

Characterization of Chromosomal Abnormalities in Acute Myeloid Leukaemia Patients at the University Teaching Hospital, Lusaka, Zambia

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ABSTRACT

Introduction: Acute myeloid leukaemia (AML) is a highly malignant clonal hematopoietic disease caused by both inherited and acquired genetic alterations (Song et al, 1999). Current AML classification and prognostic systems incorporate genetic information but are limited to known abnormalities that have previously been identified with the use of cytogenetics, array comparative genomic hybridization (CGH), gene-expression profiling, and the resequencing of candidate genes. At diagnosis, most patients with AML harbour at least 1 chromosome aberration in their marrow blasts. Numerous recurrent structural and numeric cytogenetic aberrations have been identified and many of them not only are diagnostic markers for specific AML subtypes but also constitute independent prognostic factors for attainment of complete remission (CR), relapse risk, and overall survival (OS) (Mro'zek et al, 2007). With the targeted cytogenetic therapy, 30% of the patients achieve long-term cure. At University Teaching Hospital (UTH) however, the current diagnostic approach of acute leukaemia involves mainly cytomorphology and occasional flow cytometry. The cytomorphological blast characterization is not enough to provide a critical determination of prognosis and developing a treatment plan. Most of the AML patients at the UTH die within few months after diagnosis despite being put on chemotherapy. Cytogenetic analysis is not done despite the cytogenetic abnormalities being the major predictors of favourable, intermediate or adverse prognosis.

Aim: To characterize acute myeloid leukaemia (AML) according to WHO 2008 revised classification in patients at the University Teaching Hospital.

Design and Methods: This was a descriptive cross-sectional study conducted to characterize acute myeloid leukaemia (AML) according to WHO 2008 revised classification in patients at the UTH. Patients with AML were simultaneously analyzed for the presence of 4 genetic abnormalities, PML/RAR α for t(15;17), AML1/ETO for t(8;21), CBF β /MYH11 for inv(16)/t(16;16) and

rearrangements of the MLL gene for 11q23 abnormalities. AML was classified using the new World Health Organization (WHO) classification for haematologic malignancies. The techniques used were standardized according to the recommendations of the European BIOMED-1 Concerted Action.

Results: The overall frequency of leukemia displaying one of the four recurrent cytogenetic translocations were 13 cases (46.5%) of which PML/RAR α transcript was present in six(6) patients (21.4%) (3 were bcr1, 1 bcr2 and 2 bcr3). The AML1/ETO fusion transcript was detected in only one(1) case (3.6%) with M2 morphology, but other cases with M2 morphology were negative. CBF β /MYH11 transcript was present in 2 cases (7.1%) and some of them displaying M4Eo morphology. Finally, 4 cases (14.3%) showed rearrangements of the MLL gene. By contrast, the frequency of AML not otherwise characterized which was 15 cases (53.6%) increased with age (13% for 6-35years age group, 20% for 36-65years age group and 67% for above 66years age group). Our results differ from those reported from the United States and North/Central Europe, particularly regarding the incidence of t(15;17) and t(8;21) translocations. In Zambia the frequency of t(15;17) is higher while that of t(8;21) is lower. This supports the view that geographic variations in tumor-associated aberrations in hematologic malignancies exist.

Conclusions: Our study showed that chromosomal alteration PML/RAR t(15;17) which was 21.4% ,was the commonest, whereas AML1/ETO t(8;21) which was 3.6%,was the least common among patients presenting at UTH, Lusaka, Zambia. Our study showed that chromosomal aberration detected in our patients make them less responsive to cytotoxic drugs. The use of molecular technique at point of diagnosis would assist in identifying AML with better prognosis by administering appropriate treatment. The results support the existence of chromosomal abnormalities of AML in our Zambian patients. Awareness of these chromosomal abnormalities and morphology could contribute to the design of cost-effective screening strategies,

adapted by our National Health systems according to the prevalence of locally detected genetic aberrations. Acquired genetic alterations such as balanced and unbalanced chromosome aberrations and submicroscopic gene mutations and changes in gene expression strongly affect pre-treatment features and prognosis of patients with acute myeloid leukemia (AML).

