Abstract number: P01/02
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Reduced proportion of peripheral CD4+ T-cells expressing IL-7 receptor (IL-7R, CD127) as peculiar feature of Late Presentation of HIV infection

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HIV in Europe
Working Together for Optimal Testing and Earlier Care

Introduction

In Europe 15-51% of HIV positive patients are diagnosed late [1]. Compared to patients diagnosed with HIV early in the course of infection, late presenters are at higher probability of clinical progression and treatment-related adverse events and introduce HAART with a CD4+ T-cell count under the defined threshold recommended by current antiretroviral therapy guidelines [2]. Furthermore, late presentation impacts on healthcare system and community in terms of resource use and higher risk of HIV transmission to sexual partners [3].

The definition of Late Presentation is based on immunological status at presentation, previously it was defined as CD4+ T-cell count < 200 cells/mmc, though the cut-off has been raised to CD4+ < 350 cells/mmc, based on the evidence form large cohort studies, demonstrating that initiation of HAART with CD4 counts less than 350 cells/mmc is associated to increased risk of AIDS or death [4].

Demographic factors associated with Late Presentation are well known; in fact, patients diagnosed late with HIV are more commonly heterosexuals, older and migrants [5]. A more specific characterization of immune system perturbations associated with Late Presentation are not still well understood.

Thus, early identification of HIV infected individuals and a better comprehension of immunological parameters characterizing different stages of immune depletion in HIV positive patients could allow an optimization of the choice of antiretroviral therapy time and regimen.

Rational and Objectives

Detailing the alterations of immune system homeostasis associated with late presentation would allow the identification of early immunological markers to complement CD4+ T-cell count as predictors of clinical outcome. Further, a better knowledge of T lymphocytes alterations characterizing late presentation could help the choice of optimized antiretroviral therapy of patients diagnosed late with HIV. Thus, we aimed to describe immune phenotypes associated with newly HIV diagnoses at different degrees of immune depletion.

Methods

We enrolled patients newly diagnosed with HIV at out Clinic in the period 2007-2011. Patients were stratified by CD4+ T-cell count at presentation in:

- **Late Presenters (LP)**: patients with CD4+ T-cell count < 350 cells/mmc and/or an AIDS disease at presentation
- **Intermediate Presenters (IP)**: patients with CD4+ T-cell count between 350-500 cells/mmc at presentation
- **Early Presenters (EP)**: patients with CD4+ T-cell count >500 cells/mmc at presentation

T lymphocyte immune-phenotypes were evaluated by flow cytometry: CD8/CD4/CD127, CD8/CD4/CD95, CD8/CD38, CD8/CD38/CD45R0.
ANOVA with Dunnett's T3 test for multiple comparisons and Chi square tests were used to analyze eventual differences between LP, IP and EP.
The identification of peripheral T lymphocytes immune phenotypes independently associated with LP was explored by two models of multivariate logistic regression, adjusted for age, risk factor for HIV infection and viral load:
- **model 1:** we performed the logistic regression selecting patients IP and LP, excluding EP patients from the analysis (covariates: CD95+CD8+%, CD38+CD8+%, CD45RO+CD38+CD8+%, CD127+CD4+%);
- **model 2:** we performed the logistic regression selecting patients EP and LP, excluding IP patients from the analysis (covariates: CD38+CD8+%, CD127+CD4+%).
Given the recommendation to start cART with CD4+=350-500/mmc, we performed a third model of logistic regression to analyze immunological parameters associated with IP (**model 3** conducted selecting patients EP-IP, excluding LP patients from the analysis, covariates: CD127+CD4+%, CD38+CD8+%, CD45RO+CD38+CD8+%).
Factors with p<0.05 in univariate analysis entered the multivariate models.

Results

260 patients were newly diagnosed with HIV between 2007 and 2011 at our Clinic. The majority of patients were LP (144, 55%), 36 (14%) were IP and 80 (31%) were EP.
Compared to EP, LP were characterized by older age, acquired HIV infection more frequently by heterosexual contacts and displayed higher HIV-RNA loads (Table 1).

