Case report

Early transformation event from follicular lymphoma: our experience of four cases

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Follicular lymphoma (FL) is a mature B-cell lymphoma that can transform into a more aggressive disease such as diffuse large B-cell lymphoma, Burkitt lymphoma, or precursor B-lymphoblastic leukaemia/lymphoma. The process of transformation of FL occurs by the acquisition of additional genetic alterations, e.g. c-MYC rearrangement, TP53, and cyclin D1 inactivation. Herein, we describe four such cases of FL that transformed into more aggressive B-cell non-Hodgkin lymphomas within six months of their initial diagnosis. Subsequent testing of c-myc, P53 and cyclin D1 by immunohistochemistry and fluorescence in situ hybridization was done to further analyse their role in the process of transformation.

Key words: diffuse large B-cell lymphoma, follicular lymphoma, precursor B-lymphoblastic lymphoma, transformation.

Introduction

Follicular lymphomas (FL) constitute 20% of all lymphomas. FL is an indolent lymphoma that arises from the germinal centre B-cell and is driven by t (14; 18) (q32; q21) rearrangement in 85% of cases with corresponding overexpression of Bcl2 [1]. About 2–3% of cases of FL per year get transformed into high-grade lymphomas with a poor response to therapy and an aggressive clinical course. As with other B-cell lymphomas, FL transforms into diffuse large B-cell lymphoma (DLBCL) and less commonly into other lymphomas such as Burkitt lymphoma, high-grade B-cell lymphoma with *c-MYC* rearrangement along with *BCL2* and/or *BCL6* rearrangement (double hit high-grade B-cell lymphoma), and plasmablastic lymphoma. Infrequently these FL get transformed to precursor B-lymphoblastic lymphoma [1, 2]. Such cases of transformation involve several mechanisms such as *c-MYC* rearrangements, alterations of cell-cycle control (mutation or deletion of cyclin-dependent kinase 2A/B) and impairment of DNA damage response mechanisms such as loss of *TP53* [3].

Herein, we evaluate four cases of histological transformation of FL to high-grade lymphoma and analyse c-myc, P53, and cyclin D1 by immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH) testing.

Material and methods

All cases of FL were reviewed for the period 2018–2021 from the archives at the Department of Pathology. A total of 72 cases of FL were diagnosed during this period, of which 4 (5.5%) cases showed transformation to high grade lymphoma. Tissue microarray (TMA) of these four cases was prepared using representative paraffin blocks. Supplementary IHC and FISH staining beyond that needed for the diagnosis was performed on this TMA. Immunohistochemistry was done on 4 μm sections using an automated staining platform (Ventana Benchmark XT, Tucson, Arizona USA). The clones mentioned below were used for IHC evaluation, cyclin D1 (anti-cyclin D1, clone EP12, cell marque, Marseilles France), P53 (anti-P53, clone D0-7, cell marque, Marseilles

Table I. Probe details used in fluorescent in situ hybridization analysis for *TP53, c- MYC* and *CCND1*

Type of probe	Manufacturer	Locus	Interpretation
Spec TP53/CEN17	Zytovision, Germany	TP53-17p13.1 (orange) CEN17-centromeric region of chromosome 17 (green)	Two orange and two green- normal One orange and two green- TP53 deletion One orange and one green- monosomy of chromosome 17 More than two orange signals indicate copy number gain of TP53
Spec MYC Dual colour break apart probe	Zytovision, Germany	MYC- 8q24.21 5′ (orange 475 kb) and 3′ (green 560 kb)	Two orange/green fusion signals-normal One orange/green fusion signal and one orange and a separate green signal indicate one 8q24.21 normal locus and one 8q24.21 locus involved in translocation More than two orange/green fusion signals indicate copy number gain of MYC
Spec CCND1/IGH Dual colour Dual fusion probe	Zytovision, Germany	CCND1-11q13.3 (orange) IGH-14q32.33 (green)	Two orange and two green signals-normal One orange/green fusion signal and one orange and one green signal indicate one chromosome involved in translocation More than two orange signals indicate copy number gain of CCND1

France) and c-myc (anti-c-myc, clone EP121, cell marque, Marseilles France).

TP53 deletion, *c-MYC* and *cyclin D1* amplification and rearrangement were evaluated using FISH. The details of probes used are shown in Table I [4].

Analysis of interphase FISH was done using an Olympus B × 63 automated FISH Microscope and image capture was done using digital FISH scanning provided by Applied Spectral Imaging Ltd., Israel. The threshold for copy number alterations was set at 5% and rearrangement at 15% as per the institutional laboratory cut points. Principles outlined in the Declaration of Helsinki were followed for ethical consideration.