Keywords- Acute myeloid leukaemia, Chromosomal Abnormalities

I. INTRODUCTION

Acute myeloid leukaemia (AML) is a highly malignant clonal hematopoietic disease caused by both inherited and acquired genetic alterations (Song et al, 1999). Current AML classification and prognostic systems incorporate genetic information but are limited to known abnormalities that have previously been identified with the use of cytogenetics, array comparative genomic hybridization (CGH), gene-expression profiling, and the resequencing of candidate genes. There are distinct differences between sub-Saharan Africa and the industrialized countries as to the age and gender distributions of the Leukaemias.

AML is diagnosed with equal frequency as ALL in children in tropical Africa, and is especially common in boys aged 5 to 14 years, who present often with chloromas, usually arising in the orbit (AF Fleming et al, 1999). The karyotyping of AML cells remains the most powerful predictor of the outcome in patients with AML and is routinely used by clinicians (Byrd JC et al, 2002, Grimwade D et al, 2001). As an adjunct to cytogenetic studies, small subcytogenetic amplifications and deletions can be identified with the use of genomic methods, such as single-nucleotide-polymorphism (SNP) array and array CGH platforms. However, these techniques remain investigational, and studies suggest that there are few recurrent acquired copy-number alterations in each AML genome. Gene-expression profiling has identified patients with known chromosomal lesions and genetic mutations and subgroups of patients with normal cytogenetic profiles who have variable clinical outcomes (Bullinger L et al, 2004 and Valk PJ et al, 2004).

Expression profiling has yielded single-gene predictors of outcome that are currently being evaluated

for clinical use. Candidate-gene resequencing studies have also identified recurrent mutations in several genes — for example, genes encoding FMS-related tyrosine kinase 3 (FLT3) and nucleophosmin 1 (NPM1) — that can help to stratify patients with normal cytogenetic profiles according to risk and to identify patients for targeted therapy (e.g., those with mutated FLT3). However, the revised classification systems are imperfect, suggesting that important genetic factors for the pathogenesis of AML remain to be discovered (Elaine R et al, 2009). We believe that the data generated by this research, will allow us to propose as a starting point for future clinical studies the molecular-risk classification in our country.

II. MATERIALS AND METHODS

Study design, site and period

This was a descriptive cross-sectional study. It was done at The University Teaching Hospital (UTH), Lusaka, Zambia. This site was purposely chosen owing to the fact that, the UTH is the largest hospital in Zambia and offers both inpatient and outpatient care and is a Centre for specialist referrals from across the country. Furthermore, the site was used because of the availability of the facilities and equipment needed to conduct this study.

Target Population

Patients with Onco-haematological diseases being managed at the Onco-Haematology Clinic of the University Teaching Hospital in Lusaka.

Sampling frame

The study population was all patients with Onco-haematological disease being managed at the Onco-Haematology Clinic of the University Teaching Hospital in Lusaka.

Sample size

Prevalence of AML at The UTH is unknown. Since the prevalence is unknown, a conservative estimate of 50% was used. In order to estimate the prevalence within 5% (or 0.05) and considering 95% confidence level, a convenient sample size of 28 was analyzed, as shown by the calculation. Then every kth case will be selected (Castillo et al, 2009).

Applying correction for finite population size formula (Dell et al, 2002), the new sample size was:

$$\text{New ss} = \frac{n}{1 + (n - 1)/\text{pop}}$$

Where, ss = sample size; pop = population,

$$\text{We calculate the new } n = \frac{n}{1 + (n - 1)/N} = \frac{400}{1 + (400 - 1)/48} = 43$$

n= sample size 400 as calculated above, N=Total number of AML cases from January 2011 to December 2014. Therefore, the minimum sample size (n) was 43.

