Original Article

Causes of lymphocytosis in Abidjan: a preliminary study

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ABSTRACT

AIM We set out to identify the causes of lymphocytosis in adults in Abidjan by the use of cytology combined with flow cytometry.

METHODS We recruited 39 patients with either an organomegaly or a persistent lymphocytosis above 4 x 10^9 lymphocytes/L lasting more than 3 months or both presentations. We performed full blood count and immunophenotyping by flow cytometry. We also calculated the Matutes score with the following markers: CD5, CD23, CD22 or CD79b, FMC7 and the surface immunoglobulin (sIg).

RESULTS The mean age of the study patients was 60.28±9.93 with a mean absolute lymphocyte count of 64.5±69.5 10^9/l. The commonest cause of lymphocytosis in our study was circulating phases of non-Hodgkin lymphoma followed by chronic lymphocytic leukaemia.

CONCLUSION Patients presenting with lymphocytosis in Abidjan are likely to have chronic B-cell lymphoproliferative disorder. This finding will inform guidelines for diagnosis and management of chronic lymphoproliferative disorders in Sub-Saharan Africa.

Keywords: Lymphocytosis; Lymphoma; Immunophenotyping; Cote d'Ivoire; Africa.

INTRODUCTION

Lymphocytosis is an increase in absolute lymphocyte count greater than 4 x 10^9/L. [1-2] It can be seen in reactive and lymphoproliferative disorders. In acute and chronic lymphoproliferative disorders the increased lymphocytes are malignant. They are caused by conditions such as acute lymphoblastic leukaemia, chronic lymphocytic leukaemia (CLL)/ small lymphocytic leukaemia, monoclonal B lymphocytosis and non Hodgkin lymphoma (NHL). As reported by Echimane et al. in 2000, [3] in West Africa, the principal cancers in men were liver cancer (15%) and prostate cancer (15.8%), with modest rates of NHL (10.5%) and gastric cancer (4.5%). In women, breast cancer was the most frequent tumor (25.7%), followed by cervical cancer (24.0%) and NHL (7.3%). Haematological malignancies are supposed to represent 14.02% of cancers in Côte d'Ivoire. Burkitt 's lymphoma was the commonest NHL. [3]

Lymphoma can cause lymphocytosis when it spreads to the bone marrow and peripheral blood. Early and definitive diagnosis of haematological malignancies is essential for timely and effective management. Immunophenotyping allows definitive diagnosis of haematological malignancies but flow cytometry is not readily available in Sub-Saharan African countries.

The aim of this work was to describe the main causes of lymphocytosis in Abidjan using cytology combined with flow cytometry.

METHODS

We recruited adults above 18 years of age who came to the Central Laboratory of the Yopougon University Hospital (YUH) over a period of 18 months. Those patients presented either an organomegaly or a persistent lymphocytosis defined by a value above 4 x 10^9 lymphocytes/L lasting more than 3 months [1-2] or both presentations. The study was approved by the YUH ethics committee. After obtaining a written informed consent, we collected epidemiological and clinical data. Then we collected peripheral blood samples in ethylene diamine-tetra-acetatic acid (EDTA) tubes. This reagent protects blood cell morphology and is recommended for full blood count (FBC). FBC was performed using the Sysmex XT – 2000i in the Central Laboratory of YUH.

For immunophenotyping, specimens were sent, the same day, to Cerba Laboratory in Paris France. Flow cytometry samples were processed within 24 to 48 hours on the F500 cytometer of Beckam using monoclonal antibodies to identify membrane markers for: leukocytes (CD45); T lymphocytes (CD2, CD3, CD4, CD5, CD7); NK lymphocytes (CD56); B lymphocytes (CD19, CD20, CD79b or CD22, CD25, CD103, CD11c, CD38, CD43, CD10, and surface immunoglobulin-sIg). We also calculated the Matutes score [4-5] from the following markers: CD5, CD23, CD22 or CD79b, FMC7 and surface immunoglobulin (sIg). Results were interpreted following Schwock[1] and Van Dongen. [2] Patients with chronic lymphocytic leukemia (CLL) presented the phenotype $CD5^+$, $CD19^+$, $CD23^+$, $FMC7^-$, $sIg^{+/-\ low}$ with a Matutes score superior or equal to 4. [4-5] In circulating phases of NHL, the Matutes score was lower or equal to 3. [4-6] The marginal zone lymphoma was characterized by the phenotype $CD5^-$, $CD\ 23^-$. In the mantle cell lymphoma the profile was $CD\ 5^+$, $CD\ 23^-$. [1-2] In the reactive hyperlymphocytosis, the phenotype was usually $CD\ 8^+$. [1-2, 4-5] Final diagnosis was arrived at by integrating clinical, morphological and immunophenotypic information.

