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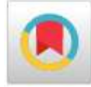
Research 

No association between *BCR-ABL1* fusion genes and clinical features of acute lymphoblastic leukaemia in Ghanaian patients

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No association between *BCR-ABL1* fusion genes and clinical features of acute lymphoblastic leukaemia in Ghanaian patients

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Abstract

Introduction: *The BCR-ABL1 fusion gene has been associated with poor prognosis in acute lymphoblastic leukaemia (ALL). This study was designed to determine the presence, frequency and associated laboratory and clinical features of the BCR-ABL1 gene in Ghanaian patients diagnosed with ALL.* ***Methods:*** *this was a cross-sectional study using archival methanol-fixed bone marrow aspirate slides of morphologically diagnosed ALL patients. Presence of the BCR-ABL1 fusion gene was determined by fluorescent in situ hybridization. Clinical features and haematological parameters were extracted from the patients´ medical records.* ***Results:*** *seventeen patients were studied, 13 (76.5%) males and 4 (23.5%) females. Median age was 24 years (range 15 to 67 years). A frequency of 29.4% was obtained for the BCR-ABL1fusion gene. There was no significant association between presence of BCR-ABL1and selected clinical features (lymphadenopathy, splenomegaly and hepatomegaly). All patients had moderate to severe anaemia with median haemoglobin concentration of 7.6 g/dL (range 3.7 to 8.7 g/dL). Median haemoglobin concentration for BCR-ABL1 positive patients was higher than that for negative patients (7.6 vs. 7.4 g/dL, P = 0.506); who also had higher median white blood cell counts (24.76 vs. 13.02 X 10^9/L), but lower median platelet counts (58.0 vs. 64.5 X 10^9/L, P = 0.721) and bone marrow blast percentages (78.5 vs. 98.0%, P = 0.851) compared to negative patients.* ***Conclusion:*** *BCR-ABL1 fusion gene was detected in nearly one third of adult ALL patients in this study, with no significant association with common haematological parameters and clinical features of the disease.*

Introduction

Acute lymphoblastic leukaemia (ALL) is characterised by the presence of more than 20% leukaemic blasts in the bone marrow and specific leukaemic cytogenetic or/and molecular genetic abnormalities [1]. The chimeric *BCR-ABL1* fusion gene results from translocation of the ABL cellular oncogene on chromosome 9 to the BCR gene on chromosome 22 [2]). This results in synthesis of either a 210 kD or 190 kD protein with enhanced tyrosine kinase activity compared with the normal 145 kD protein [3,4]. The frequency of the *BCR-ABL1* fusion gene in patients with ALL ranges from 1-5% to 11-29% in children and adults respectively [5]. The two main types of the *BCR-ABL1* fusion transcripts (p190 and p210) occur in ALL but the p190 is more prevalent [6,7]. In a study of 56 adult ALL patients in the United States by the Cancer and Leukaemia Group B (CALGB), the p190 variant accounted for 77% of patients [8]. The *BCR-ABL1* fusion gene is a poor prognostic indicator in ALL and it has been associated with decreased overall and event free survival rates [9,10], with varying frequencies across different populations. The presence and frequency of this mutant gene in Ghanaian ALL patients is unknown, patients diagnosed with ALL in Ghana are not routinely screened for its presence. Treatment is therefore not modified to include tyrosine kinase inhibitors (TKIs), which are available for those who may have the mutation. Their incorporation into therapeutic protocols in other parts of the world has been associated with improved survival in *BCR-ABL1* positive ALL [11,12]. This study was designed to determine the presence, frequency and the associated laboratory and clinical features of the chimeric *BCR-ABL1* gene in patients diagnosed with ALL at the Department of Haematology, Korle Bu Teaching Hospital (KBTH).

Methods

Study design: this was a cross-sectional study using methanol-fixed archived bone marrow slides of patients diagnosed with ALL.

Study site: the study was carried out at the Department of Haematology, Korle Bu Teaching Hospital. The department provides laboratory and clinical services to patients with various haematological disorders from all over Ghana and neighbouring West African countries. About 4,800

patients are seen in the department each year, with about 10 patients diagnosed with ALL per year. Further laboratory analysis with fluorescent in situ hybridisation (FISH) was carried out at the Queen´s Laboratory for Molecular Pathology at Queen´s University, Canada.

