Leah Shepherd
Research Department of Infection and
Population Health
University College London
Tel: +44 20 7794 0500
ext 34570
e-mail: leah.shepherd@ucl.ac.uk

The Extent of B-cell Activation and Dysfunction Preceding Lymphoma Development

L Shepherd[1], ÁH Borges[2], R Harvey[3], M Bower[4], A Grulich[5], M Silverberg[6], J Weber[7], M Ristola[8], J-P Viard[9], J Bogner[10], P Gargalianos-Kakolyris[11], C Mussini[12], K Mansinho[13], I Yust[14], D Paduta[15], D Jilich[16], T Smiatacz[17], R Radoi[18], J Tomazic[19], P Plomgaard[21], R Frikke-Schmidt[21], J Lundgren[2], A Mocroft[1] on behalf of EuroSIDA in collaboration with **The Binding Site**

[1]Research Department of Infection and Population Health, University College London, London, UK. [2]CHIP, Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark. [3]Charing Cross Oncology Laboratory and Trophoblastic Disease Centre, Charing Cross Hospital Campus of Imperial College Healthcare National Health Service Trust, London, UK. [4]National Centre for HIV Malignancy, Chelsea and Westminster Hospital NHS Foundation Trust, London, UK. [5]Kirby Institute, The University of New South Wales, Sydney, NSW, Australia. [6]Kaiser Permanente Northern California, Oakland, CA, USA. [7]Imperial College London, London, United Kingdom. [8]Division of Infectious Diseases, University of Helsinki and Helsinki University Hospital, Helsinki, Finland. [9]AP-HP, Centre de Diagnostic et de Thérapeutique, Hôpital de l'Hôtel-Dieu, Paris, France. [10]Department of Internal Medicine IV, University of Munich, Munich, Germany. [11]First Department of Internal Medicine and Infectious Diseases Unit, General Hospital of Athens "G. Gennimatas," Athens, Greece. [12]Clinic of Infectious and Tropical Diseases, University of Modena and Reggio Emilia, Azienda Policlinico, Modena, Italy. [13]Department of Infectious Diseases, Hospital Egas Moniz – Centro Hospitalar Lisboa Ocidental (CHLO), E.P.E., Lisboa, Portugal. [14]Ichilov Hospital, Israel. [15]GOCGEIOZ (Gomel Regional Centre for Hygiene), Gomel, Belarus. [16]Department of Infectious and Tropical Diseases, First Faculty of Medicine, Charles University, Prague, Czech Republic. [17]Infectious Diseases Department, Medical University of Gdańsk, Smoluchowskiego; 18 PL-80-214 Gdańsk, Poland.[18]Clinical Hospital of Infectious and Tropical Diseases "Dr. Victor Babeş",Bucharest, Romania.[19]Department of Infectious Diseases, University Medical Center Ljubljana, Ljubljana, Slovenia. [20]Department of Clinical Biochemistry Rigshospitalet, Copenhagen, Denmark

BACKGROUND

- Despite effective treatment, NHL and HL incidence in HIV+ people remains around 10 and 11 fold higher than in the HIV- population respectively.
- B-cell dysfunction and/or activation is thought to contribute to lymphoma development in HIV+ people, however the mechanisms driving pathogenesis of lymphomas in the HIV+ setting is poorly understood.

AIM

To describe the kinetics and predictive value of markers of B-cell dysfunction prior to lymphoma diagnosis.

METHODS

Design

A nested-case-control study of 73 HIV+ people with lymphoma (52 non-Hodgkin lymphoma and 21 Hodgkin lymphoma) and 142 lymphoma free HIV+ matched-controls were selected from the EuroSIDA cohort. Cases and controls were matched on date of first and last sample preceding lymphoma diagnosis (or equivalent date in controls), age and CD4 cell count at first sample, gender and region of Europe. Incidence-density sampling of controls was used to select controls.

Measurements

Prospectively stored plasma samples before lymphoma (or matched date in controls) were measured for markers of B-cell dysfunction and activation:
- Free light chain[FLC]-κ, and FLC-λ.
- Immunoglobulin [Ig]G and IgM.