RESULTS

We recruited 39 patients with an average age of 60.28±9.93 (range 34-79) years. The most common age group was 50-70 years (69.4%). We noted a female predominance with a sex ratio of M/F of 0.77 (Table 1). The majority of patients (76.9%) presented with splenomegaly and/or non-specific symptoms (Table 2). The FBC levels were: haemoglobin 8.7 ± 2.1 g/dl, white blood cell (WBC) 88.7 ± 94.3 x 10^9/L, absolute lymphocyte count 74.9± 80.6 x 10^9/L, and platelet count 119.7 ± 54.2 x 10^9/L (Table 3).

Reactive lymphocytosis was 12.8 % of cases and haematological malignancies were 87.2 % (Table 4). B-cell markers namely CD19, CD 20, CD79b with κ or λ light chain restriction were the most common findings (Tables 5-8). Chronic lymphoid malignancies represented 76.9 % (Table 4). Myeloma, Waldenström disease, Natural Killer proliferation were rare (Tables 4 and 6). The CLL patients were in Binet stage A (Table 7). The prognostic marker CD 38 was expressed in 62.5%. Leukemic phase of B NHL was the main cause of lymphocytosis (Tables 4 and 8).

DISCUSSION

The patients presented with advanced disease burden of chronic lymphoid malignancies represented by high lymphocytosis. These results were in agreement with those of Koffi [6] who pointed out that chronic lymphocytosis in elderly patients, with or without organomegaly, were in general malignant. Reactive lymphocytosis in the adults was unusual (Tables 4 and 5).

Sometimes it was difficult, only by cytologic criteria, to differentiate between a blast cell and a lymphocyte. This explains why acute leukemia patients were included in our study (Tables 4 and 6). We did not find any T cell NHL and only 1 NK proliferation (Tables 4 and 6). The majority of our cases were leukemic phase of B NHL (Table 4 and 8). We did not check the HIV status of our participants. A number of our cases may well have had HIV-associated lymphoma. [7]

Burkitt's lymphoma which mainly affects children and young male adults is the most common lymphoma in Côte d'Ivoire. [3] This was not reflected in our study. This difference can be explained by the inclusion criteria. The diagnosis of Burkitt's lymphoma is made mainly by fine-needle aspiration

Table 1. Study patient's age and sex

Parameter		n	%
Age	30-40	1	2.6
	40-50	4	10.3
	50-60	13	33.3
	60-70	14	35.9
	≥ 70	7	17.9
	Mean age ± SD	60.28 ± 9.93	
Sex	Male	17	43.6
	Female	22	56.4

Abbreviation: SD, standard deviation

Table 2. Clinical features of study patients

Clinical features	n	%
Fever	22	56.4
Acutely ill	30	76.9
Splenomegaly	30	76.9
Adenopathy	5	12.8
Hepatomegaly	1	2.6

Table 3. Full blood count parameters

Parameters	Mean ± standard deviation	Minimum	Maximum
RBC (10^{12}/L)	3.1 ± 1.0	1.7	4.9
Hb (g/L)	87 ± 21	61	129
Hematocrit (%)	28.3 ± 7.5	18	41.9
MCV (fl)	91.1 ± 7.9	79.3	107.1
MCH (pg)	29.1 ± 4	24.7	37.6
MCHC (g/L)	32.0 ± 2.9	29.0	40.6
Platelets (10^9/L)	119.7 ± 54.2	58.0	243.0
WBC (10^{19}/L)	88.7 ± 94.3	3.1	945.9
Lymphocytes (10^9/L)	74.9 ± 80.6	2.9	910.5

Abbreviations: Hb, haemoglobin; MCH (pg), mean cell hemoglobin (picograms); MCHC, mean cell hemoglobin concentration; MCV (fl), mean cell volume (femtoliters); RBC, red blood cells; WBC, white blood cells.

