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Case report

Primary T-cell rich B-cell lymphoma of the common bile duct

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Abstract. A 34-year-old woman was hospitalized for the investigation of a one-month history of intestinal disorders, gastric heaviness and transitory icteric episodes. Extensive clinical investigations suggested the diagnosis of gall bladder carcinoma or sclerosing cholangitis. At laparotomy, the proximal part of common bile duct was markedly thickened by a white, firm, fish-flesh like tumour extending in to the cystic duct, gall bladder wall and to the liver. Histological study showed a diffuse lymphoid proliferation of the common bile duct mainly composed of small cells mixed with scattered large atypical cells. Immunohistochemistry revealed that most of the small cells expressed T-cell markers with predominant CD 4 and α-β T-cell receptors and without phenotypic gap, whereas large atypical cells showed monotypic B phenotype with co-expression of μ and δ heavy chains and light λ chain restriction. No evidence of primary nodal lymphoma was found during extensive clinical, radiological, sonographic or scanographic examinations. Sequential chemotherapy (MACOP-B) was instituted and the patient was still alive 4 years after diagnosis. Morphological and immunohistochemistry findings fulfilled criteria for a primary high grade B-cell lymphoma (centroblastic type, Kiel classification) from common bile duct concealed by numerous small reactive T-cells, so called T-cell rich B-cell lymphoma, not previously described in this location.

Key words: Primary B-cell lymphoma – Common bile duct – Reactive T-cells – Paraffin section immunohistochemistry – T-cell rich B-cell lymphoma

Introduction

Primary non-Hodgkin's lymphomas (NHL) of the extrahepatic bile ducts are extremely rare. Most lymphomas involving the hepatobiliary tract are considered to be a secondary extension of a disseminated lymph node NHL. Only two cases have been recorded in the English medical literature (Kosuge et al. 1991; Nguyen 1982). T-cell rich B-cell lymphomas were described in 1988 by Ramsay et al. (1988) as B-cell lymphomas displaying morphological features similar to peripheral T-cell lymphomas and containing few neoplastic B-cells amid numerous reactive T-cells. In this brief report, morphological and immunohistochemical study on deparaffinized and on frozen sections of a primary T-cell rich B-cell lymphoma originating in the common bile duct is detailed. To our knowledge, no previous case has been described in the medical literature.

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Case report

A 34-year-old white woman was referred to Lariboisière Hospital in July 1989 for the investigation of a one month history of intestinal disorders, gastric heaviness, nausea, anorexia, weight-loss and transitory icteric episodes. She had no history of hepatobiliary disease. Physical examination was unremarkable. Laboratory investigations demonstrated cholestasis (alkaline phosphatase: 384 UI/l (N: 30–90); γ-GT: 348 UI/l (N < 40)) and cytolysis (SGOT: 46 UI/l (N < 40); SGPT: 171 UI/l (N < 40)). LDH value was 181 UI/l (N < 330). Other biological findings were unremarkable. Hepatitis-B serology was negative.

Ultrasound examinations and abdominal CT-scan showed a thickened gall bladder wall without stones and multiple hepatic nodules mainly located in the right lobe. Intrahepatic bile ducts were distended whereas the common bile duct was of normal diameter. Pancreas and spleen were within normal limits. The first diagnosis was of infiltrative gall bladder carcinoma extending to the common bile duct and to the liver.

Percutaneous fine needle biopsy of an hepatic nodule was not diagnostic, showing only normal hepatic parenchyma. In August 1989, a laparotomy was performed: the liver was increased in size without any sign of cholestasis. A white, firm tumour involved the origin of common bile duct and extended to cystic duct and gall bladder. The confluence of hepatic bile ducts and the pancreas was free of tumour. Multiple palpable intrahepatic nodules were found, two of which bulged around the gall bladder. Perperative frozen sections of two tumour samples showed evidence of malignant lymphoma.

