Pediatric follicular lymphoma – a clinico-pathological study of a population-based series of patients treated within the Non-Hodgkin's Lymphoma - Berlin-Frankfurt-Münster (NHL-BFM) multicenter trials

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ABSTRACT

Background

Pediatric follicular lymphoma has recently been recognized as a novel variant of follicular lymphoma in the World Health Organization classification of lymphomas. Given the rarity of the disease, histopathological and genetic data on this type of lymphoma are still scarce.

Design and Methods

We analyzed 25 cases of pediatric follicular lymphoma (patients aged ≤18 years) by morphology, immunohistochemistry and interphase fluorescence *in situ* hybridization. All patients analyzed were treated within Non-Hodgkin's Lymphoma - Berlin-Frankfurt-Münster (NHL-BFM) multicenter trials, and the cohort was representative of the German population.

Results

The genetic hallmark of adult follicular lymphoma, t(14;18)(q32;q21), was not detectable in any of the pediatric cases, although BCL2 protein was expressed in 55% of the latter cases. No correlation was found between BCL2 protein expression and outcome. Chromosomal breaks in the immunoglobulin heavy chain gene (*IGH*) and the *BCL6* locus were detected in 5 of 17 and 1 of 18 cases, respectively. Patients with pediatric follicular lymphoma had long event-free survival and, in contrast to adult follicular lymphoma, the clinical course was not dominated by relapses. A simultaneous diffuse large B-cell lymphoma was frequently detected at initial diagnosis in children but did not indicate an aggressive clinical course.

Conclusions

Our data suggest that pediatric follicular lymphoma is a disease that differs from its adult counterpart both genetically and clinically.

Key words: pediatric follicular lymphoma, childhood lymphoma, pediatric diffuse large B-cell lymphoma, t(14;18).

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Introduction

The vast majority of B-cell non–Hodgkin's lymphomas in children and adolescents are aggressive lymphomas, predominantly Burkitt's lymphomas and diffuse large B-cell lymphomas (DLBCL). Follicular lymphomas (FL), although frequent in adults, are rare in children and adolescents and account for not more than 2% of non-Hodgkin's lymphomas in this age group.[1] Moreover, pediatric FL differ genetically from their adult counterpart.[2] The translocation t(14;18)(q32;q21), juxtaposing *BCL2* next to the immunoglobulin heavy chain gene (*IGH*) is detectable in more than 80% of adult FL, but was reported to be rare in pediatric FL.[2] The rarity of pediatric FL and the absence of the typical diagnostic features of adult FL, such as *BCL2* aberrations, render the diagnosis of pediatric FL challenging.[3,4] In adults the majority of FL are indolent, low-grade lymphomas of grade 1 or 2 according to the World Health Organization (WHO) classification.[1,5] In adults transformation of a low-grade lymphoma to a high-grade lymphoma, usually a DLBCL, is accompanied by rapid clinical progression and an unfavorable prognosis.[6,7] However, clinicopathological studies addressing the question of a simultaneous DLBCL component and outcome of FL in children have not been published so far. We have recently shown that pediatric DLBCL differ from adult DLBCL with regard to prognosis, immunophenotype and genetics.[8]

In this study, we characterized a population-based series of 25 FL in patients aged 18 years or under, using morphological examination, immunohistochemistry studies and fluorescence *in situ* hybridization (FISH). We examined genetic aberrations such as breaks in the *IGH*, *MYC* or *BCL6* loci which, remarkably, had not been studied in a larger series of pediatric FL. We evaluated patients who were treated uniformly within clinical trials of the Non-Hodgkin's Lymphoma – Berlin-Frankfurt-Münster (NHL-BFM) group.

Design and Methods

Patients

All pediatric patients (≤18 years) from Germany with a diagnosis of FL treated in the three consecutive NHL-BFM group multicenter trials, NHL-BFM 90, NHL-BFM 95 and B-NHL BFM-04, were identified. The treatment protocols of these three trials included a common backbone of chemotherapy and outcomes over the last 20 years within these trials were comparable.[9-11] The patients' disease was staged according to the St. Jude's Hospital system for childhood non-Hodgkin's lymphomas.[12] All biopsies were performed at first diagnosis before any treatment. The size and quality of the biopsy specimens, the tissue processing protocols and the paraffin blocks were heterogeneous. All cases were reviewed by at least two expert pathologists and classified according to the WHO classification.[5] Since virtually all pediatric patients with lymphoma in Germany are registered and followed by the BFM group, the samples we analyzed can be considered to have been a representative population-based cohort for Germany.[13]

The study was carried out in compliance with local ethical guidelines and the ethical guidelines of the studies in which the patients were treated. The scientific studies were done with informed consent of all parents.