Study population: the population consisted of ALL patients diagnosed at the study site from January 2013 to May 2017. Patients who were 15 years or older, with morphologically diagnosed ALL for whom unstained, fixed, bone marrow aspirate slides were available were included in the study. Twenty five of the 37 ALL patients seen within the study period met the inclusion criteria and were selected for the study.

Selection of samples and data collection: unstained bone marrow aspirate slides of patients that qualified for the study were retrieved from storage using laboratory numbers obtained from the haematology laboratory log book. A data abstraction form was used to obtain the following information from the patients´ medical records: clinical features at presentation: hepatomegaly, splenomegaly, lymphadenopathy and/or presence of mediastinal mass; the following laboratory variables: haemoglobin (Hb) concentration, white cell and platelet counts were obtained from full blood count (FBC) performed at the time bone marrow aspirate was taken for diagnosis. The blast cell percentages were obtained from morphology report of bone marrow aspirate smears.

Materials and methods: fluorescence in-situ hybridisation technique was used for the detection of the *BCR-ABL1* fusion gene in the archived bone marrow aspirate slides.

Procedure: the fluorescent in-situ hybridisation technique was performed using the protocol at Queens Laboratory for Molecular Pathology (Queens University, Canada) as described below.

Preparation of positive and negative control smears: a positive control smear was prepared from a cell culture of a commercially prepared *BCR-ABL1* positive cell line (K-562 (ATCC ® CCL -243)). A peripheral blood smear prepared from an anonymous *BCR-ABL1* negative volunteer provided by the Queens Laboratory for Molecular Pathology was used as a negative control slide. Both slides were fixed in methanol for 3 minutes.

DNA unmasking: the slides were immersed in methanol for 1 minute followed by incubation in 2 x Saline-Sodium Citrate (SSC) at room temperature for 5 minutes. The slides were then incubated in 0.2N HCl for 5 minutes. A total of 500 uL of pepsin was added to the pre-warmed 49.5 mL of 0.01N HCl in water bath at 37°C. The resulting mixture was mixed well. The slides were taken from the 0.2N HCl solution and excess liquid removed with a paper towel and immediately immersed into the pepsin/HCl solution incubating at 37°C for 7 to 15 minutes. The slides were washed in double distilled H2O for 10 minutes followed by fixation in 1% formaldehyde for 5 minutes, immersion in 1% phosphate buffered saline for 5 minutes and sequential dehydration in 70%, 85% and 100% ethanol for 2 minutes in each solution. The slides were observed under phase contrast microscope for digestion progress (unfinished digestion is signified by the appearance of white shiny cells with undefined nuclei whereas cells with clear blue nuclei shows complete digestion). If digestion was not finished, the slides were re-immersed in pepsin/HCl solution for a longer period and the subsequent steps followed until the phase contrast microscope shows finished digestion.

Denaturation and hybridization: the slides were air dried completely prior to the addition of the probe in the hybridisation steps which is described below: the ThermoBrite Denaturation/Hybridisation system was turned on and the program set for the following parameters: denaturation time: 2 minutes; denaturation temperature: 73°C; hybridisation time: 24 hours; hybridisation temperature: 37°C. The DNA probe (Vysis LSI *BCR/ABL DC/DF* translocation probe), Vysis LSI/WCP hybridisation buffer and the purified water were removed from storage and the reagents allowed to reach room temperature. The

*BCR/ABL*DNA probe and the hybridisation buffer were vortexed for 2 to 3 seconds followed by centrifugation for 2 to 3 seconds. Seven microlitres (7 uL) of the hybridisation buffer, 2 uL of purified water and 1uL of the *BCR/ABL*DNA probe were transferred into a micro-centrifuge. The mixture was vortexed and centrifuged for 2 to 3 seconds each. A micropipette was used to apply 10 uL of the probe mixture to the target area of each slide. A cover slip was immediately applied without introducing bubbles. The coverslip was sealed using a syringe filled with rubber cement. Two ThermoBrite humidity cards saturated with distilled water were inserted into the slot positions in the unit lid of the ThermoBrite hybridisation/denaturation system. The slides were placed on the heating surface of the ThermoBrite Hybridisation/Denaturation system when prompted and was ensured that, the frosted edge of the slide hanged over the heating surface, lay flat and properly aligned into the marked positions in the slide locator. The ThermoBrite lid was closed and the program was started for denaturation and hybridisation to occur overnight.