A convenient sample size of 28 was used in this study based on the leukaemia survival record reviewed at the oncology ward that had a cohort of 48 acute myeloid

leukaemia patients as at December 2014. Of this cohort, some were lost to follow up and others had died. Our convenient sample size was based on the availability of cases within the study period.

Inclusion Criteria

All available confirmed cases of AML both newly diagnosed patients based on morphology and those already on treatment for AML bearing in mind that genetic abnormalities can still be detected in the early induction & consolidation phases of treatment at The UTH was enrolled in our study. This included both male and female patients of all age groups diagnosed with or suspected to have AML.

Exclusion Criteria

Patients with Onco-haematological diseases other than AML was excluded.

III. MATERIALS AND METHODS

Identification of AML patients participants were recruited at Onco-haematology Clinic during the haemato-oncology clinic day. As the participants were being seen, the attending clinician would inform and explain the study to them, and also provide each participant with the study information sheet. Patients that are referred to the clinic were also included in the study. Parents/guardians whose children/wards are enrolled in this study were duly informed on the benefits of this study to their children/wards. Patients that concur to be part of the study will be required to sign the consent form, and assigned a serial number. Thereafter, the patient's demographic data, medical and drug history was collected and compiled using a questionnaire. The data collected was summarized in a frequency table. The variables of interest included; age, sex, location of residence and clinical manifestations at diagnosis.

Specimen Collection, Preparation, Analysis and Storage

Blood samples (4ml) was collected from the consenting research participants via venipuncture from the ante-cubital vein using the Evacuated Tube System (ETS) into EDTA containers for determining full blood count, peripheral smear examination and the PCR analysis. Samples were well mixed to avoid clotting.

Complete Blood Count

A well-mixed blood sample was processed on the Sysmex XT 2000i and 4000i machines (Sysmex Corporation, Kobe, Japan). Whole blood will be stored at 2 to 8°C for 4 days in case the tests are not run the same day. EDTA samples were analyzed using either Sysmex XT 4000i or 2000i. The machine counts the numbers and types of different cells (WBC, RBC, platelets) within the blood. The results were authorized and printed via the laboratory information management system (LIMS).

Peripheral Smear Procedure

A drop of blood was placed on one end of a clean glass slide and holding a spreader at 45°C, a single layer of cells was made for easy visualization. The film was then air dried. The smear was labelled on the frosted

end of the slide using an HB pencil. The film was fixed in absolute methanol for 5 minutes and then transferred to a jar containing May-Grunewald stain freshly diluted with an equal volume of Soresen's buffer. It was then allowed to stain for 15 minutes and then transferred to a jar containing Giemsa's stain freshly diluted with 9 volumes of the buffer. The slides were allowed to stain for 15 minutes and then transferred to a jar of buffer for 5 minutes. Slides were then allowed to drain dry in an upright position and later examined under oil immersion for malignant cells to determine the phenotype.

Bone Marrow Aspirate Smear Procedure

The bone marrow aspirate sample for the study were obtained from that meant for clinical work-up. A drop of bone marrow aspirate with particles was placed on one end of a clean glass slide and holding a spreader at 45°C, a single layer of cells was made for easy visualization. The film was then air dried. The smear was labelled on the frosted end of the slide using an HB pencil. The film was fixed in absolute methanol for 5 minutes and then transferred to a jar containing May-Grunewald stain freshly diluted with an equal volume of Soresen's buffer. It was allowed to stain for 15 minutes and then transferred to a jar containing Giemsa's stain freshly diluted with 9 volumes of the buffer. The slides were allowed to stain for 15 minutes and then transferred to a jar of buffer for 5 minutes. Slides were then allowed to drain dry in an upright position and later microscopic examination under oil immersion for malignant cells to determine the cytomorphological phenotype.

Bone Marrow Cell Collection

We pipetted the solution containing cells into a 50 ml conical tube (for optimal results the strain cell material was strained through a cell strainer to remove any large particles).

