

Chronic lymphocytic leukemia in Kenya: an immunophenotypic and clinicopathologic study

La leucémie lymphoïde chronique au Kenya : étude immunophénotypique et clinicopathologique

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Abstract Objective: To define cases of chronic lymphocytic leukemia (CLL) by immunophenotypic criteria and describe the associated clinical features in patients diagnosed at Aga Khan University Hospital, Nairobi.

Background: Rising to the growing cancer challenge will require improved diagnostic services. CLL is common in elderly patients. The current international standard in diagnosis incorporates findings of immunophenotyping. Facilities for immunophenotyping have generally been unavailable in Kenya.

Method: A cross-sectional survey was conducted between August 2011 and April 2012. Potential cases were identified based on morphologic criteria. Consecutive samples were obtained and subjected to 3 colour immunophenotyping on a Cytomics FC 500 cytometer. CLL was defined using the Royal Marsden Hospital scoring system. Baseline clinical and diagnostic data were also obtained.

Results: Forty nine cases met the eligibility criteria. Thirty one were known CLL cases, and 18 were newly diagnosed. Median age at diagnosis was 62 years. Male:female ratio was 1.3:1. Black patients (42/49) were more likely to present with high risk disease (Rai stages III–IV) and with higher lymphocyte counts than non-blacks at diagnosis. Twenty six point five percent of patients in this study were diagnosed in Rai stage 0. The prevalence of CD5/CD23 co-expression was found to be 95.9%. CD5 was universally expressed, whereas CD23 was present in all but 2 cases. Both were associated with atypical morphology. Complete absence of light chain expression using a monoclonal antibody was found in 12.2% of cases. Five patients had their diagnosis revised. Of 31 patients on follow-up for

CLL, only 5 had had any form of immunophenotyping done.

Keywords Immunophenotyping · Clinical features · CLL · Kenya

Résumé Objectif : Définir des cas de la leucémie lymphoïde chronique (LLC) selon les critères immunophénotypiques et décrire les caractéristiques cliniques associées aux patients diagnostiqués à l'hôpital universitaire d'Aga Khan de Nairobi.

Contexte : Combattre l'accroissement du cancer nécessite les services diagnostiques avancés. La LLC est fréquente chez les âgés. Une étude de l'immunophénotype, dont la capacité manque généralement au Kenya, est recommandée pour son diagnostic.

Méthode : Une enquête transversale fut faite entre août 2011 et avril 2012. Les échantillons des cas potentiels, selon les critères morphologiques, furent collectionnés et soumis à la cytométrie en flux 3 couleurs utilisant le cytomètre Cytomics FC 500. La LLC était définie par le Score Royal Marsden Hospital. Les données de base cliniques et diagnostiques furent aussi prises.

Résultats : Quarante-neuf cas furent inclus dont 31 déjà diagnostiqués et 18 de nouveaux cas. L'âge médian au diagnostic était de 62 ans. Le ratio homme/femme était 1,3/1. Les patients noirs (42/49) présentaient au diagnostic une maladie plus avancée (stades III–IV de Rai) et une lymphocytose plus élevée que chez les non-Noirs. 26,5 % des patients présentaient un stade 0. Tous les cas exprimaient le CD5. Tous exprimaient le CD23, sauf deux qui montraient une morphologie atypique : 95,9 % avaient le double marquage CD5/CD23, 12,2 % n'exprimaient pas des chaînes légères. Le diagnostic de cinq patients fut révisé. Seuls 5 des 31 patients avaient déjà profité d'une étude de l'immunophénotype.

Mots clés Immunophénotype · Caractéristiques cliniques · LLC · Kenya

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Introduction

The 2008 International Workshop on chronic lymphocytic leukemia (CLL) criteria and the 4th edition of the WHO classification of hematolymphoid neoplasms incorporate immunophenotyping results into the diagnostic criteria for CLL [1,2]. A combined approach of immunophenotyping plus morphological evaluation improves diagnostic accuracy and hence appropriate therapy and prognostication [2,3]. CLL is known to occur in Kenya, but the occurrence of CLL when accurately defined by morphological evaluation plus immunophenotyping has not been described [4]. The typical CLL immunophenotype is a co-expression of CD5 and CD23 in monoclonal B cells with dim expression of surface immunoglobulin (sIg) and absent/dim CD79b (or CD22), and the absence of expression of FMC7 [1,5]. The Royal Marsden Hospital (RMH) scoring system, described by Matutes et al. [5] allows the distinction between CLL (RMH = 3–5) and other B-cell chronic lymphoproliferative disorders (RMH = 2 or less). This study provides data on the diagnostic and clinical features of CLL at a tertiary referral hospital in Kenya.