Immunohistochemistry and interphase cytogenetics

Immunohistochemical studies were performed as described previously.[8] Lymphoma samples were scored positive for BCL2, BCL6, CD5 and CD10 if more than 25% of the tumor cells stained positive. Immunohistochemical staining for Ki-67 was assessed as percent of positive tumor cells. Interphase FISH for the detection of breakpoints affecting the *IGH*, *BCL2*, *BCL6* and *MYC* loci or fusions of *BCL2* and *IGH* was carried out on paraffin sections of tumor tissues using commercially available probes (Abbott/Vysis, Downers Grove, IL,USA). In one case with a simultaneous *IGH* and *BCL6* break, a home-made *IGH-BCL6* double-color double-fusion probe was also applied. FISH was performed according to recently published protocols.[14]

Statistical analysis

The duration of event-free survival is defined as the time from diagnosis until the date of the first adverse event (tumor failure, death from any cause or the development of a second malignancy), or, if no such event occurred, until the date of latest contact. Probabilities of event-free survival were estimated by the method of Kaplan and Meier, with standard errors according to Greenwood, and were compared using the log-rank test.[15] Differences in the distribution of individual parameters among subsets of patients were analyzed using the χ^2 test or Fisher's exact test. The statistical analyses were carried out using SAS (SAS-PC, Version 9.1, Cary, NC: SAS Institute Inc.).

Results

Clinical characteristics

The patients with pediatric FL in our cohort were predominantly male [17 of 25 (68%)] with a median age of 11 years (range, 1-17 years) (Table 1). Pediatric FL presented frequently as localized disease (36% stage I, 40% stage II, 20% stage III and 4% stage IV), and in six patients the diagnostic biopsy represented a complete resection of the lymphoma. Cervical lymph nodes (21 of 25) were the most frequent nodal presentation and the tonsil the most frequent extranodal manifestation. Interestingly, we did not detect any case with testicular involvement. In no cases was bone marrow or bone involved. Central nervous system involvement occurred in one patient with simultaneous DLBCL. Relapse of the disease was described in only one patient, a girl who suffered from the Nijmegen breakage syndrome. Two patients had lactate dehydrogenase concentration greater than 500 IU/L. The major clinical findings are summarized in Table 1.

Histopathology

All biopsy specimens were re-evaluated by two hematopathologists according to the 2008 WHO lymphoma classification.[5] The pediatric FL were usually composed of large expanded follicles, which displayed architectural features of atypia such as reduced follicle mantles, missing compartmentalization of the follicles into a dark and a light zone or discordant number of large cells and tingible body macrophages (Figure 1). In contrast to reactive follicular hyperplasia, in which a spectrum of variably

sized follicles in different functional stages is usually seen, follicles were often homogeneous in size, crowded, frequently back to back and sometimes confluent. In same cases a so-called floral pattern was present (Figure 1). The "starry sky picture", normally a feature of reactive follicles and caused by tingible body macrophages, was often preserved, especially in areas of high-grade disease. The follicular growth pattern was seen throughout the infiltrated tissues in most cases. Thus, in infiltrated lymph nodes or the tonsils the neoplastic follicles exceeded the physiological B-cell compartments, i.e. the cortical area in lymph nodes and the subepithelial area in the tonsils.

In several cases small, pre-existing reactive follicles were detectable at the borders of the infiltration. Within the neoplastic follicles cytologically typical centroblasts and centrocytes without atypical features were seen. The criteria of the current WHO classification were used to grade FL according to the number of large cells and to assess a DLBCL component. In all our cases a predominant grade 3 pattern (> 15 centroblasts per high power field of the microscope) was present. Of the 25 FL evaluated, 15 (60%) were classified as grade 3a (FL 3a) and 10 (40%) as grade 3b (FL 3b). Areas of grade 1 or 2 FL as a second lymphoma component were detectable only in cases of FL 3a (4 cases, representing 16% of all FL and 27% of all FL 3a, Table 1).