To maximize the number of cells collected, the cell strainer was rinsed in a Petri dish with extra Phosphate Buffered Saline (PBS). This was centrifuged at low speed at 1,400 rpm (400 x g) for 5 minutes at room temperature. The supernatant was discarded without disturbing the pellet. Centrifuge again at 400 x g for 1 minute and carefully remove all traces of remaining supernatant.

Cell Lysis

The lysis of cells was done by resuspending the cell pellet with 300 µl of Solution G1 (RBC Lysis Solution) and transferring it into a clean 2.0 ml microcentrifuge tube, then incubating for 1 minute at room temperature, inverting gently two times during this incubation. Centrifuge for 30 seconds at 13,000x g removing 280 µl of supernatant afterwards using a pipette tip leaving behind 20 µl of supernatant. The vortex was used to mix by vibrating the resuspended pellet completely. Thereafter, adding Solution G2 (Cell Lysis Solution). If precipitated, heat to 55-65°C for 5 minutes to dissolve and adding 300 µl of Solution G2. This was followed by pipetting up and down to lyse the cells. This

was done with caution to avoid excessive pipetting as it could lead to sheared genomic DNA.

Note: Samples were stable at room temperature in Solution G2 for extended periods; up to 8 months. Sample should be at room temperature before proceeding. About 1.5 µl of Proteinase K Solution (20mg/ml) will be added and followed by incubation at 55°C for 15-30 minutes with inversion at least once during the incubation.

Protein Precipitation

The protein precipitation was done by adding 100 µl of Solution G3 (Protein Precipitation Solution) and vortex to mix. Then centrifugation at 13,000-16,000x g for 5 minutes.

DNA Precipitation

The clear supernatant was removed and transferred to a clean 2 ml microcentrifuge tube. Thereafter, add 300 µl of 100% Isopropanol, mix the sample by inverting 15 times and incubating at room temperature for 3 minutes. Then Centrifugation at 13,000 x g for 5 minutes and carefully discard the supernatant. A small DNA pellet should be visible. We added 300 µl of 70% Ethanol and Invert tubes 5 times to wash the pellet. It was then centrifuged at 13,000 x g for 1 minute. Lastly, inverted and drained the tube on a clean absorbent paper and air dried it for 1-2 minutes.

DNA Hydration

The DNA hydration was done by adding 50 µl of Solution G4 (DNA Hydration Solution). Then, incubating at 65°C for 1 hour. DNA samples were incubated at room temperature overnight. We tapped the tube at least twice during the incubation. Thereafter, Centrifuged the tube containing DNA sample for 20 seconds. The Genomic DNA in the tube was now ready to be used for any application.

Note: DNA can be stored at 4°C. For long term storage, store at -20°C or -80°C.

We used the UltraClean™ Bone marrow DNA Isolation Kit.

DNA Estimation

The DNA concentration was estimated by ultraviolet spectroscopy at 260nm. A DNA sample with an optical density (OD) of 1 at 260nm corresponds to a DNA concentration of 50 µg/ml of double-stranded DNA. The purity of the DNA was determined by a DNA/protein

absorbance ratio of 260nm/280nm. The DNA was stored at -20°C until required.

AML Detection

DNA Extracted from bone marrow as described above, was used and AML gene sequences was targeted for t(8;21), t(16;16) and t(15;17) by PCR amplification using the following AML primers: NK and NPM11/FLT3 ITD-; NK and CEBPA1/1. These primers would amplify a t (16; 16) and t (8; 21). The primer needed to amplify t (15; 17) is still under consideration i.e. not yet available. PCR was performed in a 100 µl reaction containing 1 x UTM a reaction buffer, 1mM each primer, 40mM dNTPs, 1.5mM MgCl₂ and 3 units of DNA polymerase for 40 cycles. Each cycle consisted of denaturation at 94°C for 30 seconds, primer annealing at 60°C for 20 seconds and primer extension at 72°C for 45 seconds. After all cycles are completed, a final elongation step at 72°C for 7 minutes was done. Amplification products were subjected to electrophoresis on a 1.5% agarose gel.