Case reports

Case 1

A 63-year-old man presented with generalized lymphadenopathy. His systemic and physical examinations were unremarkable. Laboratory investigations revealed Hb of 8.4 g/dl, WBC 9840/mm^3, platelets 413 000/mm^3 and high serum lactate dehydrogenase (LDH) of 1518 U/l. The Follicular Lymphoma International Prognostic Index (FLIPI) score was in the high-risk group 3. Bone marrow was uninvolved. Excisional biopsy from the submandibular lymph node exhibited a nodular growth pattern with nodules composed of centrocytes and centroblast, the latter being > 5 and < 15 per high power field. On IHC, the lymphoid cells expressed LCA, CD20, Bcl2, Bcl6, and LMO2 but were negative for CD10 and CD3. MIB-1 was 10% in the nodules. A diagnosis of low-grade FL (grade 2) with a predominant follicular pattern was made. Positron emission tomography (PET) scan showed multiple metabolically active enlarged lymph nodes in the cervical region, left axillary, paraoesophageal, pericaval, common iliac and left external iliac region. Discrepant histology concerning high LDH level and massive retroperitoneal lymphadenopathy with necrosis prompted another computed tomography guided core biopsy from the left para-aortic lymph node. Histopathological examination of this biopsy showed sheets of large neoplastic lymphoid cells with brisk mitoses and apoptosis. On IHC, these large cells expressed LCA, CD20, CD10, Bcl2, Bcl6, MUM1, and LMO2, but were negative for CD3. The MIB-1 index was 70% and Epstein-Barr virus (EBV)-encoded RNA (EBER) by *in situ* hybridization was negative. The large neoplastic cells were also positive for c-myc IHC, which was later applied on the TMA section. Hence, this large cell morphology was a triple expressor high grade B-cell lymphoma, arising from the transformation of the previously diagnosed FL because of a similar immunophenotypic profile. The patient was given the 1st cycle of R-CVP (rituximab, cyclophosphamide, vincristine sulfate and prednisone) based chemotherapy followed by six cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine, and prednisone) based chemotherapy. The patient was on regular follow-up, but he died due to COVID infection. Further IHC was applied in TMA sections where neoplastic cells in FL were negative for c-myc and cyclin D1, while large neoplastic cells after transformation showed significant c-myc expression and few cyclin D1 positive cells are present. P53 staining was heterogenous both before and after

Table II. Immunohistochemistry findings of c-MYC, P53 and Cyclin D1 before transformation and immunohistochemistry and gene analysis after transformation

Cases	At initial diagnosis			After transformation IHC and gene analysis					
	c-MYC	P53	Cyclin D1	c-MYC		P53		Cyclin D1	
				IHC	FISH	IHC	FISH	IHC	FISH
Case 1	Negative	Wild	Negative	Significant expression	Negative	Wild	Negative	Positive	Negative
Case 2	Negative	Wild	Negative	Negative	Negative	Wild	Deletion	Negative	Negative
Case 3	Negative	Wild	Negative	Significant expression	Negative	Mutated protein	Deletion	Negative	Translocation
Case 4	Negative	Wild	Negative	Significant expression	Translocation	Wild	Negative	Negative	Translocation

FISH – fluorescent in situ hybridization, IHC – immunohistochemistry
Significant expression: > 40% of tumour cells are positive.

transformation (Table II, Fig. 1). On FISH analysis, no *TP53* deletion, *c-MYC* and *cyclin D1* copy number gain or rearrangements were observed (Table II). Fluorescent *in situ* hybridization testing on DLBCL also did not reveal *BCL2* or *BCL6* rearrangement.

Case 2

A 62-year-old man presented with enlargement of multiple groups of lymph nodes. On laboratory investigation, Hb 14.6 g/dl, WBC 6910/mm^3, platelets 195 000/mm^3 and serum LDH of 280U/l were noted. The FLIPI score was two (intermediate risk). USG guided incisional biopsy was obtained from the right cervical lymph node, which revealed a low-grade FL with expression of CD20, CD79a, CD10, Bcl6, LMO2, and Bcl2. The neoplastic lymphoid cells were negative for CD3 and CD5. D2-40 highlighted the preserved follicular dendritic meshwork. The MIB-1 index was 10%. Positron emission tomography scan revealed metabolically active lymph nodes in the right side of the neck and in the supra- and infra-diaphragmatic regions. The patient was treated with R-Bendamustine (rituximab and bendamustine) based chemotherapy. After completion of the 4th cycle of chemotherapy, almost after 5 months of initial diagnosis, the patient presented again with a mass in the right lower alveolus, the incisional biopsy of which showed diffuse sheets of large cells with prominent nucleoli which on IHC were positive for CD20, CD10, Bcl2, Bcl6 and negative for CD5, CD3 and CD30. MIB-1 labelling was 90%. Expression of EBER by *in situ* hybridization (ISH) was negative. These findings were diagnostic of DLBCL, the germinal centre immunophenotype arising on a background of FL. The patient was started on R-CHOP based chemotherapy and on re-evaluation after the 5th cycle of chemotherapy, a PET scan showed progressive metabolically active infradiaphragmatic lymph nodes, alveolar lesions, and lung infiltrates. Further treatment was switched to R-DHAP (rituximab, dexamethasone, cytarabine, and cisplatin) based chemotherapy and was assessed after 2 cycles, which showed progressive disease. Magnetic resonance imaging showed an intensity enhancing lesion along the left tentorium cerebelli and left upper eyelid suggestive of lymphomatous deposits. Cerebrospinal fluid examination also showed atypical lymphoid cells. After a few weeks the patient died due to progressive disease. The subsequent IHC was done on a TMA slide which showed negative c-myc and cyclin D1 both before and after transformation. P53 staining was wild type (Table II, Fig. 2). Fluorescent *in situ* hybridization analysis showed *TP53* deletion in areas of high-grade histology (Fig. 3). *c-MYC* and *cyclin D1* were negative for copy number alterations and rearrangement (Table II).