Methods

Study design

A cross-sectional survey was conducted between August 2011 and April 2012. Patients were recruited serially.

Study site

AKUH,N department of pathology. The department serves the hospital plus a variety of outreach laboratories, independent laboratories and hospitals spread across Kenya.

Study population

All patients with persistent lymphocytosis ≥ 3 months, consistent morphology and who gave consent were eligible for inclusion. Patients whose samples were unsuitable for immunophenotyping or with inadequate supplementary clinical information were excluded. Samples that obtained RMH scores ≤ 2 were also excluded.

Procedure

Morphologic assessment was based on FAB criteria [6]. Cases were then categorized as typical or atypical morphology based on a consensus diagnosis between 2 pathologists. Consecutive samples were obtained and subjected to 3 colour

immunophenotyping on a Beckman Coulter Cytomics FC 500 cytometer. A limited panel of antibodies was used. These included CD5, CD19, CD22, CD23, FMC7, kappa and lambda. CLL was defined using the scoring system described by Matutes et al. [5].

CD19 versus side scatter was the gating strategy used to identify the B lymphocyte cluster. Histograms were evaluated, and antigens were interpreted as positive/negative and as dim/bright positive where the intensity of expression mattered. Antigen positivity was defined as reactivity above isotype controls on $> 20\%$ of B cells as identified by flow cytometry. Dim expression was defined when the B-cell cluster fell within 1 decalogue of the isotope control. Bright positivity was when the cell cluster fell ≥ 2 decalogues of the isotope control [5]. Light chain restriction (marker of clonality) was defined by an abnormal kappa-lambda ratio of $> 3:1$ (kappa restricted) or $< 1:2$ (lambda restricted) [7]. Cases of CLL were defined as those with a score of ≥ 3 [5]. The baseline clinical and diagnostic data were also obtained. Approval for the study was obtained from the institution's research and ethics committees.

Data management

Descriptive data are presented as tables, bar and pie charts. Statistical differences in medians were sought using the Mann–Whitney test. Differences in proportions were analyzed using the Chi Square (χ^2) test. A P value < 0.05 was considered significant.

Results

A total of 49 patients were included in the final analysis. Thirty one patients known to have CLL/SLL and 18 patients with a new diagnosis of CLL/SLL were included. Of the 31 patients with an established diagnosis of CLL/SLL, only 5 patients (16.1%) had had any form of immunophenotyping done. For patients already known to have the disease, all the parameters recorded other than morphologic category and immunophenotyping results were those found at baseline. These patients were included since CLL is currently considered an incurable disease and guidelines for diagnosis mandate that immunophenotyping be performed as part of the diagnostic workup (Table 1).

The mean, median and age range were 62.1, 62 and 45–95 years, respectively (Fig. 1).

The majority of patients had a score of 5 and a minority scored 3 which are similar to what is described [5,8]. The relative proportion of scores obtained was, however, distorted by the fact that 6 cases were scored on 4 points

Table 1 Baseline features at diagnosis.	
Characteristic	Number (%)
Gender ($N = 49$; M:F ratio = 1.3:1)	
Male	28/49 (95% C.I 43.3–71%)
Female	21/49 (95% C.I 29–56.7%)
Race	
African	42 (85.7)
Asian	4 (8.2)
Caucasian	3 (6.1)
Lymphadenopathy	29/49 (59.2)
Splenomegaly	25/49 (51.0)
Hepatomegaly	8/49 (16.3)
Presence of any B symptom	
Night sweats	10/49 (20.4)
Weight loss	5/49 (8.5)
Extreme fatigue	1/49 (2.0)
Haemoglobin level (g/dL)	
Mean = 11.4 (SD = 2.67)	
< 11g/dL	18/49 (36.7)
≥ 11g/dL	31/49 (63.3)
Thrombocytopenia ($\times 10^9/L$)	
Mean=172.97 (SD = 85.01)	
< 100	9/49 (18.4)
≥ 100	40/49 (81.6)
Absolute lymphocyte count ($\times 10^9/L$)	
Median = 40.5	
Range (5.08–324.2)	
Rai stage	
0	13 (26.5)
I–II	17 (34.7)
III–IV	19 (38.8)
Binet	
A	23 (46.9)
B	7 (14.3)
C	19 (38.8)