AML Subtyping

PCR was performed on DNA extracted from bone marrow tissue described above. PCR was carried out by using AML primers indicated in the fore-going section. DNA fragments were extracted from bone marrow for direct use in nucleotide sequencing reactions. Detection of t (8; 21) and t (16; 16) was performed by using standard PCR assays specific primers: NK and NPM11/FLT3 ITD-; NK and CEBPA1/1 for AML in two sets of amplification tubes. After denaturation of the template DNA at 94°C for 5 minutes, the PCR was performed for 25 cycles. Each cycle consisted of denaturation at 94°C for 30 seconds, primer annealing at 60°C for 20 seconds and primer extension at 72°C for 45 seconds. After all cycles are complete, a final elongation step at 72°C for 7 minutes was done. Ten microliters of the PCR product was electrophoresed in 2% agarose gel containing ethidium bromide. The products of the type-specific primers were visualized under ultra violet light. A DNA marker was used as a molecular size marker. A translocation specific band would show the test is positive for a translocation. The target translocations are shown in table 1. The identity of this translocation was established by the PCR.

Table 1: Chromosomal alteration targets.

Chromosomal Alterations	Genes Involved	Primer Sequence (5'-3')
t(8;21)(q22;q22)	RUNX1	AGCCGAGTAGTTTTCATCATTGC
	RUNX1T1	TCTCCTATCTCGGGTCAAATGTC
t(15;17)(q24;q21)	PML	GTGCGCCAGGTGGTAGCTC
	RARA	AAGCCCTTGCAGCCCTCAC
inv(16) (p13;q22)	CBFB	TTTGAAGGCTCCCATGATTCTG
	MYH11	AGGTCCCCTTCCAGCTTCTTCT

IV. RESULTS

The distribution of the 28 cases that were finally included in the study, according to the FAB classification was as follows: M0 in two(2) cases, M1 in four(4), M2 in five(5), M3 in seven(7) (6 hypergranular and 1 microgranular), M4 in five(5), M5 in four(4), M6 in one(1) and M7 zero(0) case. There were 17 males and 11 females, with a median age of 63 years (range 6-90). Four patients were aged <14 years. The relative frequencies of each group according to the WHO classification within the 28 consecutive valid cases are shown in Table 3. Six(6) patients (21.5%) were found to have the PML/RAR- α fusion transcript (three with type bcr1, one bcr2 and two bcr3) that identifies the presence of t(15;17). Of these, 5 (83%) corresponded to cases identified as having a M3 morphology (4 hypergranular, 1 microgranular), while there was one case initially diagnosed as having an M0 morphology. The presence of the AML1/ETO fusion transcript which results from the t(8;21) was detected in only one case (3.6%). This case displayed a M2 morphology, while the remaining other cases with such morphology were negative. The presence of the CBF- β /MYH11 fusion transcript was detected in two cases (7.1%). The CBF- β /MYH11 positive cases was identified as a FAB-M4, but without a sufficient number of eosinophils to be classified as M4Eo. The MLL gene was rearranged by Southern blot analysis in four cases (15.4%). These included one M1, one M2, one M4 and one M5.

The most frequent chromosome/molecular rearrangements, that is, t(15;17)(q22;q12 ~ 21)/ PML-RARA characteristic of acute promyelocytic leukemia (APL), AML with 11q23 abnormalities(MLL), inv(16)(p13q22)/t(16;16)(p13;q22)/ CBFB-MYH11 characteristic of core-binding factor (CBF) AML and t(8;21)(q22;q22)/ RUNX1-RUNX1T1 and, confer favourable clinical outcome when patients receive optimal treatment, that is, regimens that include high-dose cytarabine for CBF AML and all- trans - retinoic acid and/or arsenic trioxide for APL. Recently, mutations in such genes as KIT in CBF AML and FLT3 in APL have been correlated with clinical features and/or outcome of patients with these AML subtypes, and microarray gene expression profiling has been successfully used for diagnostic purposes and to provide biologic insights.