Case 3

A 52-year-old man presented with multiple enlarged cervical lymph nodes. General physical and systemic examinations were unremarkable. Pancytopenia was observed and serum LDH was raised against the normal value. The FLIPI score was 3 (high risk). Excisional biopsy examination of the right cervical lymph node exhibited a follicular pattern with follicles composed of centrocytes. On IHC, CD20, Bcl2, CD10 and Bcl6 were positive and negative for LMO2, CD5, and CD3. CD23 highlighted the follicular dendritic meshwork. The MIB-1 index was less than 10%. The diagnosis of low-grade FL was made. A positron emission tomography scan revealed metabolically active extensive generalized lymphadenopathy above and below the diaphragm, hepatosplenomegaly with multiple metabolically active parenchymal lesions, and extensive involvement of the skeletal system. Because of pancytopenia and extensive skeletal system involvement, bone marrow biopsy was done, which showed marrow spaces dif-

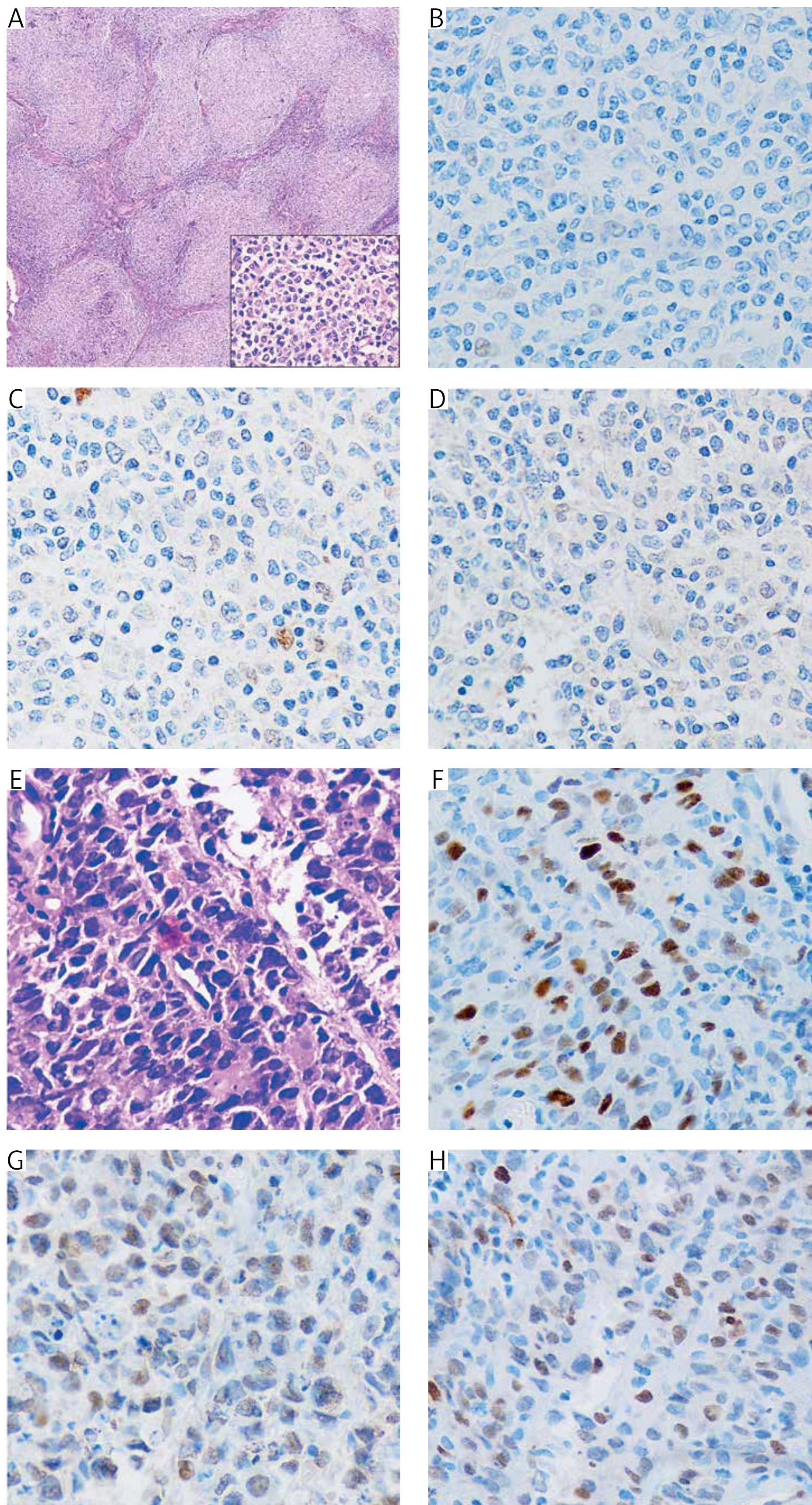

Fig. 1. Case 1. Follicular lymphoma, grade 2, showing follicular pattern (haematoxylin and eosin – H&E; 100×). A) Inset showing centrocytes with a few centroblasts (H&E; 200×). B) On immunohistochemistry (DAB; 200×) neoplastic cells are negative for c-myc. C) P53 staining shows wild type. D) P53 staining shows negative type for cyclin D1. E) After transformation to diffuse large B-cell lymphoma (H&E; 200×), there is loss of follicular pattern and large neoplastic cells. F) On immunohistochemistry (DAB; 200×) neoplastic cells are immunopositive for c-myc. G) P53 staining wild type. H) P53 staining positive type for cyclin D1