Although all biopsy specimens were obtained at initial diagnosis before any treatment, a simultaneous DLBCL component, indicated by a diffuse growth pattern[5] within the same biopsy, was noted in nine (36%) of the cases. In the majority of our cases of FL with a DLBCL the follicular component dominated the biopsy and usually there was a gradual transition from follicular growth into the diffuse pattern with ill-defined borders between the two components (Figure 1). Nevertheless, in order for a simultaneous DLBCL to be diagnosed, a considerable area with diffuse lymphoma growth had to be present.[5] The DLBCL component was more frequently associated with a FL 3b (6 of 10) than a FL 3a (3 of 15) although this association did not reach statistical significance (P=0.09, Fisher's exact test). There were no statistically significant differences in clinical, immunhistochemical or genetic data between FL 3a and FL3b (Table 1). The DLBCL component was classified as centroblastic subtype in all cases (Table 1).

Table 1. Characteristics of the patients.

Patient N.	Age at diagnosis	Gender	Diagnosis	2nd lymphoma component	Stage	Localization
1	14	m	FL 3a	–	I (completely resected)	LN (c)
2	6	m	FL 3a	DLBCL	III	LN (c, med)
3	9	m	FL 3a	DLBCL	I (completely resected)	LN (c)
4	8	m	FL 3a	–	II	LN (c)
5	9	f	FL 3a	DLBCL	III	T, LN (c, ax, ab), liver, intestine
6	11	m	FL 3a	FL 1	I	LN (c)
7	10	f	FL 3a	–	I (completely resected)	LN (c)
8	12	m	FL 3a	–	II	T, LN (c)
9	12	f	FL 3b	–	II	T, LN (c)
10	15	m	FL 3b	DLBCL	III	LN (ab), intestine
11	1 (nearly 2)	f	FL 3b	DLBCL	II	LN (unknown)
12	7	m	FL 3b	DLBCL	IV	CNS, LN (c)
13	8	m	FL 3b	DLBCL	II	T, LN (c)
14	11	f	FL 3b	DLBCL	I (completely resected)	LN (c)
15	16	m	FL 3a	–	II	LN (c)
16	10	f	FL 3a	–	III	LN (c, ax, med, ab, ing)
17	8	m	FL 3a	FL 2	II	Lk (ab, ing)
18	16	m	3a	FL 2	I (completely resected)	LN (c)
19	13	m	FL 3b	–	II	LN (c), parotic gland
20	8	m	FL 3b	–	I (completely resected)	LN (c)
21	17	m	FL3a	–	I	LN (c)
22	15	m	FL3b	–	II	LN (supra- and infraclavicular, ax)
23	6	f	FL3b	DLBCL	II	LN (c)
24	15	m	FL3a	–	III	LN (c, ing), liver, spleen
25	15	f	FL3a	FL2	I	LN (c)

Patient 12 suffered from a Nijmegen-breakage syndrome; m: male; f:female; FL: follicular lymphoma; DLBCL: diffuse large B-cell lymphoma; LN: lymph nodes; T: tonsil; c: cervical, including nuchal and submental; ing: inguinal; med: mediastinal; n: nuchal; s: sumbental; ax: axillary; ab: abdominal.

Immunophenotype

In order to distinguish a pseudo-follicular growth pattern occasionally observed in DLBCL from the follicular growth pattern of a FL, the presence of follicular dendritic cell meshworks was demonstrated in all cases by staining for CD23 (*data not shown*). Interestingly, in cases with simultaneous FL and DLBCL, both lymphoma components generally had the same immunophenotypic profile (*data not shown*).

All pediatric FL in our series were positive for CD20. CD5 expression was only detected in one case (Table 2). The expression of Ki-67 was variable, but overall high, ranging from 40 to 95% (Table 2). The germinal center markers CD10 and BCL6 were expressed in the majority of cases [CD10 in 17 of 21 evaluable cases (81%) and BCL6 in 15 of 16 evaluable cases (94%)].

We used staining for CD5 to highlight small, reactive T cells, present in the interfollicular areas and within some neoplastic follicles. The amount of physiologically BCL-2 expressed by T cells within the lymphoma follicles was considered when estimating the percentage of BCL2-positive lymphoma cells. This was generally easily applicable as only BCL2-positivity in large cells was evaluated whilst the CD5 staining was confined to small T-cells. Using this approach BCL2 expression of the lymphoma cells was detectable in roughly half of the samples (12 of 22, 55% of evaluable cases, Table 2).