As far as the age distribution was concerned, the study sample was representative of the population of our country, characterized by advanced age (mean 63 years). Upon dividing into three age groups (6-35, 35-65 and >60 years), we observed that the overall frequency of leukemia displaying one of the four recurrent cytogenetic translocations was much higher in the youngest age group. By contrast, the frequency of AML not otherwise characterized increased with age (13%, 20% and 67%) ($p < 0.05$, Table 4). It should be noted that this difference was mainly due to the higher incidence of PML/RAR α positive cases among young AML patients, while AML with 11q23 abnormalities (MLL) abnormalities were more frequent in the advanced age group.

Table 2: Relative frequencies of the different AML subtypes in patients

Acute myeloid leukemias	Cases n = 28	%
AML with recurrent cytogenetic translocations	13	46.5%
AML with t(15;17) PML/RAR- α	6	21.4%
AML with t(8;21) AML1/ETO	1	3.6%
AML with inv(16) or t(16;16) CBFB- β /MYH11	2	7.1%
AML with 11q23 abnormalities (MLL)	4	14.3%
AML not otherwise characterized	15	53.6%
AML minimally differentiated	1	3.6%
AML without maturation	3	10.7%
AML with maturation	5	17.9%
Acute myelomonocytic leukemia	2	7.1%
Acute monocytic leukemia	3	10.7%
Acute erythroid leukemia	1	3.6%
Acute megakaryocytic leukemia	0	0.0%
Acute basophilic leukemia	0	0.0%
Acute pan-myelosis with myelofibrosis	0	0.0%

Table 3: Distribution of chromosomal translocations in AML according to patients' age.

<i>Acute myeloid leukemias</i>	<i>6-35 years</i>	<i>36-65 years</i>	<i>>66 years</i>	<i>Total</i>
AML with t(15;176) PML/RAR-	4 (67%)	1 (17%)	1 (17%)	6 (21.4%)
/MYH11®AML with inv(16) or t(16;16) CBF-	0 (0%)	0 (0%)	1 (100%)	1 (3.6%)
AML with 11q23 abnormalities (MLL)	1 (50%)	1 (50%)	0 (0%)	2 (7.1%)
AML with 11q23 abnormalities (MLL)	1 (25%)	1 (25%)	2 (50%)	4 (14.3%)
AML not otherwise characterized	2 (13%)	3 (20%)	10 (67%)	15 (53.6%)
Total	8 (29%)	6 (21%)	14 (50%)	28

AML high in > 66 and < 35years.

t(15,17) PML/RAR was the commonest chromosomal translocation in our patients mostly below 35years of age and AML1/ETO t(8,21) the least common.

V. DISCUSSION

In our study we analyzed, a number of AML patient representatives of the population from our country, the chromosomal abnormalities in AML patients at the University Teaching Hospital according to WHO 2008 revised classification. Two important pre requisites of this study were:

- The patients were recruited from a national referral hospital as based on AML cases and
- in all cases the four genetic abnormalities were simultaneously explored, using well-standardized molecular techniques according to BIOMED-1 protocols.

Our data show that in Zambia the PML/RARA t(15;17) is more frequent and the AML1/ETO t(8;21) is rarer than in other countries (USA and North-Central Europe). The PML/RAR t(15;17) and AML1/ETO t(8;21) in USA was found to be equal at 10% (Kumar et al,2011). The PML-RARA protein binds to corepressor/histone deacetylase (HDAC) complexes with higher affinity than the wildtype RARA, leading to aberrant chromatin acetylation and alterations of chromatin conformation inhibiting the normal transcription of genes regulated by RARA. This blocks cell differentiation and leads to the accumulation of abnormal promyelocytes (Jing et al,2004). Notably, therapeutic doses of all- trans -retinoic acid (ATRA) change conformation of the PML-RARA protein and release corepressor/HDAC complexes resulting in transcriptional activation of downstream target genes. Additionally, both ATRA and arsenic trioxide, a compound also used to treat APL, induce proteolysis of PML-RARA protein resulting in granulocytic differentiation of the leukemic blasts (Jing et