Kits for measurements were provided by **The Binding Site.**

Statistical methods

- Mixed models were used to describe kinetics in the time before diagnosis (or equivalent date in controls).
- Conditional logistic regression investigated associations between markers and lymphoma <2 and >2 years prior to diagnosis.

RESULTS

Baseline

- 215 men were included, with a median of 2.0 (interquartile range [IQR]: 0.4–4.3) years between first and last sample, and 9 (IQR 4 - 32) months between last sample and lymphoma in cases.
- Baseline characteristics for cases and controls are shown in table 1. Cases differed from controls according to current HIV-Viral load (HIV-VL) and Cart use. Cases and controls were well matched on other demographic variables.

Trajectories

- The trajectories of immunoglobulins and free light chains prior to lymphoma diagnosis is shown in **figure 1**. After adjustment, the largest difference was observed for IgM, in which cases were declining by 7% (95%CI: -10, -3) per year, but levels were stable in controls (%Change per year: 0 95%CI-3, 3%, P for difference<0.01). Levels of IgG were also declining in cases, but stable in controls (P for difference was 0.10).

Factors associated with marker levels

- Associations between demographic and HIV-related factors and marker levels in the control population are shown in **figure 2**.
- Higher FLC-κ, FLC-λ, and IgG were all associated with lower current CD4 and higher FLC-κ and IgG and levels were also associated with not being on treatment.
- IgM was associated with higher HIV-VL and not being on cART, and borderline associated with higher Nadir CD4 and lower current CD4.

Odds of developing a lymphoma during prospective follow-up

- The odds of lymphoma development for a 2 fold-higher marker level ≤2 and > 2 years before lymphomas are shown in **Figure 3**.
- Associations were not evident ≤ 2 years prior to diagnosis, except for FLC- λ where the association was borderline significant.
- Proportionately high levels of both FLC-κ and FLC-λ (a marker of polyclonal expansion[1]) was associated with lymphoma >2 years prior to diagnosis (OR: 4.74 95%CI: 1.71 – 27.56), but not ≤ 2 years prior (1.62 94%CI: 0.54, 5.05). Having a disproportionately high level of one FLC (a marker of monoclonal expansion[1]) was not associated at either time point.
- Of the HIV related markers – HIV-VL AUC was associated with higher risk in sample ≤ 2 years prior to diagnosis, however, HIV-VL was predictive in the long term (**Figure 4**).
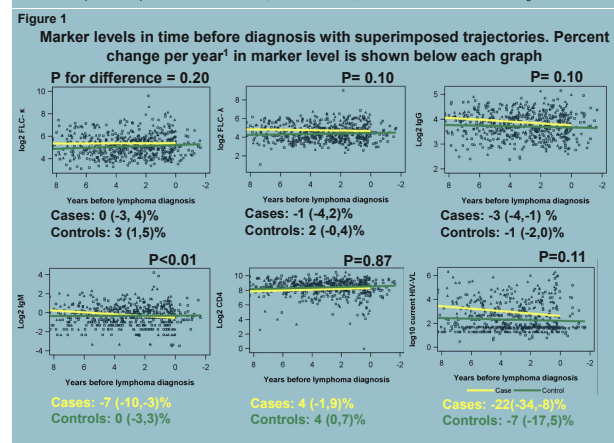
CONCLUSIONS

- FLC-lambda, FLC-kappa and IgG were higher >2 years before lymphoma diagnosis, but the difference diminished nearer diagnosis, which is in keeping with other studies.
- B-cell dysfunction, as demonstrated by polyclonal hyperglobulinemia, occurred many years prior to lymphoma development.
- The association between FLC- κ, FLC- λ, and IgG and lymphomas was weak and unlikely to be a strong candidate for identifying those at highest risk of developing lymphomas.
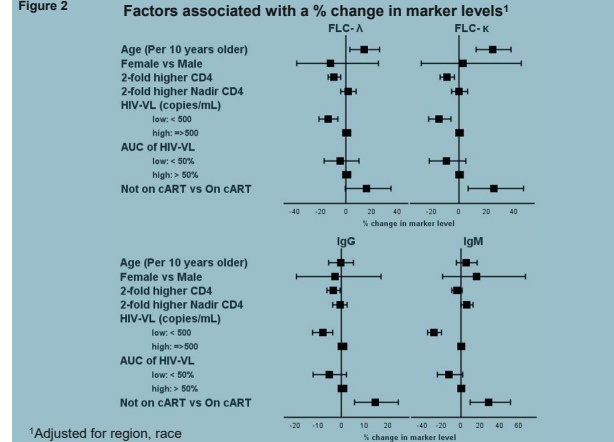
[1]Monoclonal elevations: FLC-kappa >19.4 mg/L or FLC-lambda > 26.3 mg/L and FLC-K/L not between 0.26 – 1.65. Polyclonal elevations: FLC-kappa >19.4 mg/L or FLC-lambda > 26.3 mg/L and FLC-K/L between 0.26 mg/L – 1.65 mg/L. Normal levels: FLC-κ <19.4 mg/L and FLC-λ <26.3 mg/L. A ratio of FLC-κ to FLC-λ [FLC- κ/ λ] outside of the range of 0.26 – 1.65 is considered abnormal