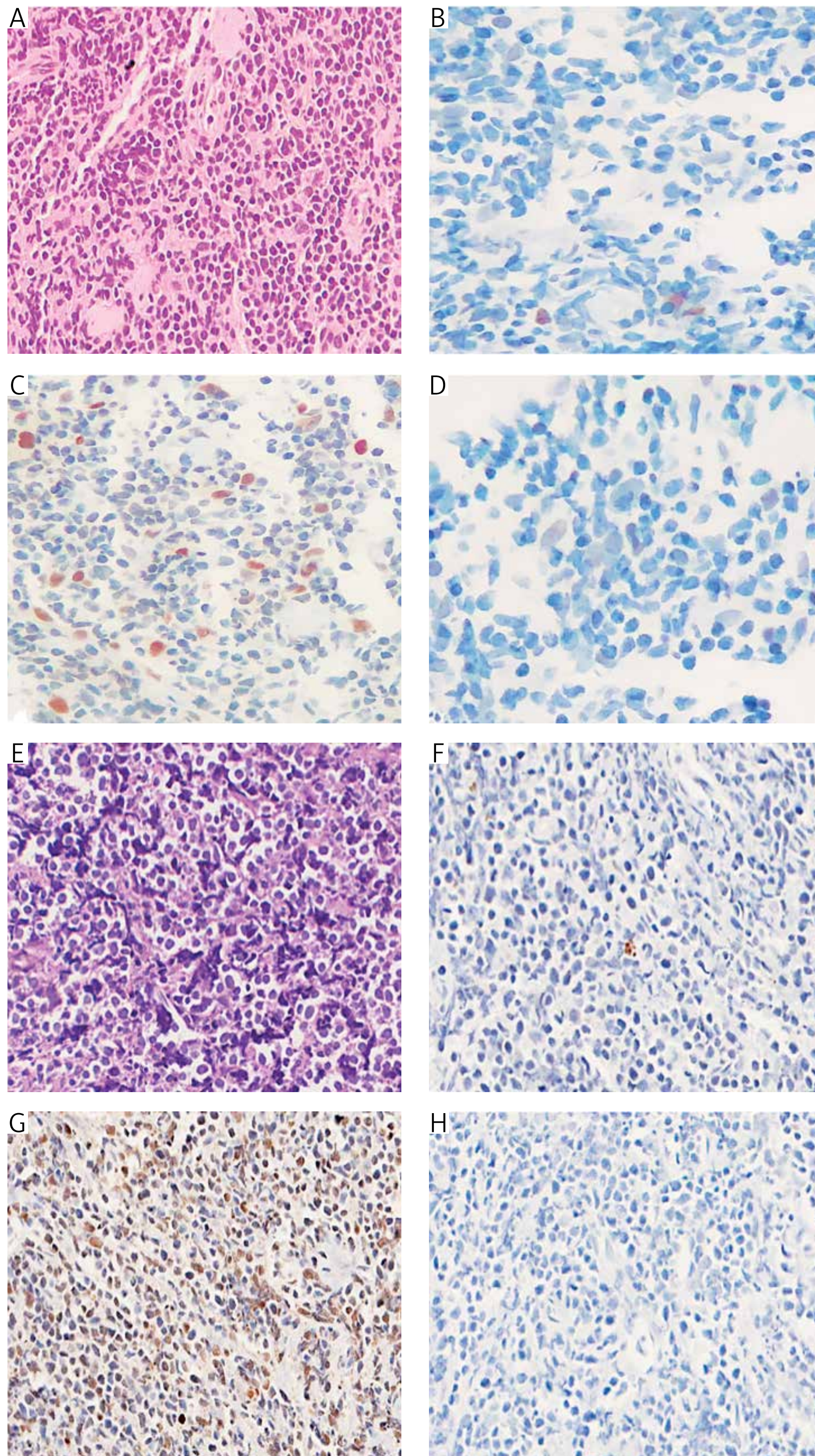

Fig. 2. Case 2. Follicular lymphoma, grade ½. A) Predominantly centrocytes are seen (haematoxylin and eosin – H&E; 200×). B) On immunohistochemistry (DAB; 200×) tumour cells are negative for c-myc. C) P53 staining is wild type. D) P53 staining is negative for cyclin D1. E) After transformation into diffuse large B-cell lymphoma, the neoplastic cells are large and show a diffuse pattern (H&E; 200×). F) On immunohistochemistry (DAB; 200×) tumour cells are negative for c-myc. G) On immunohistochemistry (DAB; 200×) tumour cells are wild type P53. H) On immunohistochemistry (DAB; 200×) tumour cells are negative for cyclin D1