Molecular cytogenetics

Genetic aberrations were studied using FISH to detect breaks in *IGH*, *BCL2*, *BCL6* and *MYC*. We did not detect any fusions of *BCL2* and *IGH* (0 of 17 evaluable cases, data not shown) or breaks in *BCL2* (0 of 18 evaluable cases), indicating that the translocation t(14;18)(q32;q21) was absent in this series. However, a gain (3-4 copies) of *BCL2* was detected in one case (case 25, Table 2). We found no breaks of *MYC* in our series (0 of 15 evaluable cases), whereas breaks in *IGH* occurred in 5 of 17 (29%) evaluable cases (Table 2, Figure 1). One of 18 evaluable cases showed a *BCL6* break but a fusion with the *IGH* gene was not detected although this case did show a *IGH* break (case 16, Table 2). Although there are reports of the presence of trisomy 3 and/or *BCL6*/3q27 gains in t(14;18)-negative FL, we detected no gains of *BCL6*/3q27 in our series using a BCL6 break-apart probe. Considering the cases in which any break was found, 5 of 17 FL with evaluable FISH results for at least the *IGH* and *BCL6* assay displayed chromosomal aberrations (Table 2). The tumor cell content in the cases with detectable chromosomal aberrations did not differ from that in cases without aberrations (*data not shown*).

Correlations with clinical characteristics and outcome

In our series of 25 pediatric FL followed for a median of 6.1 years (range, 0.2 to 10.2 years), only one patient (case 12, Tables 1 and 2) suffered from a relapse. The estimated probability of 5-year event-free survival was 96±4%. Given this excellent outcome of the whole series of patients, with only one event, no significantly different outcomes for FL with a DLBCL component, with chromosomal aberrations or with BCL2 positivity could be demonstrated (*data not shown*). However, four of 12 (47%) of the BCL2-positive lymphomas were in an advanced stage (stage III or IV), whereas only one of the ten BCL2-negative lymphomas was stage III. In addition, FL with detectable FISH aberrations more often presented in a higher stage (> stage II) than FL without detectable chromosomal aberrations (3 of 5 and 2 of 12 cases, respectively). However, neither association was statistically significant (Fisher's exact test).

Discussion

Pediatric FL is a rare disease. A recently published review,[4] and a review by our group of the literature indicated that fewer than 100 cases of FL in children have been reported so far.[2,3,16-21] To our knowledge, our analysis represents the largest series of pediatric FL published so far and our cohort can be considered representative for Germany. In addition, all patients in this study were treated similarly within trials of the NHL-BFM group.

Pediatric FL often present in localized stages with involvement of cervical lymph nodes and the tonsil, a feature that has been recognized previously.[2,5] Interestingly,

