle regulators and oncogene HRAS for their growth. Notwithstanding, these cases also show overexpression of some important NF-\( \kappa \)B pathway genes such as BIRC4, TNFRSF11A and TNFSF4 and TNFRSF17 and TNFSF9 with lower significance. Cluster 2 cases tend to exhibit a more aggressive phenotype and show overexpression of a large number of genes associated with NF-\( \kappa \)B activation including BIRC2 (IAP1) and TNFRSF12 (APO3L1) and HFKBL2, BIRC1 (NAIP), BIRC5, TNFSF10 (TRAIL) and TNFSF13 (APRIL) with lower significance. Additionally a large number of potent oncogenes are upregulated some of which also play a role in NF-\( \kappa \)B activation while tumour suppressor genes are downregulated as compared to cluster 1 cases. Overall a stronger NF-\( \kappa \)B activation or distinct mechanisms of NF-\( \kappa \)B activation in combination with strong oncogenic pathway activation may help explain the tendency for cluster 2 cases to show a more aggressive phenotype.

Conclusions

MF tumorigenesis combines endogenous TNF production and NF-\( \kappa \)B activation

Microarray analysis revealed 27 genes whose expression is significantly different between MF and non-tumoural ID cases. MF tumorigenesis is associated with NF-\( \kappa \)B activation through TNFR2 and other TNF family receptors such as CD40 and LT\( \beta \), in addition to inhibition of the pro-apoptotic pathways of TNFR1 and other death receptors. IL2 may also play a critical role in MF tumorigenesis by inducing expression of oncogenes such as MYC, LYN and HCK. LYN and HCK participate in an autocrine feedback loop by promoting endogenous TNF production and thereby auto-stimulating TNFR1 and TNFR2 NF-\( \kappa \)B activation pathways and creating a feedback loop of TNF survival pathway activation.

A 6-gene MF prediction predicts MF diagnosis with unprecedented accuracy

A 6-gene prediction model constructed provides an unprecedented accuracy in diagnostic prediction, correctly classifying 97.3% of the original series and 97.0% of the MF blind set. Further weight to the validation of this prediction model is provided by the exclusive presence in the blind set of cases with early stages of MF, whose histology more closely mimics that of ID cases.

MF consists of two cases clusters, one of which is characterised by a more aggressive phenotype

Hierarchical clustering of 53 MF samples revealed 2 main MF clusters. Cluster 1 cases tend to be less aggressive and these cases are more dependent on overexpression of growth factors, interleukins and positive cell cycle regulators and oncogene HRAS for their growth. Cluster 2 cases tend to exhibit a more aggressive phenotype and show overexpression of a large number of genes associated with NF-\( \kappa \)B activation. Additionally a large number of potent oncogenes are upregulated some of which also play a role in NF-\( \kappa \)B activation while tumour suppressor genes are downregulated as compared to cluster 1 cases.

References