al,2004).While more APL patients carry t(15;17) or its complex variants, some of the patients harbor an insertion of chromosomal material from 17q with the RARA gene into 15q22, the PML gene locus (Grimwade et al, 2000). Most of these insertions are cryptic, associated with a normal karyotype, and detectable only using reverse transcription – polymerase chain reaction (RT – PCR) and/or fluorescence in situ hybridization (FISH). In about one-third of APL patients with t(15;17), this translocation is accompanied by at least one secondary aberration, most commonly +8 or a partial trisomy of 8q (Hernández et al,2001). The presence of secondary chromosome abnormalities does not impact on prognosis of APL patients treated with chemotherapy and ATRA (Sanz et al,2005).The rearrangements of PML/RAR t(15;17) is very strongly correlated with characteristic marrow morphology in which abnormal promyelocytes predominate (FAB M3).The diagnosis should always be supported by results of cytogenetic, FISH, and/or RT – PCR analyses. RT – PCR determination of the particular PML-RARA isoform in each case is important for disease monitoring because the probability of relapse is increased in patients who after 3 – 4 cycles of ATRA and chemotherapy are still positive for the presence of the PML-RARA transcript, whereas persistent negative RT – PCR results correlate with long-standing remissions in most, but not all, patients (Lo-Coco et al,2006). The historically very poor prognosis of APL patients with t(15;17)/ PML-RARA , with a median survival of 2 weeks in the 1960s, has become favorable with the use of therapies containing anthracyclines, ATRA, and/or arsenic trioxide, with a cure rate exceeding 80% in recent studies (Frankfurt et al,2006). Recently,

gemtuzumabozogamicin, which is a monoclonal antibody directed against CD33, an antigen present on leukemic blasts of almost all patients with APL, has been shown to be effective in producing molecular remissions in both newly diagnosed and relapsed patients (Lo Coco et al, 2006). In one study, a combination of gemtuzumab, ATRA and arsenic trioxide was successfully used to induce prolonged second CRs in APL patients who had experienced first hematologic recurrence (Aribi et al, 2007).

Translocation (8;21) is the first reciprocal translocation in AML that was identified using banding techniques (Rowley et al, 1973). Both the standard t(8;21) and its relatively rare variants, that is, complex translocations involving three or four different chromosomes that consistently affect bands 8q22 and 21q22 or the insertions ins(8;21)(q22;q22q22) or ins(21;8)(q22;q22q22) (Mrózek et al, 2001), disrupt the RUNX1 gene that encodes subunit alpha of core-binding factor (CBF), and lead to the creation of a chimeric gene RUNX1-RUNX1T1 (AML1-ETO) (Miyoshi et al, 1993). The presence of t(8;21)/ RUNX1-RUNX1T1 is associated with AML with maturation in the neutrophil lineage (FAB M2). It has been suggested that characteristic pink-colored cytoplasm of neutrophils and an increased number of eosinophil precursors without abnormalities typical for AML with inv(16)/t(16;16) morphologically distinguish patients with t(8;21) from other patients with AML M2 who do not harbor t(8;21)/RUNX1-RUNX1T1 (Nakamura et al, 1997). The prognosis of patients with inv(16)/t(16;16) and those with t(8;21) is relatively favorable (Bloomfield et al, 1994). Because of these similarities in response to treatment and of involvement of subunits of CBF at the molecular level, many clinical trials and reports have combined patients with inv(16)/t(16;16) with those with t(8;21) into one, favorable risk prognostic category of AML (Mrózek et al, 2008). Nevertheless, despite similarities, patients with t(8;21) differ from those with inv(16)/t(16;16) with respect to many pretreatment features. In univariable analyses, neither relapse risk nor overall survival (OS) differed significantly between t(8;21) and inv(16)/t(16;16) groups (Schlenk et al, 2004). However, after adjusting for age, log(WBC), and log(platelets), the OS of t(8;21) patients was significantly shorter than OS of those with inv(16)/t(16;16) (Marcucci et al, 2005). The difference may be in part explained by a dissimilar response to salvage treatment because t(8;21) patients had a significantly shorter survival after relapse than inv(16)/t(16;16) patients in three large, independent studies (Appelbaum et al, 2006).