Post-hybridisation wash: the post-hybridisation wash was carried out according to the following procedure: The room was darkened and the coverslip removed from the slide by peeling off rubber cement. The slides were incubated in 2x SSC/0.3% IgePal solution (180 uL IgePal in 60 mL 2 x SSC) at 73°C for 2 minutes. The temperature was increased by 0.5°C for each slide if the slides were more than one. The slides were washed in 2x SSC for 5 minutes and air-dried in upright position under foil cap.

Counterstaining: counterstaining was carried out by the application of 10 uL DAPI to the middle of each slide. Coverslip was applied and air bubbles pushed out. They were stored in the dark at - 20°C until fluorescent microscopy was carried out in the following step.

Fluorescent microscopy: the fluorescent microscopy room was darkened and immersion oil added to the slides. They were then observed under the fluorescent microscope using the spectrum orange, spectrum green and the dual filter which allows the visualisation of ABL1, BCR and BCR/ABL1 gene respectively. A total of 100 interphase nuclei were scored for each slide. Images of the slides were captured using the imaging software GenASIs FISH view and processed using Case Data Manager (CDM). The cut-off point for *BCR-ABL1* positivity was 3% and 15% for double fusion and single fusion respectively (values for fluorescent signals were compare to normative cut-off database at the QLMP). Representative images are shown in Figure 1 and Figure 2.

Data handling: study cases were assigned unique identification numbers, these were used to label the respective bone marrow aspirate slides and also for subsequent data processing. Data was stored on a password protected computer. The names of subjects were not used in the data but were however kept in a different file. Only the Investigators had access to data obtained from the study.

Statistical analysis: the data was entered into Microsoft Excel and exported to Statistical Package for the Social Sciences (SPSS version 20) for analysis. Data was expressed using summary and descriptive statistics such as frequency, percentages and median as appropriate and presented in tabular form. Chi Square, Fisher exact test and non-parametric test (Mann-Whitney U-test) were used to assess the association between categorical factors. The study received approval from the ethical and protocol review committee, College of Health Sciences, University of Ghana, No. CHS-Et/M.S-P 4.2/2016/2017.

Results

Bone marrow aspirate slides from 25 patients morphologically diagnosed as ALL were available for study. FISH was successfully carried out on 17/25 (68%) slides as 8/25 (32%) slides were not suitable for processing due to poor storage and deterioration.

FISH results and frequency of the *BCR-ABL1* gene in samples: five out of seventeen patients (29.4%) were positive for the *BCR-ABL1*gene. Of the 5 positive cases, 4 were double fusion and one had single fusion. The percentage scores for positive cases of the gene were 46%, 37%, 9% and 8% for double fusion and 20% for single fusion (Table 1).

Selected FISH images: Figure 1 shows a fluorescent photomicrograph of the negative control slide (A) and a negative patient (B). No fusion signals are present. Figure 2 shows the fluorescent photomicrograph of a positive control slide (A) and a positive patient slide (B). Double fusion signals which appear yellow are seen in the lower left and topmost nuclei (A). Double fusion signals (either yellow dots or orange and green dots in juxtaposition) are seen in the two nuclei in the middle (B).

Key to interpretation of images: *BCR-GENE* (locus 22q11.2) - green signals (dots); *ABL GENE* (locus q34.1) - orange signals (dots); *BCR-ABL1* fusion gene - orange and green signals in juxtaposition or yellow signal are seen for *BCR-ABL1* positive cases. Double fusion cases have 2 signals whereas 1 signal is seen single fusion cases or may represent proximity of signals due to chance). *BCR-ABL* negative cases show separated green and orange signals but no fusion signals).

Demographics

In the study 76.5% (13/17) of the samples were from males and 23.5% (4/17) were from females. Positive cases consisted of (25%) of females and 4/13 (30.8%) of males. The ages of participants ranged from 15 years to 67 years (median age 24 year). Participants in the study were categorized into two groups; adolescent and young adult group (AYA group, 15 to 39 years) and older adult group (40 years and older) respectively based on the guidelines of the U.S. National Comprehensive Cancer Network (NCCN) [13]. All the positive participants were in the AYA group 5/12 (41.7%).

Descriptive and inferential statistics of clinical features and *BCR-ABL1* gene: Table 2 show the frequencies of clinical features and test of association with the *BCR-ABL1*gene.