Table 4. Conclusion of the immunophenotyping assays

Pathology		n	%
Reactive lymphocytosis		5	12.8
Acute Leukemia		4	10.3
Chronic lymphoid malignancies			
	Myeloma or Plasma cell leukemia	1	2.6
	Waldenström disease	1	2.6
	NK proliferation	1	2.6
	Chronic lymphocytic leukemia	8	20.5
	Circulating phase of B cell non-Hodgkin's lymphoma	19	48.6
Total		39	100

Table 5. Immunophenotyping results for patients with reactive lymphocytosis

Patient number	Ly 10^9/ L	CD 45^+ %	CD 3^+ %	CD 3^-CD56^+%	CD 19^+ %	CD 20+ %	Κ %	λ %	Κ/λ
1	6.4	++ 74	78	17	6	5	66	34	1.94
2	3.5	++ 42	62	22	10	10	69	31	2.23
3	3.2	++ 15	78	17	5	4	76	24	3.17
4	5.0	++ 63	41	19	40	25			
5	31.6	++ 23	70	15	7	2	0	0	0

Abbreviation: Ly, lymphocytes.

or biopsy of involved lymph nodes. [1-2] The disease develops aggressively and is unlikely to present with lymphocytosis per se. Therefore our inclusion criteria excluded Burkitt's lymphoma cases.

The CLL patients were diagnosed in Binet stage A. [8] In 2009, Koffi [6] found more subjects in state B or C using a larger sample size in a retrospective study from 1994 to 2006 only based on clinical and morphological data. [6] This demonstrates the usefulness of immunophenotyping in establishing early diagnosis of CLL. [1-2] Moreover, flow cytometry is also very important for the follow-up of patients during the treatment of residual disease.[1-2] A large number (62.5%) of the CLL patients expressed CD38 (Table 7). This is a bad prognostic marker**.** [9-10] According to Ibrahim [9] and Durig, [10] CD38 identifies patients with aggressive disease and poor outcome.

Three patients presented with specific B-cell

Table 6. Immunophenotyping results for the patients when Matutes Score was not available

Patient number	Ly 10^9/L	CD 45^+ %	CD 3^+ %	CD 3^- $CD56^+$ %	CD 19^+ %	CD 20^+ %	Κ %	λ%	Κ/λ	Other characteristics	Conclusion
6	10.3	++ 20	56	34	9	4	70	30	2.3	Plasma cells	Myeloma or Plasma cell leukemia
7	3.6	++ 18	51	15	34	34	97	3	32.3	Lymphocyte, Plasmocyte, Ig M	Waldenström disease
8	50.9	++ 89	8	86	5	5	59	41	1.4	CD 3^-CD16^+	NK Proliferation
9	35.2	± 65	0		0	0				c MPO^-, CD 34^+, HLA-DR^+, CD 117^-, CD 33^-	Acute leukemia
10	70.0	± 80	50		0	0				c MPO^-, c $CD3^+$, HLA-DR^+, CD 5^+, CD 7^+	T-cell ALL
11	20.5	± 15	8	-	5					cMPO$^+$, CD 34^+, HLA-DR^+, CD 33^+	AML
12	7.4	± 20	15		28					Myeloblast/ cMPO$^+$, CD 34^+, HLA-DR^+, CD 33^+	AML-M2

Abbreviation: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ly, lymphocytes.

Table 7. Immunophenotyping results for CLL patients

Patient number	Ly 10^9/L	CD 19 %	CD 5	CD 23	CD 43	Fmc7	CD 79b	CD 20	sIg	Κ %	λ%	Κ/λ	MS	CD 38
13	69.4	+ 80	+	+	+	-	+	±	+	100	0		5	-
14	30.0	+ 69	+	+	+	-	+	+	+	2	98	0.02	5	-
15	276.5	+ 88	+	+	+	+	+	+	+	5	95	0.05	4	+
16	910.5	+ 96	+	+	+	-	+	+	+	2	98	0.02	5	+
17	21.9	+ 83	+	+	+	-	±	±	±	2	98	0.02	5	+
18	33.7	+ 90	+	+	+	-	+	+	+	100	0	0	5	+
19	258.5	+ 93	+	+	+	-	+	+	+	4	96	0.04	5	-
20	188.5	+ 97	+	+	+	-	+	+	+	0	100		5	+

Abbreviations: Ly, lymphocytes; MS, Matutes score ; sIg, surface immunoglobulin.

