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Feasibility of iFISH patterns in hematologic malignancies among Congolese patients at Kinshasa University clinics

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ABSTRACT

Objective: To analyze the feasibility of detecting Ph[1] in leukemia patients in the Kinshasa University Clinics in the Democratic Republic of Congo, at KU Leuven, Belgium.
Methods: Bone marrow and peripheral blood samples with chronic myeloid leukemia, acute myeloid leukemia or acute leukocytes leukemia were obtained from 32 patients in Kinshasa University clinics in the Democratic Republic of Congo and transferred to KU Leuven in Belgium for iFISH feasibility. Ph[1] was detected by using a remote analysis of interphase fluorescence in situ hybridization (iFISH).
Results: Out of the 32 patients involved in this study, 65.6% (n = 21) of the cases were successfully tested, of which 52.4% (n = 11) were iFISH positives for the variant t(9;22) (presence of Ph[1]) in chronic myeloid leukemia samples and 47.6% (n = 10) negatives in all subtypes of hematological malignancies. However, there was a female predominance in chronic myeloid leukemia samples Ph[1]-positives by iFISH, whereas no sexual influence was observed on acute subtypes of leukemia.
Conclusions: iFISH analysis is feasible on samples obtained from remote sites in the Democratic Republic of Congo. However, the optimization of the sample storage is necessary to further improve iFISH's performance.

1. Introduction

The emergence of heamatologic malignancy (HM) is established worldwide and vastly reported in the literature [1–6]. Recently, a collaborative initiative reported evidence of HM among patients from Kinshasa, the Democratic Republic of Congo (DRC) [7]. The burden of HM is associated with morbidity, co-morbidity, mortality and disability in the DRC [8,9]. Furthermore, the DRC is facing poverty, malnutrition, malaria, tuberculosis, HIV/AIDS and a socio-political crisis without an enabling environment, prevention strategies, early diagnosis, or efficient treatment of HM. Indeed, there is a significant lack of adequate infrastructures, activities, human resources, trained experts in laboratory medicine, and precise laboratory equipment for HM management in the DRC.

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In developed countries, cytogenetic and molecular findings are cornerstones in the current WHO classification of hematopoietic malignancies [10]. Accordingly, the clinical management of HM is strongly governed by cytogenetic and molecular analysis [1,2,4,11]. Apart from this, the Philadelphia Chromosome (Ph^1), which refers to the presence of balanced translocation between the long arms of chromosomes 9 and 22 [t(9;22)(q34;q11.2)], is a characteristic of a chronic myeloid leukemia (CML) [12,13]. To confirm this, molecular methods or fluorescence in situ hybridization (FISH) are used to detect the Breakpoint cluster region-Abelson in complex variant Ph^1 [14,15]. However, these techniques are not available at Kinshasa University Clinics in the DRC. Therefore, because FISH allows to detect recurrent translocations, and as the preservation conditions are less stringent for FISH than for karyotyping and reverse transcriptase PCR [3], we set up a feasibility study in which smears from Kinshasa clinics in the DRC were sent for interphase FISH (iFISH) analysis of t(9,22) at the University Hospitals in Leuven in Belgium.

2. Materials and methods

This was a case-series report of 32 pediatric and adult patients managed in two clinics from the city of Kinshasa between the years 2015–2016: the department of chemical pathology Kinshasa University Clinics and the Ngaliema Clinic. The variables of interest were gender, age, transfusion number, hemogram ([white cell count (WCC)/mm^3]; blast, lymphocytes, plasmocytes; platelets count/mm^3) and erythrocyte sedimentation rate/1st hour. In total, 32 patients (study population) were diagnosed with HM by routine hematologic parameters [16] and specifically investigated using FISH methods. The most commonly suspected types of leukemia were the chronic myeloid leukemia (CML) and the acute leukocytes leukemia (ALL). The average age of adult patients both females and males was 42 years, while the pediatric patients were in average 10 years old.

The present study was undertaken in compliance with the Helsinki Declaration (59th WMA General Assembly Seoul, South Korea, October 2008. http://www.wma.net/en/10 policies/b3/index.html).