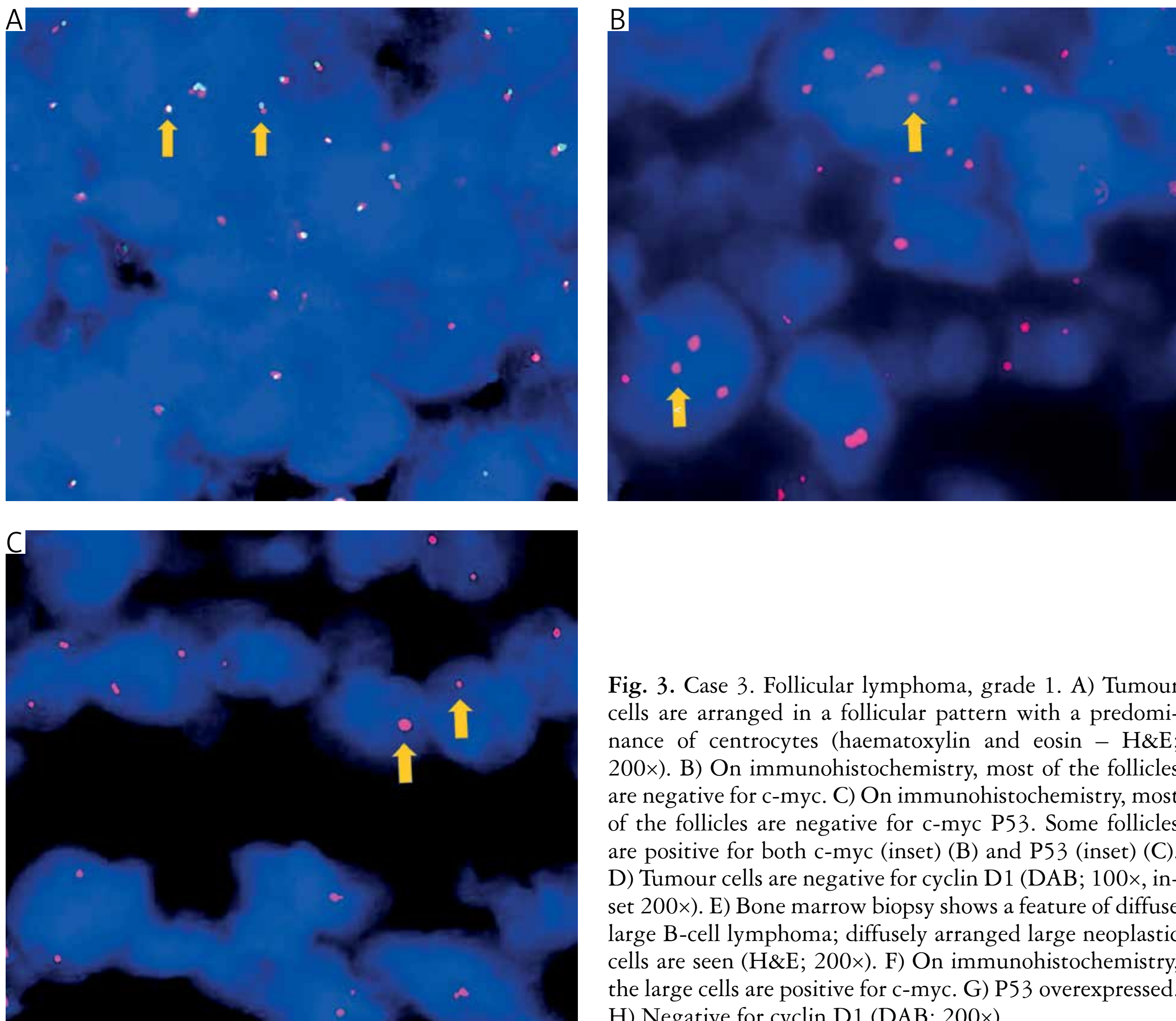

Fig. 3. Case 3. Follicular lymphoma, grade 1. A) Tumour cells are arranged in a follicular pattern with a predominance of centrocytes (haematoxylin and eosin – H&E; 200×). B) On immunohistochemistry, most of the follicles are negative for c-myc. C) On immunohistochemistry, most of the follicles are negative for c-myc P53. Some follicles are positive for both c-myc (inset) (B) and P53 (inset) (C). D) Tumour cells are negative for cyclin D1 (DAB; 100×, inset 200×). E) Bone marrow biopsy shows a feature of diffuse large B-cell lymphoma; diffusely arranged large neoplastic cells are seen (H&E; 200×). F) On immunohistochemistry, the large cells are positive for c-myc. G) P53 overexpressed. H) Negative for cyclin D1 (DAB; 200×)

fusely replaced by large atypical lymphoid cells with intense mitosis. Immunopositivity was observed for LCA, CD20, CD10, c-myc, Bcl2 and Bcl6. MIB-1 labelling was 80%. Similar high-grade histology was noted in the core biopsy of one of the liver space occupying lesions. Low-grade follicular lymphoma in the cervical lymph node with concurrent transformation into high grade B-cell lymphoma, triple expressor in bone marrow and liver was offered as the final diagnosis. The patient was given one cycle of rituximab/cyclophosphamide-based chemotherapy, which was well tolerated. Further FISH testing was done; it showed positivity for *BCL2* gene rearrangement but was negative for *BCL6* and *c-MYC*. The patient received six cycles of R-CHOP based chemotherapy and is on close follow-up. We performed additional IHC on a TMA slide, which showed negative c-myc and cyclin D1 and wild type P53 in FL. The transformed DLBCL are positive for c-myc, while cyclin D1 is negative. P53 was overtly expressed in neoplastic cells (Table II, Fig. 4). Immunohistochemistry findings also correlated with the FISH analysis, *cyclin D1* showed copy number gain, and one copy of *TP53* was deleted (Fig. 3). However, no copy number gain or rearrangement was seen in *c-MYC* (Table II).