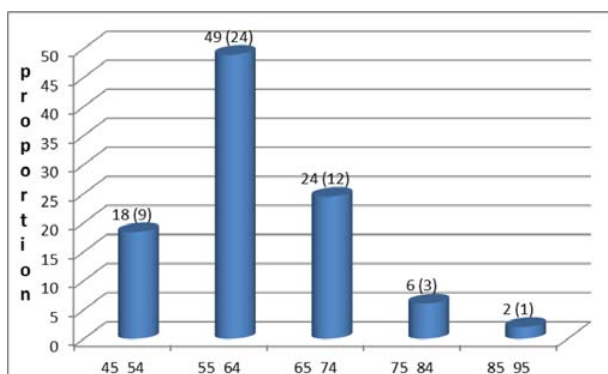


Fig. 1 Age distribution at diagnosis

only due to absence of detectable light chains. This meant that the intensity of surface immunoglobulin (sIg) expression could not be assessed and light chain restriction could not be demonstrated. Five of these were assigned a score of 4 and one was assigned a score of 3. CD5 was universally expressed, CD23 positive in 47/49 and there was bright surface immunoglobulin (sIg) expression in 4/49 cases (Fig. 2) (Tables 2, 3).

Discussion

Twenty six point five percent of patients in this study were diagnosed in Rai stage 0. In an earlier study on CLL in Kenya, no patient ($N = 42$) was found to present with lymphocytosis only [4]. This may reflect the increased use of automated complete blood counts. It may also point to an increased number of patients undergoing routine full haemogram as part of wellness checks. Similar trends have been observed in developed countries where the majority of their patients are now diagnosed as an incidental finding [9,10].

The mean/median age was comparable to other African series [12,13] but lower than that of developed nations [11,14]. This may be explained by the life expectancy of the population. The life expectancy in Africa is lower than that in Europe or North America, and this may create a selection bias that could explain the clustering of diseases at an earlier age. The youngest patient was 45 years old. An earlier series, also done in Kenya, had documented that at least 4 of 42 patients were in the age bracket 15–39 years [4]. CLL at that age is unusual [15] and their cases may represent rare cases or may reflect misdiagnosis due to unavailability of immunophenotyping facilities.

Racial differences were observed. It was noted that non-blacks were more likely to present with earlier stage disease and lower WBC counts than non-blacks and the difference was statistically significant (Tables 4, 5). Though a similar observation has been previously noted, the reason(s) for this was not clear [16]. It could be that non-blacks had easier access to health care.

CD5/CD23 co-expression was found in 95.9%. This immunophenotype excludes most reactive or clonal lymphoid disorders that could be confused with CLL. It is also reported to have a high positive predictive value in diagnosing CLL [8]. CD5 was found to be the most frequently expressed marker with 100% of cases expressing it. CD23 was expressed in all but 2 cases. Six cases (12%) were scored on 4 points only due to the complete absence of detectable light chains. This aberrancy was attributed to a genuine absence due to changes in the epitope targeted by the monoclonal antibody used. The proportion of 12% which was obtained is close to that seen by Horna et al.

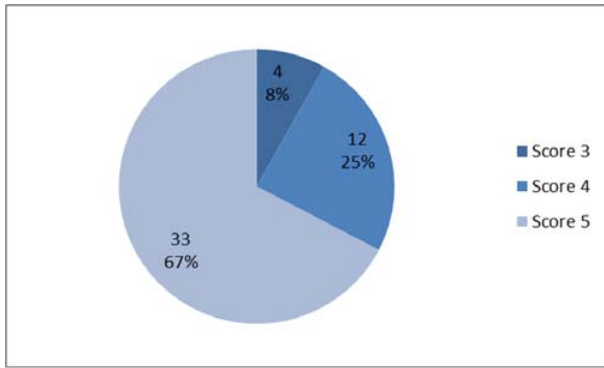


Fig. 2 Frequency of RMH scores obtained

[17] who described the absence of light chain expression in 13.2% of patients with CLL when a monoclonal antibody was used.