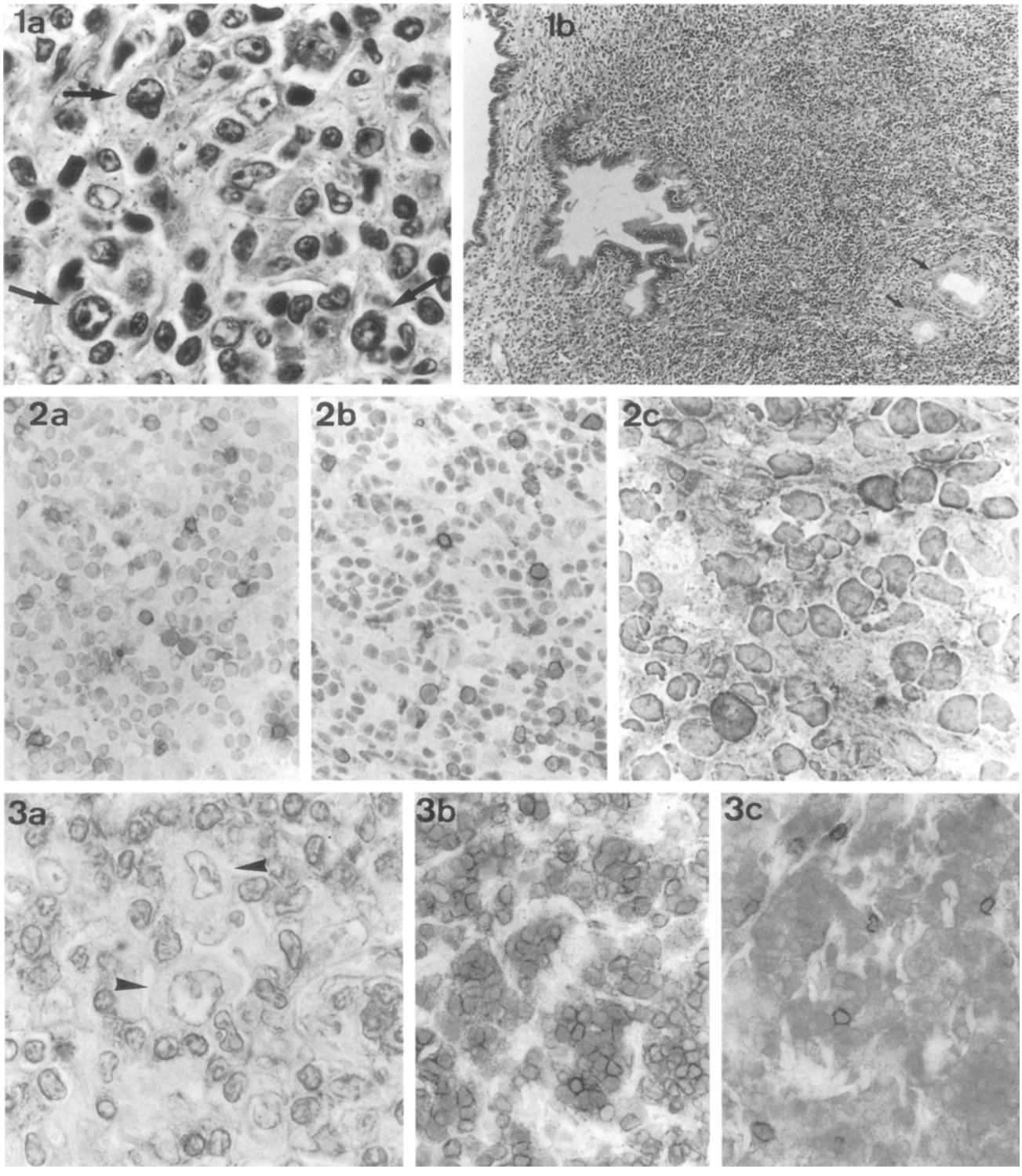

Fig. 1a, b. Common bile duct: **a** malignant lymphoma large cells (→) with large nuclei and one to three medium size nucleoli (Giemsa ×1056); **b** lymphoid infiltrate located in common bile duct wall. No evidence of lymphoepithelial lesion in glands (→) (HES, ×264)

Fig. 2a–c. Liver strip: large cell immunostaining for **a** μ, **b** δ-heavy chains and **c** λ-light chain (Frozen sections, **a, b** ×422; **c** ×1056)

Fig. 3a–c. Common bile duct: **a** staining of small cells with polyclonal CD3 antibody. No staining of large cells (►) (Paraffin embedded sections, ×1056); liver strip: **b** immunostaining for α-β T-cell receptor (βF1) compared to **c** γ-δ T-cell receptor (TCR δ1) (Frozen sections, ×422)