2.1. Laboratory data

2.1.1. Hematologic profiles

Peripheral blood samples were obtained by venipuncture in tubes with ethylene diamine tetraacetic acid for the hemogram, and on citrate for the erythrocyte sedimentation. Bone marrow aspiration was done from the sternal bone or posterior iliac spine. Ten smears were used for morphological evaluation after staining by routine May Grünwald Giemsa; and special staining by Sudan black B, Periodic acid Schiff and Perls. The subtypes of HM followed the morphological typing according to the French-American-British WHO classification system [16].

2.1.2. Cytogenetics/FISH data

Smears were preserved at tropical ambient temperature within Kinshasa University Clinics and were sent by air from the DRC to Brussels in Belgium and by road to the inner city of Leuven. iFISH analysis was assayed at KU Leuven Hospitals between February and March 2016. Pre-prepared slides of methanol/acetic fixed bone marrow and peripheral blood cells from the cytogenetic palette stored in fixative at −20 °C and dehydrated with ethanol were used for analysis. iFISH was performed as previously described [17], using the Vysis LSI BCR, ABL extra signal Dual Color Translocation Probe Kit (Abbott Molecular, Des Plaines, IL – US).

2.2. Statistical analysis

Categorical variables were expressed as frequency (count, number = n) and proportion (%). Continuous variables were presented as means ± SD. Percentages were compared between 2 groups using non-parametric Mac Nemar *chi*-square test. A non-parametric Mann–Whitney U test was used to compare means between 2 groups. P-value < 0.05 was considered for statistical difference. All analysis was performed using SPSS software version 23.0 for Windows (IBM/SPSS Inc., New York, USA).

2.3. Consent

Fully informed and written consent was obtained from all adult participants aged ≥20 years.

3. Results

Out of this study population, 65.6% (n = 21) were successfully analyzed and 34.4% (n = 11) were not. Out of the 21 leukemia patients with excellent hybridization quality, 52.4% (n = 11) and 47.6% (n = 10) were FISH positives (presence of Ph^1) and negatives, respectively.

Among the 11 FISH positives, all progressed toward severe CML, of which 7 were females versus 4 males (sex ratio of 2 female: 1 male). In the remaining 10 cases of FISH negatives, there were 5 males and 5 females (sex ratio 1 male: 1 female) of which 4, 3 and 3 patients were suffering from acute leukocytes leukemia (ALL), acute myelogenous leukemia, and CML, respectively.

Table 1 summarizes mean values of ages, blood transfusion, WCC, platelets, erythropoiesis sedimentation rate, plasmocytic cella, blasts, and lymphocytes according to positives and negative

Table 1
Age and hematologic biomarkers levels by FISH patterns.

Variables	Positive FISH	Negative FISH	P-value
Age (years)	35.1 ± 14.6	44.1 ± 25.5	0.318
Transfusion (n)	2.1 ± 0.3	2.2 ± 2.0	0.846
WCC (mm^3)	106 348.1 ± 78 414.1	52 442.0 ± 27 825.4	0.030
Platelets (mm^3)	507 727.3 ± 499 076.4	193 500.0 ± 85 753.2	0.021
ERS (1st hour)	77.4 ± 25.0	78.0 ± 24.4	0.975
Plasmocytes (%)	0.0 ± 0.0	9.0 ± 19.8	0.131
Blasts (%)	0.0 ± 0.0	22.0 ± 32.0	0.034
Lymphocytes (%)	24.3 ± 27.2	40.0 ± 9.6	<0.000 1

Data are expressed as mean ± SD.

FISH findings. Both means of age (young adults <50 years), blood transfusion, ERS (very accelerated), and plasmocytic cells were similar ($P < 0.05$) between FISH positive and FISH negatives. As expected, and compared with FISH negatives, FISH positives presented higher levels of WCC and platelets but lower levels of blasts and lymphocytes.