Table 8. Immunophenotyping results for B-cell NHL patients

Patient number	Ly 10^9/L	CD 45 %	CD 19 %	CD 5	CD 23	CD 43	Fmc7	CD 79b	CD 20	sIg	K %	λ %	K/λ	MS/5	Conclusion
21	184.0	++ 96	+ + 91	-	±	-	+	+	++	++	99	1	99	1	PLL
22	56.1	++ 97	++ 91	+	-	-	++	++	++	++	2	98	0.02	1	MCL
23	44.9	++ 90	++ 87	-	-	-	+	+	++	+	99	1	99	2	MZL
24	19.6	++ 84	++ 71	-	-	-	+	+	++	+	0	100		0	MZL/PLL
25	78.2	++ 93	++ 93	+	±	-	++	++	++	++	100	0		2	MZL/MCL
26	35.4	++ 90	++ 87	-	+	-	+	+	++	+	100	0		1	B-NHL/MZL
27	25.1	++ 77	++ 62	-	-	-	+	+	++	+	99	0		0	B-NHL/MZL
28	11.5	++ 90	++ 55	+	±	-	++	++	++	++	99	1	99	2	B-NHL/MZL
29	333.9	++ 97	++ 95	-	-	-	++	++	++	++	11	89	0.12	0	B-NHL/MZL
30	17.7	++ 84	++ 81	-	-	-	++	++	++	++	97	3	32.3	0	B-NHL/MZL
31	56.2	++ 93	++ 89	±	-	-	+	+	++	++	1	99	0.01	1	B-NHL
32	9.6	++ 78	++ 72	-	+	-	+	+	++	+	80	20	4	1	B-NHL
33	11.7	++ 69	++ 39	-	-	-	+	+	++	+	8	92	0.09	0	B-NHL
34	2.9	++ 20	+ 19	±	±	-	++	+	++	+	98	3	32.7	2	B-NHL
35	88.8	++ 95	++ 91	-	-	-	+	+	++	+	100	0		0	B-NHL
36	27.5	++ 87	++ 90	-	+	-	+	+	++	+	100	0		1	B-NHL
37	75.2	++ 96	++ 89	-	+	-	+	+	++	+	0	100		1	B-NHL
38	4.5	++ 68	+ 53	-	-	-	++	++	++	++	99	1	99	0	B-NHL
39	18.4	++ 71	++ 68	-	-	-	++	++	+		0	0		0	B-NHL

Abbreviations: Ly, lymphocyte; MCL, Mantle cell lymphoma, MS, Matutes score; MZL, Marginal zone lymphoma; NHL, non Hodgkin lymphoma; PLL, Pro-lymphocytic leukaemia; sIg, surface immunoglobulin.

lymphoproliferative disorders: marginal zone lymphoma, mantle cell lymphoma and pro-lymphocytic leukemia (Tables 4 and 8). However in 16/19 patients (84.2%) flow cytometry did not give precise details about the nature of the B-cell lymphoproliferative disorder. This result underlined the necessity to associate flow cytometry with molecular biology to achieve an accurate diagnosis. For example, we did not investigate the translocation t(11; 14) (q13; q32) leading to an aberrant expression of cyclin D1, which is not expressed in normal lymphocytes. [11] Cyclin D1 is a key finding in mantle cell lymphoma pathogenesis. [11]

In some cases flow cytometry results led to a search for marginal zone lymphoma (Table 8). This could be explained by the high prevalence of infection by hepatitis B virus (HBV) or C virus (HCV) in Cote d'Ivoire. [12] There is plenty of data confirming that HBV and HCV infection is a predisposing factor for a B-cell NHL. [13-14] Indeed the chronic stimulation of the immune system by chronic infections (*Helicobacter pylori*, HCV) and autoimmune conditions could lead to marginal zone lymphoma.

The haematological malignancies encountered in this study were mostly indolent lymphomas. Their clinical behavior is heterogeneous. With some patients the disease is aggressive; causing their death within 2 years. Whereas others stay alive for decades without any need for treatment. [15]

CONCLUSION

Patients with lymphocytosis in Abidjan are likely to suffer from chronic B-cell lymphoproliferative disorders. Immunophenotyping enables early diagnosis and prognostication for these disorders.

FOOTNOTES

Conflict of interest: The authors declare no competing conflicts of interest.

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