Table 1. Panel of antibodies used for immunohistochemistry on paraffin sections

Antibody	Cluster of Differentiation	Fixative[a]	Major specificities	Source[b]
LCA	CD45	F/B	Leucocyte common antigen	Dako
LN 1	CDw75	F	Germinal center B cells	ICN
LN 2	CD74	B	B-cells, interdigitating cells	ICN
L 26	CD20	F	B-cells	Dako
MB 2		B	B-cells, many non-lymphoid cells	Eurodiagnostics BV
CD3[c]	CD3	F/B	Mature thymocytes, peripheral T cells	Dako
Leu M1	CD15	B	Granulocytes, Reed-Sternberg cells	Becton Dickinson
Ber H2	CD30	F	Actived T- and B-cells, Reed-Sternberg cells	Dako
KP 1	CD68	B/F	Macrophages	Dako
EMA		B/F	Epithelial membrane antigen	Dako
Kl1		B/F	Epithelial cells, carcinoma	Immunotech
F VIII[d]		B	Endothelial cells, megacaryocytes	Dako

[a] B: Bouin' fluid; F: 10% formalin; [b] Becton Dickinson, Mountain View Calif., USA; Dako, Glostrup, Denmark; Eurodiagnostics BV, Apeldoorn, Holland; ICN, Lisle, Ill., USA; Immunotech, Marseille, France. [c] Polyclonal antibody; [d] Factor VIII-related antigen

Table 2. Panel of antibodies used for immunohistochemistry on frozen sections

Antibody	Cluster of Differentiation	Major specificities	Source[a]
Leu 12	CD19	B-cells	Becton Dickinson
Leu 14	CD22	B-cells	Becton Dickinson
Ig M		Ig heavy chain	Clonatec
Ig D		Ig heavy chain	Dako
Kappa		Ig light chain	Zymed
Lambda		Ig light chain	Zymed
Leu 5	CD2	T-cells	Becton Dickinson
Leu 4	CD3	T-cells	Becton Dickinson
Leu 1	CD5	T-cells, B-cell subset	Becton Dickinson
Leu 9	CD7	T-cells	Becton Dickinson
Leu 3	CD4	T-cells subset (helper/inducer)	Becton Dickinson
Leu 2	CD8	T-cells subset (cytotoxic/suppressor)	Becton Dickinson
CALLA	CD10	Common acute lymphoblastic leukaemia antigen	Dako
ß F1		T-cell receptor ß chain	T-cell sciences
δ TCS1		T-cell receptor δ chain	T-cell sciences
TCR δ1		T-cell receptor δ chain	T-cell sciences
bcl-2		*bcl*-2 gene product	Dako
IL2 R	CD25	Interleukin-2 receptor	Becton Dickinson
C3b R	CD35	Reticular dendritic cells, B-cell subsets	Dako
DRC1		Reticular dendritic cells,	Dako
KB61	CDw32	Monocytes, granulocytes and B-cells	Gift, Dr. Mason
Ki-67		Proliferating cells	Dako

[a] Becton Dickinson, Mountain View, Calif., USA; Clonatec, Paris, France; Dako, Glostrup, Denmark; T-cell sciences, Cambridge, Mass., USA; Zymed, San-Francisco, Calif., USA

Peroperative cholangiogram showed normal right and left hepatic ducts, and a narrowed proximal part of the common bile duct with a normal size below. The gall bladder, cystic duct and extrapancreatic part of common bile duct were removed. The postoperative course was uneventful. Chest radiography, thoracic CT-scan, ear nose and throat examination, gastroscopy, colonoscopy and bone marrow biopsy did not show other tumour. The final diagnosis was stage IV, high-grade NHL and sequential chemotherapy (Methotrexate, Doxorubicine, Cyclophosphamide, Vincristine, Prednisolone) was prescribed. No recurrent disease was found 4 years after diagnosis.

In the resected specimen the common bile duct was 4 cm long and 0.5 cm in diameter. It was set into a white, firm, fish-flesh like tumour extending to cystic duct (4 cm long) and gall bladder (4 cm long and 1.5 cm in diameter). The gall bladder wall was thickened (0.6 to 2 cm thick) but mucosa did not seem to be infiltrated by the tumour. One side of the gall bladder and a strip of hepatic parenchyma were firmly joined together by the white tissue. Gall bladder, cystic and common bile ducts, and liver samples were fixed and/or snap-frozen with isopentane in liquid nitrogen.