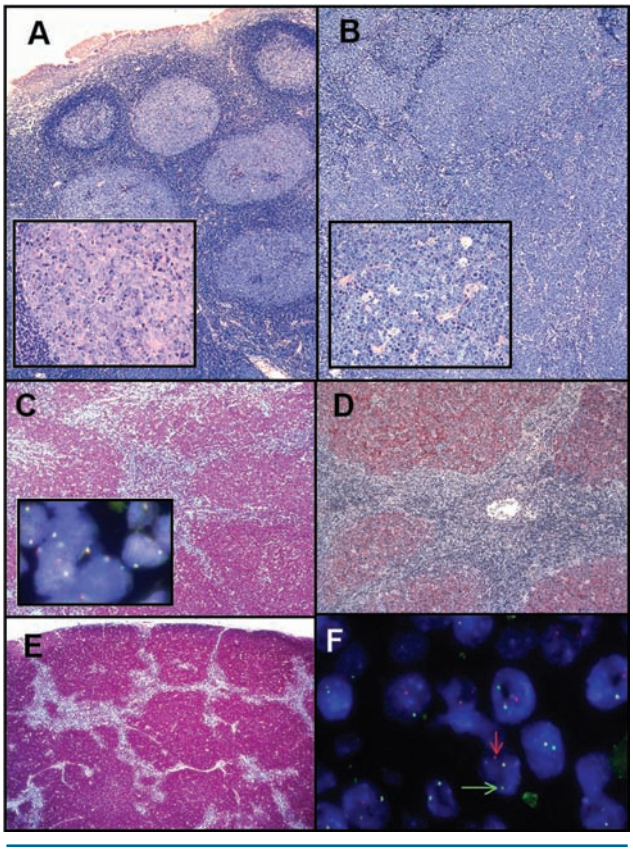

Figure 1. Follicular lymphoma grade 3a involving the tonsil (A) with a simultanous DLBCL component in deeper parts of the tumor (B) with higher magnification in the inserts, (A+B Giemsa stain). Strong expression of BCL2 (C) in the absence of *BCL2* breaks (insert in C) and dense networks of follicular dendritic cells positive for CD23 (D), A-D correspond to case 5 in Tables 1 and 2). A *floral* follicular growth pattern in case 12 highlighted by staining for CD10 (E). Breaks in the *IGH* gene of case 13 (F, arrows indicate the split signal).

although there are well documented cases of testicular FL in children,[2,21] we did not find any FL with a manifestation in the testis in our population-based series. Thus, it is possible that the frequency of testicular FL has been overestimated, since the published series might represent highly selected case series. Alternatively this specific testicular disease might be dependent on epidemiological factors that are less prevalent in western Europe.

The immunohistochemical profile of pediatric FL has been described before.[2] Our data confirm that the majority of cases of pediatric FL express the germinal center marker CD10. However, the percentage of BCL2-positive cases was higher in our series than in the series analyzed so far.[2] The differences between the studies might be due to different immunohistochemical techniques or evaluation criteria. We used a threshold of 25% positive cells, which is widely accepted for the evaluation of the BCL2-staining. In our series with an excellent outcome under BFM-therapy, we did not find a correlation between BCL2 expression and outcome as was stated in the WHO classification.[5] Nevertheless there was a trend towards higher stage disease in BCL2-positive FL, indicating more aggressive tumor growth in these cases.

Using the FISH technique, we excluded the presence of fusions between the *IGH* and *BCL2* loci, which are indicative of the t(14;18)(q32;q21) translocation, in all evaluable pediatric FL. Moreover, we excluded any variant translocations affecting the *BCL2* locus by a specific break-apart probe. A previously published study demonstrated *BCL2* translocations in two pediatric FL (patients aged 13 and 17 years) using a polymerase chain reaction technique.[2] A screening of healthy individuals indicated that t(14;18)(q32;q21)-positive B cells are detectable in the peripheral blood starting from the age of 10 years.[22] Thus, positive polymerase chain reaction results suggestive of a t(14;18)(q32;q21) in pediatric patients should be validated by an *in situ* technique, such as FISH, to prove that the translocation is a feature of the lymphoma cells.

The lack of *BCL2* translocations raises the question of the molecular mechanisms involved in the pathogenesis of pediatric FL. We were able to demonstrate recurrent aberrations of *IGH* in pediatric FL. Although, the translocation partner of *IGH* remains to be determined, our finding might point the way towards a understanding of the genetic basis of pediatric FL. Furthermore, our genetic findings might be of help in a diagnostic setting.

Table 2. FISH and immunohistochemical results.

			FISH					Immunohistochemistry			
Patient N.	Diagnosis	2nd lymphoma component	BCL2	BCL6	MYC	IGH	Ki-67 (%)	BCL2	BCL6	CD5	CD10
1	FL 3a	–	nd	nd	nd	+	80	nd	nd	–	–
2	FL 3a	DLBCL	–	–	–	–	70	+	+	–	+
3	FL 3a	DLBCL	–	–	–	–	nd	–	+	–	+
4	FL 3a	–	–	–	nd	-	60	–	+	–	+
5	FL 3a	DLBCL	–	–	–	+	50	+	+	+	+
6	FL 3a	FL 1	–	–	–	–	60	–	–	–	+
7	FL 3a	–	nd	nd	nd	nd	nd	nd	nd	nd	nd
8	FL 3a	–	–	–	nd	–	45	+	nd	–	–
9	FL 3b	–	nd	nd	nd	nd	75	–	nd	nd	nd
10	FL 3b	DLBCL	–	–	nd	+	45	–	+	–	+
11	FL 3b	DLBCL	–	–	–	–	90	+	+	–	+
12	FL 3b	DLBCL	–	–	–	nd	60	+	+	–	+
13	FL 3b	DLBCL	–	–	–	+	60	+	+	–	+
14	FL 3b	DLBCL	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	FL 3a	–	nd	nd	nd	nd	80	-	nd	–	+
16	FL 3a	–	–	+	–	+	70	+	+	–	nd
17	FL 3a	FL 2	–	–	–	–	60	+	+	–	+
18	FL 3a	FL 2	–	–	–	nd	60	+	+	–	+
19	FL 3b	–	nd	nd	nd	nd	85	–	+	–	+
20	FL 3b	–	nd	nd	nd	nd	65	–	+	–	+
21	FL3a	–	–	–	–	–	50	–	nd	nd	+
22	FL3b	–	–	–	–	–	30	+	+	–	-
23	FL3b	DLBCL	–	–	–	–	95	–	nd	nd	+
24	FL3a	–	–	–	–	–	40	+	nd	nd	–
25	FL3a	FL2	nd	–	–	–	40	+	+	–	+