Case 4

A 70-year-old man presented with abdominal pain and distension. General physical examinations were within normal limits. Laboratory investigations were within normal limits except for high serum β2 microglobulin 4089 ng/ml (normal value: 609–2366 ng/ml). The FLIPI score was 3 (high risk). A computed tomography scan of the abdomen and pelvis revealed multiple enlarged and necrotic abdominal lymph nodes which on core biopsy showed atypical lymphoid cells predominantly in follicles with centroblasts and centrocytes. On IHC the cells were positive for CD20, Bcl2, CD10 and LMO2 and negative for CD3, CD5 and CD30. CD21 + 23 highlighted the follicular meshwork. The MIB-1 index was 30%. The diagnosis of FL, grade 3A, predominantly follicular, was established. A further PET

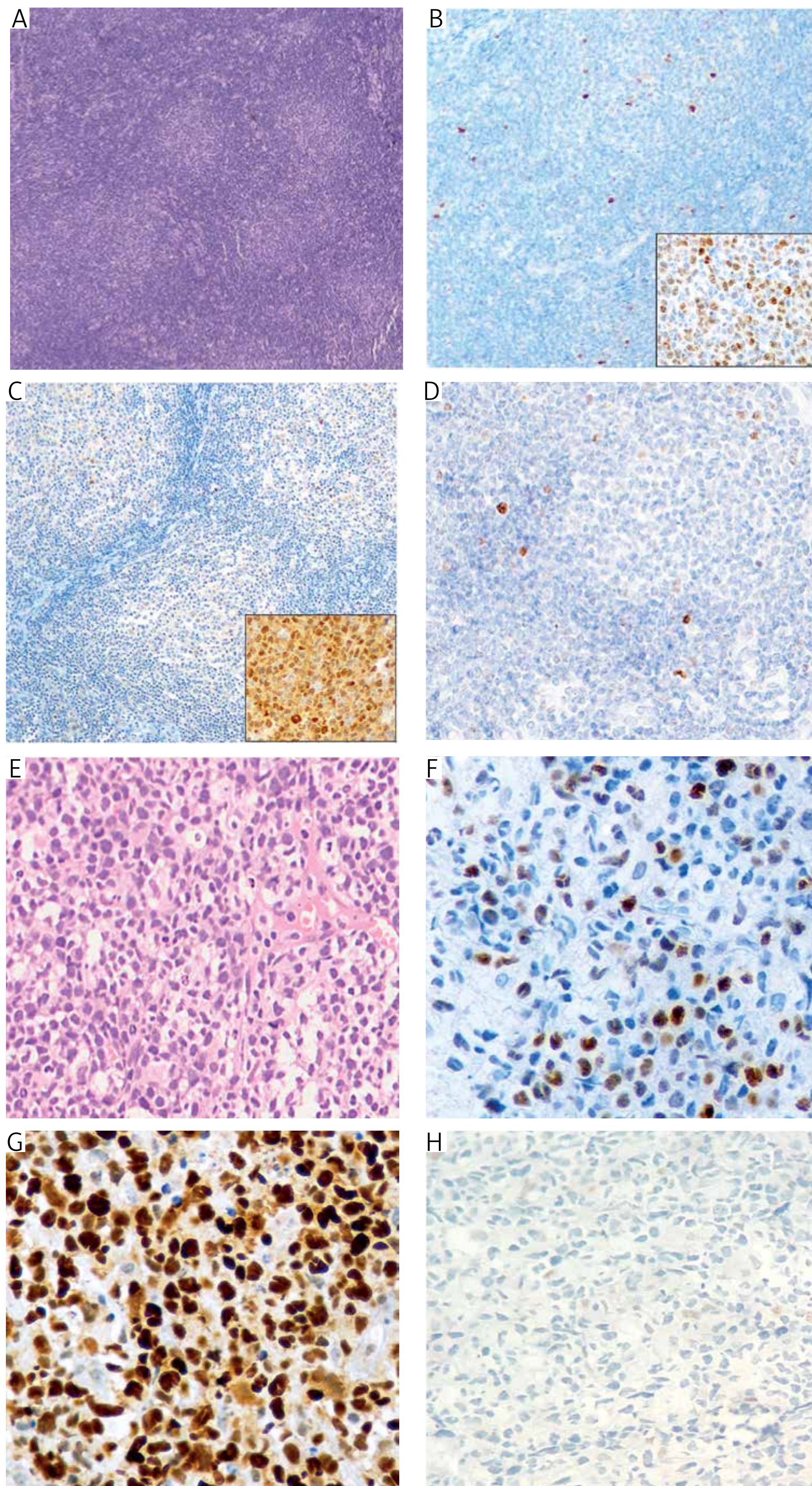

Fig. 4. Case 3. A) Follicular lymphoma, grade 1, tumour cells are arranged in a follicular pattern with a predominance of centrocytes. B, C) On immunohistochemistry (IHC), most of the follicles are negative for c-myc (B) and P53 (C) while some follicles are positive for both c-myc (inset) (B) and P53 (inset) (C). D) Tumour cells are negative for cyclin D1. E) Bone marrow biopsy shows a feature of diffuse large B-cell lymphoma, diffusely arranged large neoplastic cells are seen. F, G, H) On IHC, the large cells are positive for c-myc (F), P53 overexpressed (G), negative for cyclin D1 (H)