Microscopically the tumour was diffuse, without any nodular pattern. It was mainly composed of small lymphocytes (small cells) with round to oval nuclei with irregular outline and coarse granular chromatin without PAS-positive intra-nuclear vacuoles. The cytoplasm was not apparent on Giemsa stain. On thorough examination large atypical cells were observed, either isolated or in small clusters, mixed with lymphocytic proliferation. They exhibited large regular or irregular shaped nuclei with one to three medium size nucleoli stuck to the nuclear membrane (Fig. 1a) and a well-developed blue-grey cytoplasm on Giemsa stain. Some atypical multilobated large cells and few immunoblastic cells were seen. Scattered eosinophils and histiocytes were mixed with the lymphoid proliferation.

The tumour was mainly located in the common bile duct wall. The luminal surface was lined with residual columnar epithelial cells and no lymphoepithelial lesion was observed (Fig. 1b). In the gall bladder, lymphomatous proliferation was observed only in the subserosal connective tissue and did not involve the muscle layer or mucosa. Around cystic duct, common bile duct and gall bladder, hypertrophic nerve sheaths were surrounded and sometime infiltrated by lymphoid proliferation. Blood vessels were underlined but not invaded by lymphoma. In the hepatic parenchyma, the normal architecture was destroyed by extensive, diffuse, malignant infiltrate. Gall bladder neck lymph node exhibited a normal architecture but perinodal fatty tissue was invaded by tumorous cells.

Immunohistochemistry was performed on paraffin sections (formalin or Bouin's fluid fixed tissue according to primary antibody reactivity: Table 1) and on frozen sections (Table 2) with either an avidin-biotin-peroxidase complex (ABC-method: Vectastain ABC kit, Vector, Calif., USA) (Hsu et al. 1981) method revealed by 3–3′ diaminobenzidine, or alkaline phosphatase-anti-alkaline phosphatase complexes (APAAP method: Dako, Glostrup, Denmark) revealed by Fast-red TR salt (Cordell et al. 1984).

The most characteristic finding was the expression of B-cell markers (CD20, MB2, CDw75, CD74, CD19 and CD22) in scattered, large, atypical cells and very few small ones. The monoclonality of neoplastic cells was seen on frozen sections through the expression of a monotypic λ light chain and the co-expression of two surface μ and δ heavy chains (Fig. 2). Neoplastic large cells did not express epithelial membrane antigen (EMA), CD 30 or CD 15. Positive staining for *bcl*-2 was found in few small and large cells. CD 25 was expressed in about 10% of large atypical cells. Less than 10% of neoplastic cells were positive with Ki-67.

Most small cells, representing approximately 80% of all cells, expressed T-cell markers (Fig. 3a) without any phenotypic gap, with a large predominance of CD 4, αβ T-cell receptor and HLA-DR positive cells (Fig. 3b, c).

Both small and large cells were DRC1, CD 35, CDw 32 negative. With anti-cytokeratin and anti-EMA antibodies, no lymphoepithelial lesion was observed. Blood vessels – positive with anti-factor VIII antibody – did not show intraluminal invasion. A few histiocytes were demonstrated with CD 68. Alkaline phosphatase activity was negative in B-cells. According to morphology and immunohistochemistry, the final diagnosis was common bile duct primary high-grade B-cell lymphoma (centroblastic type according to the Kiel classification) concealed by numerous reactive T-cells, so called T-cell rich B-cell lymphoma.

Discussion

Lymphomatous infiltration of hepatobiliary tract is not uncommon and generally results from a secondary spread occuring during the course of a generalized lymphoma process. A few cases of primary extranodal NHL of pancreas, ampulla of Vater and gall bladder have been reported (Barek and Orron 1986; Botha and Kahn 1974; Mosnier et al. 1992; Webb et al. 1989). Freeman et al. (1972) recorded only six cases of non-disseminated NHL of extranodal origin, including hepatobiliary tract. Among NHL, primary extranodal NHL of common bile duct are extremely rare and only two detailed cases have been described in the English medical literature (Kosuge et al. 1991; Nguyen 1982). In these cases, jaundice was the major presenting symptom. In our report the patient presented several transitory icteric episodes probably due to localized thickening of common bile duct. This cholestasis in association with the features identified on imaging suggests the diagnosis of adenocarcinoma or primary sclerosing cholangitis. However, multiple, intrahepatic nodules were not explained by the latter diagnosis. The macroscopic and histological findings in association with radiographic, sonographic, and scanographic examinations allowed to diagnose a primary lymphoma of the common bile duct with secondary liver extension through regional subserosal tissue.