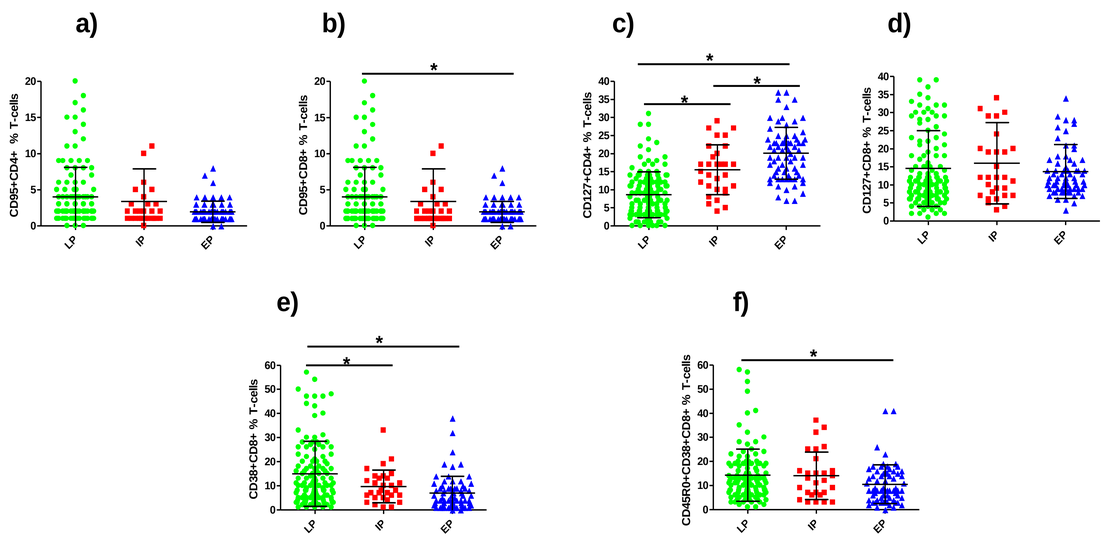
Table 1. Demographic characteristics of LP, IP and EP

Baseline characteristics	LP (n 144)	IP (n 36)	EP (n 80)	p
Age, years	43 (±12.6) [a]	38 (±9.5)	37 (±10) [a]	**0.0001**
Heterosexuals (n, %) °	80 (55%) [a, b]	12 (33%) [b]	22 (27%) [a]	**0.0001**
Log_{10} HIV-RNA cp/mL	5.01 (±0.99) [a, b]	4.58 (±0.76) [b]	4.39 (±0.97) [a]	**0.0001**
CD4+%	14 (± 0.6) [a, b]	25 (± 1) [b, c]	31 (± 0.7) [a, c]	0.0001
CD4+ cells/mmc	184 (± 10) [a, b]	438 (± 7) [b, c]	684 (± 18) [a, c]	0.0001
CD8+%	47 (± 1) [a, b]	51 (± 2)	47 (± 1)	0.09
CD8+ cells/mmc	596 (± 26) [a, b]	979 (± 72) [b]	1125 (± 56) [a]	**0.0001**

NOTE Table 1. Data are presented as mean, (SD) and ° absolute number, (%). Differences between groups were compared by ANOVA and ° Chi square test. a, b, c: p<0.05 by Dunnett's T3 test for multiple comparisons. CD4+ T-cell count are significantly different among the three groups by definition.

LP were characterized by higher percentages of apoptotic CD95+CD8+, activated CD38+CD8+ and terminal differentiated CD45R0+CD38+CD8+ T-cells and by lower percentages of central memory CD127+CD4+ T-cells, compared to EP. Further, we observed differences also between LP and IP: in fact, LP displayed higher immune activation (expressed by CD38+CD8+%) and down regulation of IL-7 receptor (CD127) on CD4+ T-cells in comparison with IP. Despite relatively conserved CD4+ T-cell count (350-500 cells/mmc), IP presented lower CD127+CD4+ percentages compared to EP. Figure 1 shows peripheral T lymphocytes characterizing LP, IP and EP.