Descriptive and inferential statistics of haematological parameters: association between *BCR-ABL1* Fusion Gene and Haematological Parameters There was no significant difference (using the Mann-Whitney-U test) in the median of the following haematological parameters: WBC count (13.02 vs. 24.76 X 10^9/L), P = 0.879), Hb concentration 7.4 vs. 7.6 g/dl, P = 0.506), blast percentage (94% vs. 78.5%, p= 0.851 and platelet count (64.5 vs. 58 X 10^9/L p = 0.721) between patients negative or positive for the *BCR-ABL1* fusion gene.

Discussion

The *BCR-ABL1* fusion gene was present in nearly one-third of adolescent and adult patients in our study. Additionally, our findings suggested no statistically significant association between the presence of the gene and clinical features or haematological laboratory parameters. The gene frequency of 29.4% for *BCR-ABL1* in this study is consistent with the rates of 28.3% reported from India by Chopra *et al*. [14], and 11-29% reported by Mrozek *et al*. [15] in a review of adult ALL studies from USA, United Kingdom and France. However, it is higher than a prevalence of 12.5% obtained by Ajuba *et al*. [16] in Nigeria. The difference between this study and that of Ajuba *et al*. (both conducted in West Africa) may arise from the fact that our study excluded children since the *BCR-ABL1* gene is more common in adults Mrozek *et al*. [15]. Racial differences may account for some of the disparities seen. For example, in Saudi Arabia, none of the 16 adult ALL patients investigated by El-Sissy *et al*. were positive for the gene [17] while Ariffin *et al*. in Singapore demonstrated variation in frequency between Indians, Malays and Chinese [18].

Age and gender: all the *BCR-ABL1* positive patients were in the (AYA) group, the exact reasons for this are not clear; although globally, ALL is commoner in

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children and adolescents. Ghana has a young population with almost 60% less than 24 years old and only 11% older than 50 years. This contrasts with the population structure of advanced countries such as the United States, Germany and Japan with relatively older populations where about 33%, 25% and 23% respectively are less than 24 years old whereas 34%, 40% and 45% are older than 50 years [19]. Thus, the lower proportions of the elderly in this study may reflect the relatively shorter life expectancy at birth (61.3 years in Ghana) compared to that of the United States, Germany and Japan which is 78.9, 80.8 and 83.6 years respectively [20].

Clinical Feature: lymphadenopathy was present in 41.2% of all the patients in this study of which 14.3% were *BCR-ABL1* positive whereas 23.5% of the patients studied had splenomegaly of which a 25% was *BCR-ABL1* positive. Hepatomegaly was evident in 35.7% of study participants with 20% showing *BCR-ABL1* positivity, no patient had a mediastinal mass. The frequencies of organomegaly are lower than reported in a study by Elbossaty *et al*. in Egypt in which lymphadenopathy, splenomegaly and hepatomegaly occurred in 54% of adult ALL patients [21]. However, it is consistent with the findings in Netherlands by Daenen *et al*. in which organomegaly (lymphadenopathy, splenomegaly and hepatomegaly) were less prevalent with a combined frequency of 40% in adult ALL [22]. As reported in our study, Westbrook *et al*. in a study by the Cancer and Leukaemia Group B in USA found no statistically significant association between *BCR-ABL1* positivity and these clinical features [8].

Haematological parameters: the mean white blood cell (WBC) count, bone marrow blast percentages and platelet counts were lower in *BCR-ABL1* positive participants than those who were negative. Contrastingly, the mean Hb concentration for *BCR-ABL1* positive participants was higher than in participants negative for the gene. These were in contrast to the findings by Gleiβner *et al*. in Germany in which *BCR-ABL1* positive patients had statistically significantly higher WBC count and Hb concentration than *BCR-ABL1* negative patients [6]. However, similar to Gleiβner *et al*. our study found no statistically significant difference in white cell count, platelet count, Hb concentration and blast percentage in *BCR-ABL1* positive and negative patients [6]. All the patients in this study had severe to moderate anaemia with Hb concentration ranging from 3.7 to 8.7 g/dL. Also, with the exception of one, all patients in the study (approximately 94%) had thrombocytopenia. These frequencies and degree of anaemia and thrombocytopenia is higher compared to earlier reports from Denmark and Italy [23,24]. This may result from delayed presentation of our patients. This challenge is common in developing countries such as Ghana due to inadequate number of health facilities as well as haematologists. Secondly, the prevalence of anaemia is higher in Ghana compared to high income countries. For instance, the proportion of non-pregnant women with Hb concentration below 12 g/dL in Ghana is 56% whereas in Canada, Germany, Japan and the United States of America it is 16, 18, 22 and 12% respectively [25]. The clinical outcome of most of the participants in our study could not be determined. Due to the fact that most of the patients studied 13/17 (76.5%) were lost to follow up. Our study was limited by the number of properly stored bone marrow slides available for study. About a third of the marrow slides were not suitable for processing due to poor storage.