4. Discussion

The present study analyzed demographics, blood transfusion, hematologic parameters and the complex variant t(9;22) Chromosome Translocation in Central Africans with leukemia. There was female predominance among CML with FISH positive findings, while there was no sex influence on acute subtypes of leukemia (ALL, CML) with FISH negative findings. In most studies in the DRC without FISH results, sex was not significantly related to acute myelogenous leukemia, ALL, CML [6,7] and this concurs with other reports in the literature [18,19]. In the DRC, female preponderance is associated with CML, while male predominance is associated with multiple myeloma and myelodysplastic syndrome [7]. Interestingly, the age group of the population (<50 years) in this study is similar to the report in the literature [6,7]. However, an exploration of complex variant t(9;22) chromosome translocations using positive iFISH patterns in this population is an enormous scientific advance.

This was also the first exploratory analysis to discover complex variant t(9;22) chromosome translocations during positive iFISH among black and Bantu Africans with a cytological diagnosis of leukemia from Kinshasa, DRC. The present study demonstrated the prognostic significance of the variant Ph^1 translocations associated with CML. Indeed, the literature confirms severe prognosis among patients with variant Ph^1 translocations [7,9,20]. During this study, the most frustrating constraint was the high proportion (34.4%) of FISH technical failures in the study population. Many reasons might explain such irrelevant results, one reason being that the smears were performed at Kinshasa University Clinics in conditions of electricity shortage. Samples and smears were therefore exposed to a tropical ambient temperature of 25 °C and high humidity from one week to three months, before being sent in batch to KU Leuven for iFISH analysis. Normally, such iFISH analysis is undertaken within days after the smear preparation, with smear storage under controlled ambient temperature [21].

Other techniques like the metaphase FISH and conventional cytogenetic analysis, which are routinely used in Western countries were not applicable in this study as they require immediate processing of the samples in a cytogenetic laboratory [22]. This study only used iFISH analysis, and was performed in a temperate climate.

For a full clinical investigation in the HM, additional applications such as the BCR/ABL extra signal dual-color technique and quantitative, real-time, reverse transcriptase polymerase chain reaction of the chimeric BCR/ABL transcript are performed. In the present study, these applications were lacking; these limitations to some degree could be useful to determine deletions of der-9 in CML [1,7,23–25]. Normally, Ph^1 translocations and deletions der-9 are associated with a worse survival [24].

The era of targeted therapies has revolutionized the treatment of HM, improving the survival of patients and at the same time their lifestyle [26,27]. In the field of targeted therapies, CML was the first hematologic disease to benefit from a targeted therapy that significantly affected the natural course of the disease with Imatinib inhibitor of activated tyrosine kinase [28]. Certainly, Imatinib is now the targeted reference therapy in CML and is systematically proposed as a first-line treatment because it is more effective, better tolerated and easier to administer (oral) than interferon [29]. Moreover, Novartis developed anti-tyrosine kinase inhibitors, a targeted therapy to be initiated early in patients with Ph^1 anomalies, and progression toward chronic blast crisis [30].

The iFISH particularly makes it possible to get rid of the cell culture of application difficult in our context. It contributes to the management of the diagnosis, and makes it possible to evaluate the prognosis of patients with HM [4,11]. Therefore, it can easily be transposed into our environment and hence be the method of choice for making a cytogenetic diagnosis. The advantages and disadvantages of these methods compared to other molecular methods are well known [22,31].

The presence of a Ph^1 in ALL is a poor prognostic factor and is a formal indication of allografting of hematopoietic stem cells (allo-HSC) in patients eligible for transplantation [32]. However, their management has been revolutionized [33] with the development of tyrosine kinase inhibitors and in this case imatinib [28]. Indeed, therapeutic strategies that combine conventional chemotherapy for ALL and a tyrosine kinase inhibitor have developed and lead to an increase in the rate of complete remissions before allografting or even to obtaining major or complete molecular responses.

The present study indicates the feasibility of interphase FISH performed at a Congolese remote site in collaboration between Kinshasa University clinics in the DRC and KU Leuven in Belgium; the importance of this collaboration for the advancement of science in the DRC cannot be overemphasized. However, this study also suggests that optimization of sample storage conditions may further improve the yield of a remote FISH analysis. This study reinforces the fact that the availability targeted and early treatment under tyrosine kinase inhibitors needs the involvement of Congolese policy-makers, pharmaceutical companies, and Non-Governmental Organizations. Further studies with a broader participation of stake holders will promote a better health to the population in the DRC.

Conflict of interest statement

There is no competing financial interest in relation to the work described.

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