Figure 1. Peripheral T lymphocytes immune phenotypes of LP, IP and EP



NOTE Figure 1. Comparison of mean CD95+CD4+% (a), CD95+CD8+% (b), CD127+CD4+% (c), CD127+CD8+% (d), CD38+CD8+% (e) and CD45R0+CD38+CD8+% (f) between LP, IP and EP (*, p<0.05 by ANOVA and Dunnett's T3 test for multiple comparisons).

In the model-1 of logistic regression (LP-EP), LP resulted significantly associated with heterosexual contacts and a reduction of CD127+ expression on CD4+ T-cells.
The model-2 (LP-IP) confirmed lower CD127+CD4+% the only parameter independently associated with LP.
Interestingly, down regulation of CD127 on CD4+ T-cells resulted associated with a trend toward statistical significance with IP (model-3, IP-EP), (Table 2).

Table 2. Parameters indepentently associated with LP and IP

Model 1 (EP-LP), factors associated with LP	AOR	95%CI	p
Age (each year more)	1.04	0.99-1.09	0.1
HIV-RNA (each log_{10} cp/mL more)	1.51	0.9-2.53	0.11
Heterosexuals (vs other HIV risk groups)	4.14	1.45-11.82	**0.01**
CD95+CD8+% (each unit more)	1.25	0.94-1.66	0.12
CD38+CD8+% (each unit more)	1.03	0.97-1.1	0.33
CD45R0+CD38+CD8+ (each unit more)	0.96	0.89-1.03	0.29
CD127+CD4+% (each unit more)	0.8	0.74-0.87	**0.0001**
Model 2 (IP-LP), factors associated with LP	**AOR**	**95%CI**	**p**
Age (each year more)	1.04	0.99-1.09	0.1
HIV-RNA (each log_{10} cp/mL more)	1.26	0.77-2.07	0.36
Heterosexuals (vs other HIV risk groups)	2.21	0.82-5.98	0.12
CD38+CD8+% (each unit more)	1.02	0.96-1.07	0.54
CD127+CD4+% (each unit more)	0.88	0.82-0.94	**0.0001**
Model 3 (IP-EP), factors associated with IP	**AOR**	**95%CI**	**p**
CD38+CD8+% (each unit more)	1.04	0.97-1.11	0.28
CD45R0+CD38+CD8+% (each unit more)	1.003	0.94-1.07	0.93
CD127+CD4+% (each unit more)	0.92	0.85-1.003	0.058

NOTE Table 3. AOR, adjusted odds ratio; CI, confidence interval. Model 1 and 2 were performed to explore factors associated with LP: model 1 was conducted on patients EP and LP, excluding IP; model 2 was conducted on patients IP and LP, excluding EP. Model 3 was performed to analyze factors associated with IP and was conducted on patients EP and IP, excluding LP.

Conclusions

Late Presentation of HIV, defined as CD4+ T-cell count < 350 cells/mmc at HIV diagnosis according to the most recent definitions, is characterized by a contraction of CD4+ T-cells expressing IL-7 receptor (CD127), suggesting an impairment in homeostatic maintenance of memory T-cells. In multivariate analyses, no association was found between immune activation and Late Presentation; in previous works CD127 down-regulation and T cell activation has been demonstrated to be tightly connected in effector memory, but not in naïve T cells; further studies investigating CD127 expression on different T cells subsets could thus explain the association of disturbances in IL-7/IL-7 receptor axis and CD38 expression with Late Presentation in order to find a peculiar hallmark of Late Presentation correlated to disease progression and response to therapy. Further, the down regulation of CD127 observed also in IP patients could strengthen the rationale of an early HAART start, despite relatively conserved CD4+ T-cell counts.

References

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