Conclusion

We describe for the first time the detection of the *BCR-ABL1* fusion gene in Ghanaian ALL patients. With a frequency of almost 30%, the *BCR-ABL1* fusion gene is an important molecular genetic lesion in adolescent and adult ALL cases in our environment. However, it has no significant association with the clinical features and haematological parameters of the disease. We recommend a larger multicentre prospective study of *BCR-ABL1* gene in both children and adults with ALL involving the characterization of the associated

molecular signatures such as the *IKZF1* and the *PAX*genes.

What is known about this topic

- *The BCR-ABL fusion gene is a poor prognostic indicator in ALL;*
- *Incorporation of targeted therapy with TKIs improves prognosis;*
- *The frequency of the BCR-ABL 1 fusion gene frequency varies across different populations.*

What this study adds

- *The detection of the BCR-ABL1 fusion gene is described for the first time in Ghanaian ALL patients;*
- *The gene was only seen in adolescents and young adults;*
- *From our findings there is no statistically significant association between presence of the gene and clinical features or haematological laboratory parameters.*

Competing interests

The authors declare no competing interests.

Authors' contributions

VOO, ABAK and EO designed the study. VOO carried out the bench work under the supervision of SC. VOO and EO analysed the data. VOO and EO wrote the first draft. All authors participated in writing subsequent drafts, revised it critically and approved the final version before submission.

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Tables and figures

Table 1: features of *BCR-ABL1* positive patients

Table 2: association between *BCR-ABL1* fusion gene and clinical features

Figure 1: fuorescent photomicrograph of a negative control slide (A) and a negative patient slide (B)

Figure 2: fluorescent photomicrograph of a positive control slide (A) and a positive patient slide (B)

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Table 1 : features of BCR-ABL1 positive patients					
	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Age (years)	16	24	36	16	15
Sex	Female	Male	Male	Male	Male
Blast percentage	36	95	-	66	99
WBC count (X 109/L)	1.60	88.4	67.04	24.76	2.33
Platelet count(X 109/L)	12	109	27	67	58
Haemoglobin concentration (g/dL)	5.8	8.5	8.5	7.6	5.9
Lymphadenopathy	No	No	No	No	Yes
Splenomegaly	No	No	No	Yes	No
Hepatomegaly	No	No	No	Yes	No
Treatment outcome	Undetermined	Undetermined	Undetermined	Undetermined	Death
Fusion pattern	Double	Double	Double	Double	Single
Percentage of fusion signal	37	8	46	9	20

Table 2: association between *BCR-ABL1* fusion gene and clinical features

Clinical Feature		*BCR-ABL1* RESULTS		Total	P-value (Fisher exact test)
		Negative	Positive		
LYMPHADENOPATHY	No	6	4	10	0.338
		60%	40%	100.0%	
	Yes	6	1	7	
		85.7%	14.3%	100.0%	
SPLENOMEGALY	No	9	4	13	0.67
		69.2%	30.8%	100.0%	
	Yes	3	1	4	
		75.0%	25.0%	100.0%	
HEPATOMEGALY	No	8	4	12	0.528
		66.7%	33.3%	100.0%	
	Yes	4	1	5	
		80.0%	20.0%	100.0%	
	Total	12	5	17	
		70.6%	29.4%	100.0%	