It is currently recognized that some B-cell lymphomas can contain numerous T-cells, mimicking some, or all, morphological features of T-cell lymphomas (Mirchandani et al. 1985). In most cases B-cell lymphomas were apparently diffuse (Osborne et al. 1990; Ramsay et al. 1988), as in our case, but others included cases with follicular nodularity (Ng et al. 1989). B-cell clonality was not proved in all cases (Ng et al. 1989). Five cases were reported by Ramsay et al. (1988) and they used the term "T-cell rich B-cell lymphoma" (TCRBCL). They noticed that TCRBCLs were initially misdiagnosed as T-cell lymphomas. In their study, TCRBCLs lacked eosinophilic infiltrate whereas in our case such an eosinophilic component was present as in other studies (Macon et al. 1992b; Ng et al. 1989; Osborne et al. 1990). Standard histological examination alone may result in misdiagnosis. Flow cytometry analysis may also support misdiagnosis since the few neoplastic B-cells can be hidden by the large number of reactive T-cells (more than 80% in some cases, Ramsay et al. 1988) and immunohistochemistry is a major help establishing the diagnosis, since it reveals the B-cell component concealed by reactive T-cells.

In the present case, the expression by large atypical cells of two μ and δ surface heavy chains in an uncommon finding since this co-expression is generally seen in mantle cell lymphome (Banks et al. 1992). Diagnosis of this type of lymphoma can be ruled out since neoplastic cells are large cells and do not express CD 5 and CDw32 (KB 61). This result cannot be accounted for. In their study of 21 cases of TCRBCLs Ng et al. (1989) found respectively two and three cases jointly expressing δ and γ chains and γ and μ chains.

The absence of lymphoepithelial lesion, confirmed by immunostaining against cytokeratin and EMA, suggests that our case is not originating in mucosa associated lymphoid tissue as has been recently described for one case of gall bladder lymphoma (Mosnier et al. 1992).

In the Kiel classification (Stansfeld et al. 1988) as in the Working Formulation (WF; National Cancer Institute 1982) TCRBCL is not recognized and most of the cases are classified as centroblastic or immunoblastic lymphoma (Kiel), or mixed small and large cell lymphoma (group F, WF) or large cell immunoblastic lymphoma (group H, WF) or unclassified lymphoma. In the present case, large atypical B-cells could be assimilated to centroblastic cell type in the Kiel classification. Positivity for *bcl*-2 in our case could support a follicular centre cell origin of the lymphoma (Gaulard et al. 1992) as suggested by *bcl*-2 rearrangement (Macon et al. 1992b). Finally, the term of TCRBCL should prevent pathologists from over-diagnosing T-cell lymphoma.

Eosinophils and/or histiocytes can be found mixed with small lymphocytes and Hodgkin's disease may be considered (Chittal et al. 1991). In our case, Hodgkin's

disease was excluded since no typical or atypical Reed-Sternberg cell was found and immunohistochemistry did not support this diagnosis (negativity of large atypical cells for CD 15 and CD 30).

The T-cell infiltrate probably represents a particular host-response to B-cell lymphoma as suggested by Ramsay et al. (1988). It might related to the secretion of cytokines, such as interleukin 4, by tumour cells and/or histiocytes as demonstrated by Macon et al. (1992a). The significance of T-cell component remains unclear. In a study of diffuse mixed lymphomas, Katzin et al. (1989) demonstrated that most of T-cells were CD 4 positive, as in our case. Similar findings were also described in studies of follicular and diffuse B-cell lymphomas (Dvoretshy et al. 1982; Garcia et al. 1986). The prognostic significance of this reactive T-cell infiltrate is suggested by Strickler et al. (1988) who have shown that patients with spontaneous regression of follicular lymphomas had significantly more T-helper (CD4 positive) non-neoplastic cells than control patients. In the present case the patient is still alive, free of recurrence and pregnant 4 years after diagnosis supporting Strickler's suggestion.

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