Although the cases were also analyzed by cytogenetics, there were a substantial number of cases lacking these data due to inappropriate storage or transportation, or due to the lack of metaphases or clonal cytogenetic abnormalities after the analysis. So, no epidemiological data can be added based on conventional cytogenetics, although we can say that no differences

were observed between the two techniques when cytogenetics was available. Using cytogenetic and morphologic data it was reported that the incidence of M3/t(15;17) positive AML was between 5% and 11% in United States and North-Central Europe (Head et al, 1995). More recent data confirm that the frequency is around 10% (Grimwade et al, 1998), as it is in Australia and Japan (Nakase et al, 2000). In a British series including 1,612 AML patients and based on cytogenetic data, the incidence of t(15;17) is 13% (Grimwade et al, 1998). However, there are some data suggesting that the frequency of the t(15;17) can be higher in some countries and/or specific populations. Thus, this frequency increases to 17% in Italian children and seems to be over 20% in countries such as Brazil, Mexico, Peru and Spain (Ruiz-Argüelles et al, 1998). Even more significantly, this frequency has been shown to be as high as 37.5% in patients of Latin origin from Los Angeles (Douer et al, 1996). However, as mentioned above, these findings are mainly based on morphologic and cytogenetic data, so some variations could be expected upon using molecular analysis. Our study confirms that AML with t(15;17) represents one quarter of all *de novo* AML, which actually means that it is the most frequent type of acute leukemia in our population. Moreover, the incidence is greater in younger patients, which makes this epidemiological data even more valuable, since such patients can take advantage of curative treatments and quick screening tests for the disease. The second observation of note in our study is the extremely low frequency of t(8;21) AML1/ETO found: only 3.6%, even when we included specially referred patients. So far, we have only seen few cases in all AMLs already screened in the haematology laboratory for this genetic abnormality. The initial reports showed a global frequency of t(8;21) of around 15%, increasing within those cases with FAB-M2 morphology. However, marked geographic variations have been shown. Thus, the reported incidence of t(8;21) within the FAB M2 subtype ranges from 58-88% in Asian patients, 19-54% in other European countries and 12-27% in USA. In addition, the most recent data from European countries based on cytogenetic or molecular studies have shown that the frequency of this aberration is around 8% of all AMLs (Grimwade et al, 1998). These data indicate that, according to the frequency detected in our study (3.6% of all AMLs; 6% of all FAB M2 morphologies), this genetic abnormality is more unusual in Zambia than in other countries, although apparently the incidence of t(8;21) is higher in young AML patients in other countries. Nevertheless, this factor is not strong enough to justify the great variation that has been obtained compared to previous reports and moreover, our analysis according to age subgroups did not show any difference. Finally, the frequencies of the CBF- β /MYH11 fusion gene and MLL rearrangements (7.1% and 14.3%, respectively) coincide with data reported in the literature (Grimwade et al, 1998).

VI. CONCLUSIONS

Our study showed that chromosomal alteration PML/RAR t(15,17) which was 21.4% ,was the commonest, whereas AML1/ETO t(8,21) which was 3.6%,was the least common among patients presenting at UTH, Lusaka, Zambia. Our study showed that chromosomal aberration detected in our patients make them less responsive to cytotoxic drugs. The use of molecular technique at point of diagnosis would assist in identifying AML with better prognosis by administering appropriate treatment. The results support the existence of chromosomal abnormalities of AML in our Zambian patients. Awareness of these chromosomal abnormalities and morphology could contribute to the design of cost-effective screening strategies, adapted by our National Health systems according to the prevalence of locally detected genetic aberrations. Acquired genetic alterations such as balanced and unbalanced chromosome aberrations and sub microscopic gene mutations and changes in gene expression strongly affect pre-treatment features and prognosis of patients with acute myeloid leukaemia (AML).

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