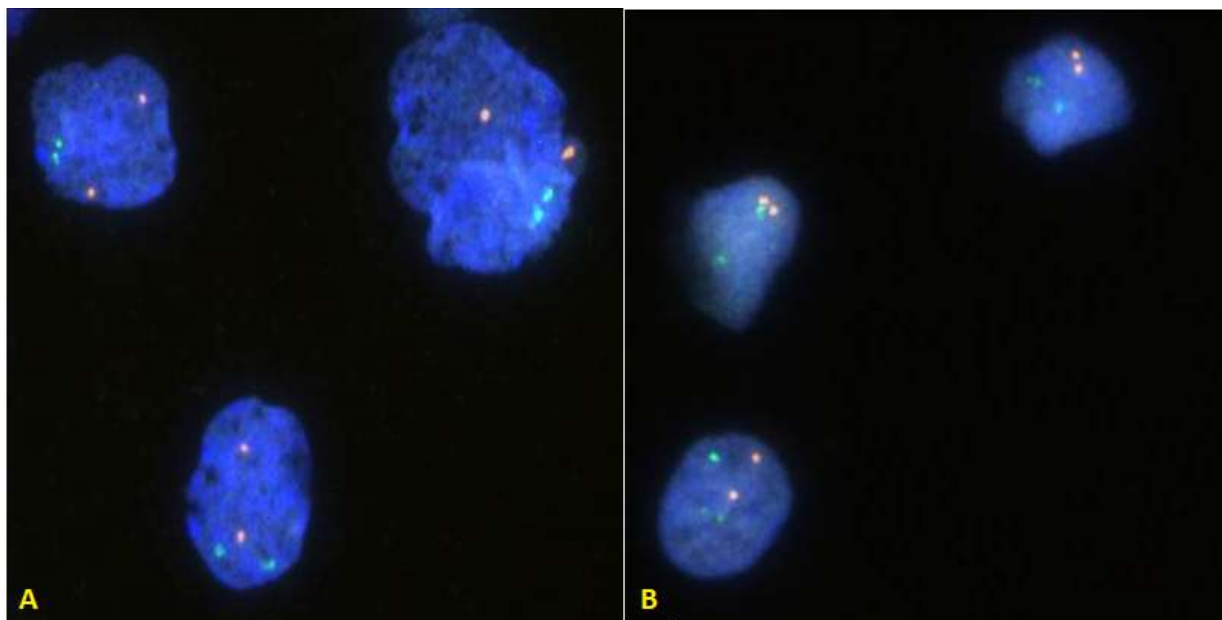

Figure 1: fluorescent photomicrograph of a negative control slide (A) and a negative patient slide (B)

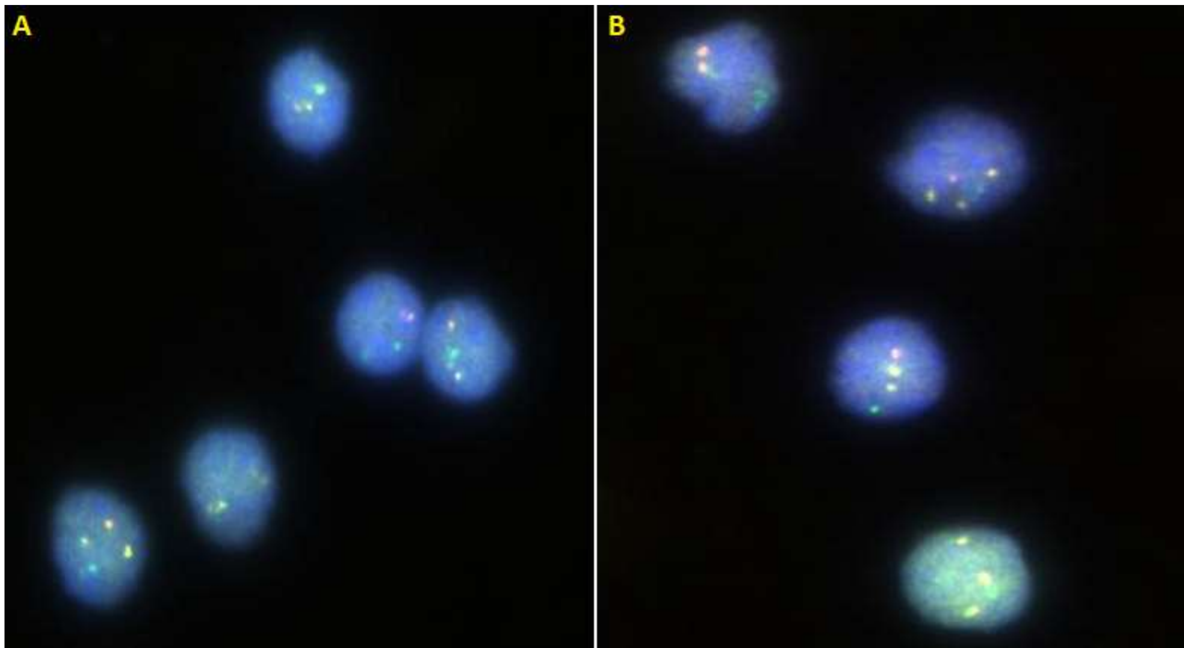

Figure 2: fluorescent photomicrograph of a positive control slide (A) and a positive patient slide (B)