Table 1	Baseline[1] characteristics of cases and controls			
Factors	Total	Lymphoma diagnosis		P
		Cases	Controls	
	N (%)			
Total	216 (100)	73 (100)	143 (100)	-
Male gender[2]	193 (89.4)	65 (89.0)	128 (89.5)	-
Risk group				
Homosexual	114 (52.8)	41 (56.2)	73 (51.0)	0.39
Other	102 (47.2)	32 (43.8)	70 (49.0)	
Non-white Ethnicity	180 (83.3)	56 (76.7)	124 (86.7)	0.06
On cART[2]	158 (73.1)	46 (63.0)	112 (78.3)	0.02
	Median (IQR)			
Age (years)[3]	42 (37,50)	43 (37,51)	42 (37,49)	0.47
CD4 count (cells/mm3)	318 (208,477)	316 (180,500)	319 (213,461)	0.06
Log10 HIV VL (copies/ml)	2.3 (1.7,3.3)	2.8 (1.9,4.0)	2.1 (1.6,2.9)	<.01
Log10 AUC of HIV VL (copy years/ml)	4.3 (3.6,5.0)	4.5 (3.6,5.1)	4.2 (3.6,5.0)	0.51
FLC-λ (mg/L)	21.8 (14.5,31.4)	27.7 (19.2,45.0)	18.6 (13.7,27.2)	<.01
FLC-κ (mg/L)	32.0 (23.4,50.5)	39.9 (28.1,62.1)	29.4 (20.8,41.0)	<.01
IgG (g/L)	14.3 (11.1,17.8)	15.8 (12.5,19.6)	13.2 (10.3,16.9)	<.01
IgM (g/L)	0.8 (0.5,1.3)	1.1 (0.7,1.7)	0.8 (0.5,1.1)	0.02

[1]Date of first plasma sample was considered baseline, [2]Matched variable, [3]ART defined as ≤2 antiretroviral drugs

Figure 1

Marker levels in time before diagnosis with superimposed trajectories. Percent change per year[1] in marker level is shown below each graph

Figure 2

Factors associated with a % change in marker levels[1]

[1]Adjusted for region, race

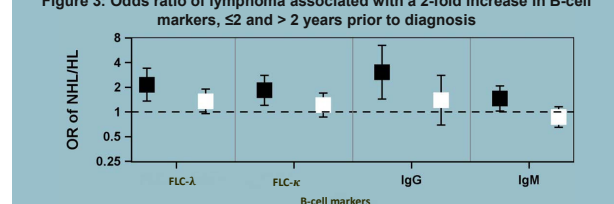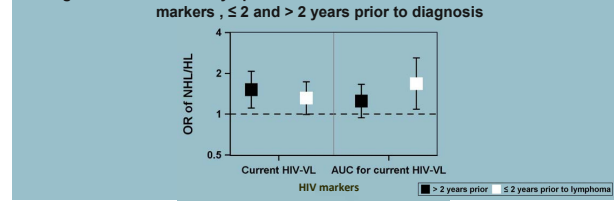Figure 3: Odds ratio of lymphoma associated with a 2-fold increase in B-cell markers, ≤2 and > 2 years prior to diagnosis

Figure 4: Odds ratio of lymphoma associated with a 10-fold increase in HIV markers , ≤ 2 and > 2 years prior to diagnosis