scan showed mildly metabolically active supra- and infradiaphragmatic lymphadenopathy with metabolically active lesions in the liver, spleen, and bilateral lung nodule. The patient underwent 6 cycles of chemotherapy with an R-CHOP based regimen.

After 7 months he presented with multiple non-tender subcutaneous nodules over the abdomen. Laboratory findings were within normal limits except for high serum LDH of 369 U/l. The punch biopsy from one of the skin nodules showed diffuse proliferation of atypical lymphoid cells with individual cells having blastoid morphology. On IHC, these cells were positive for CD10, Bcl2, PAX5, MUM1 with weak expression of LCA and CD79a. Terminal deoxynucleotidyl transferase showed nuclear positivity in a significant number of cells (Fig. 5). CD3, CD4, CD8, CD20, Bcl6, CD43, LMO2 and CD34 were negative. EBER by ISH was negative. Cyclin D1 was negative and P53 was wild type in both FL and transformed biopsy, but c-myc showed significant expression in transformed neoplastic lymphoid cells. Subsequent, FISH analysis showed rearrangement of *c-Myc* (Fig. 3). *Cyclin D1* was also positive for translocation, but *Tp53* showed no copy number alterations (Table II). The final diagnosis of precursor B-cell lymphoblastic lymphoma as a transformation event from previously diagnosed FL following *c-Myc* rearrangement was made. The patient was started on the UKALL regimen and was given up to the 5th block of ALL induction therapy (daunorubicin and cytocristine) followed by induction II (cyclophosphamide, 6-mercaptopurine, cytarabine) and consolidation (cytarabine and etoposide). The patient died after 3 weeks.

Discussion

Transformation of FL to high-grade lymphoma requires confirmation by biopsy supported by immunophenotype. However, when a biopsy is not feasible, the diagnosis of transformation is often made on clinical grounds. The clinical features of transformation have been mentioned with the presence of at least one of the following: sudden rises in LDH, rapid increase in the size of lymph node, new lesion in unusual extranodal sites, new B symptoms, and new hypercalcemia [5]. Associated high-risk factors for transformation have also been mentioned in several studies, including advanced disease stage, presence of B symptoms and bulky disease, high β2 microglobulin and low albumin levels, and higher FLIPI score [6]. Three of our cases (cases 1, 3, 4) also presented with high-risk factors such as a sudden rise in LDH, low albumin level, high β2 microglobulin, multiple lymph node enlargements, multiple new lesions over the skin, subcutaneous plane, and liver with FLIPI score ≥ 3 (high risk).

The average risk of transformation is 20% at 5 years and 30% at 10 years [3]. The duration of transformation has been reported from 2 months to 25 years from the time of diagnosis [5]. Similarly, around a 12-month interval is noted between the initial FL diagnosis and B-LBL transformation [4]. In contrast, all four of our patients showed transformation within a short period of 2 weeks to 6 months. This form of early transformation might be due to the presence of high-risk factors and high FLIPI scores at diagnosis in these patients, suggesting concomitant transformation at an unbiopsied site.

Follicular lymphoma can show transformation to more aggressive lymphomas, such as DLBCL or triple expressor high grade B-cell lymphoma. In a rare instance, precursor B-cell lymphoblastic lymphoma transformation has been documented in the literature. One of our patients (case 4) is of such a rare type. All the reported cases of initial diagnosed FL were of grade 1 or 2 [2]. In contrast, our patient (case 4) showed lymphoblastic transformation from grade 3A FL.

Our findings show c-myc, P53 and cyclin D1 immunopositivity in biopsy after transformation, indicating its role in high-grade histologic transformation. However, one of the patients (case 2) did not show any immunopositivity in these markers despite developing high-grade large cell lymphoma transformation, which suggests that high stage, high-risk FLIPI score, underlying predisposing factors, tumour microenvironment, and many other underlying genetic alterations collectively play a role in the process of histologic transformation [6].

Subsequently, we performed *c-MYC*, *TP53* and *cyclin D1* gene analysis by FISH testing in biopsy of high grade histology Tables I and II, Fig. 4). As seen in the present case series, not all the cases which were positive for c-myc, P53 and cyclin D1 on IHC correlated with *c-MYC*, *cyclin D1* or *TP53* alteration in FISH analysis and vice versa (Table II). This finding indicates that the genetic alteration at the molecular level does not necessarily correlate with expression of the respective protein; for example, not all double expressor lymphomas are double hit lymphomas [7]. We did not perform FISH testing in FL cases.

The survival of patients after the development of transformation is poor and associated with major morbidity and mortality [8, 9]. Two of our patients (cases 2 and 4) developed progressive disease and did not respond well to chemotherapy and died after a short interval of high-grade transformation.