Of the 31 patients with an established diagnosis of CLL, only 5 patients had had any form of immunophenotyping done. This likely reflects the limited availability of these services in Kenya. Five cases had their diagnosis revised

on the basis of morphologic and/or immunophenotypic criteria (Table 6).

Conclusion

Immunophenotyping is diagnostically productive and should be part of the diagnostic workup when a chronic lymphoproliferative disorder is suspected.

Ethical standards Approval for the study was obtained from the institution’s research and ethics committees.

Conflict of Interest: E. Mulwa-Babu and D. Paresh do not have any conflict of interest to declare.

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Table 2 Association between antigen expression patterns and morphologic subtype.

Antigen expression		Typical, N (%)	Atypical, N (%)	χ^2	P value
sIg	Absent	6 (17)	0 (0)	3.39	0.18
	Bright	2 (6)	2 (14)		
	Dim	27 (77)	12 (86)		
FMC7	Absent/partial	32 (91)	12 (86)	0.36	0.55
	Positive	3 (9)	2 (14)		
CD22	Absent/dim	33 (94)	13 (93)	0.04	0.85
	Bright	2 (6)	1 (7)		
CD5/CD23	5+/23+	35 (100)	12 (86)	5.21	0.02
	5+/23-	0 (0)	2 (14)		

Table 3 Association between treatment status, morphology and immunophenotype.

		Treated	Untreated	P value
Morphology	Typical	15	20	0.365
	Atypical	8	6	
sIg ^a	Absent	3	3	0.655
	Bright	1	3	
	Dim	19	20	
FMC7	Absent/partial	22	22	0.203
	Positive	1	4	
CD22	Absent/dim	23	23	0.093
	Bright	0	3	
CD5/CD23	CD5+/CD 23+	23	24	0.174
	CD5+/CD 23-	0	2	

^a sIg: surface immunoglobulin as evaluated using monoclonal antibodies against kappa and lambda immunoglobulin light chains.

Table 4 Median hematologic parameters against race and clinical stage.				
	Median (IQR)			
Race	Africans (42/49)	Non-Africans	P value	
Haemoglobin	11.6 (9.4–13.3)	13.9 (12.7–14.1)	0.02	
WBC count	54.2 (32.1–96.7)	24.6 (17.7–35.4)	0.01	
ALC	44.8 (25.7–76.1)	18.6 (9.6–23.9)	0.005	
Platelets	138.0 (106.0–218.0)	265.0 (198.0–312.0)	0.003	
Rai stage	0 (13/49)	I + II (17/49)	III + IV (19/49)	P value
Haemoglobin	13.5 (11.8–14.1)	13.0 (12.4 – 13.9)	9.4 (8.6–9.8)	< 0.01
WBC count	32.3 (21.8–40.7)	46.1 (19.6 – 97.4)	66.0 (44.0–97.7)	0.03
ALC	25.7 (16.6–35.0)	34.2 (13.2–92.5)	51.4 (37.7–93.9)	0.02
Platelets	221.0 (174.0–265.0)	164.0 (141.0–238.0)	108.0 (69.0–144.0)	< 0.01

Table 5 Association between gender, race and clinical stage.				
Rai stage	0	Male (%) 7 (25)	Female (%) 6 (29)	P value 0.42
	I + II	8 (29)	9 (43)	
	III + IV	13 (46)	6 (29)	
Gender		African (%)	Non-African	P value 1.00
	Male	24 (57)	4 (57)	
Rai stage	Female	18 (43)	3 (43)	< 0.01
	0	7 (17)	6 (86)	
	I + II	16 (38)	1 (14)	
	III + IV	19 (45)	0 (0)	

Table 6 Patients with altered diagnosis.	
Initial diagnosis	New diagnosis
1 patient with CLL	Hairy cell leukemia
2 patients with chronic lymphoproliferative disorders (CLPD)- ?follicular lymphoma, ? CLL	CLL
1 case of CLL ^a	Essential thrombocythemia (ET)
CLL	1 case of acute lymphoblastic leukemia

^a This was a new patient who was referred as a case of CLL. It was unclear how the diagnosis had been made. The clinical presentation and further evaluation resulted in a diagnosis of ET.

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