FISH: fluorescence in situ *hybridization; - : no break, +: break for FISH results, -: negative, +: positive for immunohistochemistry for BCL2, BCL6, CD5, CD10 with 25% of tumor cells as a cut-off; nd: not determined.*

Considering the fact that some lymphomas are immunohistochemically negative for BCL2, the diagnosis of pediatric FL, especially the distinction from atypical hyperplasia, can be quite challenging.[3,4] Analyses of clonal rearrangements of *IGH* or monoclonal light chain expression are useful tools for the differential diagnosis of FL from atypical hyperplasia. However, clonality does not definitely distinguish between atypical hyperplasia and lymphoma.[3,23]

The diagnosis of FL is based primarily on the recognition of atypical follicles, as described above. In addition to the morphological criteria several features assessed in our study can be considered as reliable markers of malignancy that are useful for the differential diagnosis: (i) the presence of any chromosomal aberration, (ii) a DLBCL component, and (iii) immunohistochemical expression of BCL2 in centroblasts or centrocytes (using any level of expression, including cases with <25% positive cells). In our cohort, 23 cases showed at least one of these markers of malignancy, and the diagnosis of FL was based on morphological features alone in only two cases (cases 7 and 21). Unfortunately, in these two cases VDJ rearrangement analysis of *IGH* was not assessable.

In adults FL is considered an incurable disease and thus its clinical course differs from that of DLBCL if treated with comparable therapy.[24-27] However, in children, the outcome of both diseases, FL and DLBCL, is equivalent if the patients are treated according to the NHL-BFM protocols for mature B-cell non-Hodgkin's lymphoma.[8] Relapses, which are frequent in adult FL, are rare in children. More differences in the clinical course between children and adults became evident in our study. In our cohort of pediatric FL a large proportion (36%) of patients had a DLBCL as a second lymphoma component at the time of the primary biopsy. In adults the presence of a DLBCL component is considered to be a transformation of the FL. The rate of transformation of adult FL is low and was estimated to be in the range of 3% per year.[7] Transformation in adults is usually associated with a very poor outcome.[6,7] Here again, pediatric FL differs from the adult counterpart, in that the presence of a DLBCL component does not indicate an adverse outcome.

In summary, our data suggest that pediatric FL is a disease that differs from the adult counterpart genetically and clinically. The genetic hallmark of adult FL, the translocation t(14;18)(q32;q21), is typically not detectable in pediatric FL. The molecular mechanisms involved in the pathogenesis of pediatric FL still remain to be elucidated. The outcome of pediatric FL is excellent and, in contrast to adult FL, the clinical course is not dominated by relapses, if the pediatric FL are treated according to NHL-BFM protocols. Furthermore, in pediatric FL a simultaneous DLBCL can frequently be detected at initial diagnosis but does not indicate a more aggressive clinical course in children.

Authorship and Disclosures

IO, RS, and WK contributed to the conception and design of the study, acquisition, analysis and interpretation of the data, drafting the article and revising it critically for important intellectual content. IS, FM, MK and SG analyzed and interpreted FISH data and revised the draft of the article. BB, WW, AM MZ and AR provided clinical data, performed the statistical analyses and revised the draft of the article. The order of authorship reflects the contribution of each author to the design of the study, data interpretation and writing of the manuscript. All authors approved the last version of the manuscript.

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