Conclusions

The present case highlights the transformation of FL to high-grade lymphomas. These lymphomas usually occur in adults, carry a poor prognosis, and show a poor response to therapy. A histopathological

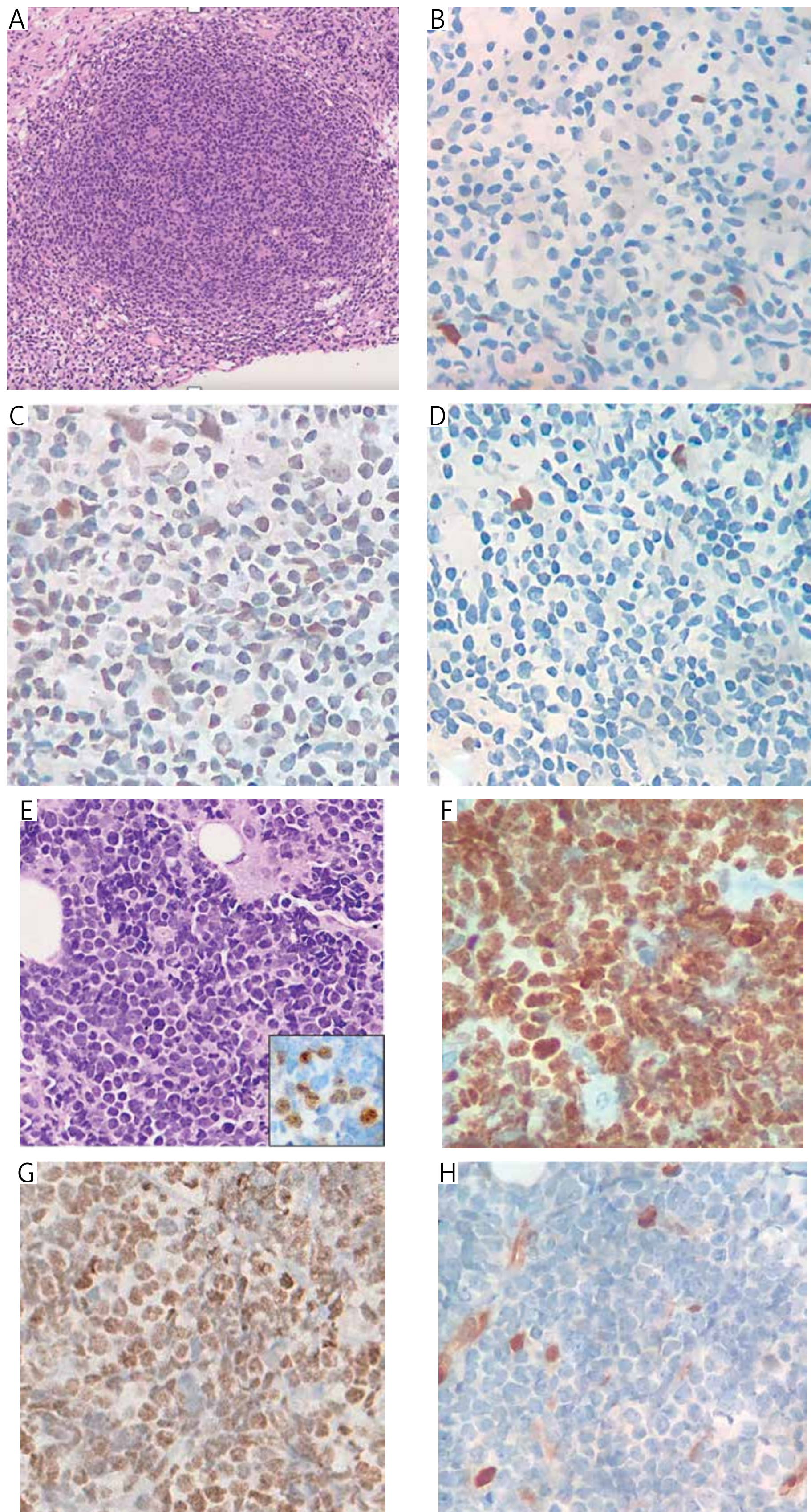

Fig. 5. Case 4. Follicular lymphoma, grade 3A. A) Predominantly centroblasts are seen with a few centrocytes (haematoxylin and eosin – H&E; 200×). B) On immunohistochemistry, neoplastic cells are negative for c-myc. C) P53 is wild type. D) Negative cyclin D1 (D) (DAB; 200×). E) Transformation to precursor B-cell lymphoblastic lymphoma showing lymphoblast with scant cytoplasm and tiny nucleoli which on immunohistochemistry are positive for terminal deoxynucleotidyl transferase (inset) (H&E; 200×, inset-DAB; 200×). F) These lymphoblasts show significant c-myc expression. G) P53 staining in wild type. H) Negative cyclin D1 (DAB; 200×)

diagnosis not aligned to clinical and radiological profiles should be carefully studied and the possibility of a transformation be kept in mind, and an additional biopsy from another site may reveal the high-grade transformation. Molecular events as described herein define the molecular basis for such transformation and may pave the way in future to engage those with targeted therapies.

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The authors declare no conflict of interest.

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