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Pulmonary Function in HIV-1 Vertically Infected Children

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Abstract

Background: Despite reports of an increasing incidence of asthma in HIV-infected children, exploration of pulmonary function by spirometry has never been reported in this population in the HAART era.

Objective: The aim of this study was to determine the prevalence of spirometric abnormalities in HIV-1 infected children. We conducted a cross-sectional study of pulmonary function tests (PFT) in HIV-1 vertically infected children.

Methods: Spirometric values were measured in 17 HIV-1 chronically infected children and compared to matched healthy children. In HIV-1 infected children, the correlations between PFT and the determination of the single breath carbon monoxide diffusing capacity of the lung (TL_{CO}) and immunological and virological values were assessed.

Results: Overall, 11 of the 17 PFT were normal. Four showed mild distal obstruction. Two were considered restrictive. When compared with matched healthy children, the only difference was the FEV1/FVC ratio that was significantly lower in the patients' group (91.9% of predicted value versus 97.1%, $p<0.0001$). We found a positive correlation between TL_{CO} and the CD4/CD8 T-cell ratio ($p=0.012$) and viral load ($p=0.05$). We observed that FEF_{25-75} values increased with age and weight in healthy children ($p= 0.006$ and $p=0.007$ respectively), but not in infected patients.

Conclusions: Our results showed that chronic HIV-1 infection and/or continuous HAART exposure induce a specific response of the pulmonary immune system which may compromise its function with time. If confirmed, it may justify a careful follow-up of pulmonary function in vertically infected children.

Keywords: Pediatric HIV/AIDS; Pulmonary function; HAART

Introduction

Pulmonary complications have been a major cause of morbidity and mortality in children with HIV infection [1]. Before the introduction of highly active antiretroviral therapy (HAART), opportunistic pulmonary infections, mainly *Pneumocystis jiroveci (carinii)* pneumonia and, to a lesser extent, *Streptococus pneumoniae* pneumonia, were frequent, potentially lethal complications [1-4]. Introduction of HAART in the mid-eighties and improvements in prophylaxis resulted in an impressive decrease of their incidence [2-4]. Besides infections, pulmonary complications include a large number of clinical and radiological conditions in which the lung immune system plays a central role, such as lymphocytic interstitial pneumonia (LIP) or immune reconstitution inflammatory syndrome (IRIS) [1,4]. In the pre-HAART era, HIV-infected subjects had a high prevalence of respiratory complaints [5] and HIV infection was associated with accelerated development of emphysema, airway obstruction, reduced diffusing capacity for carbon monoxide and airway hyperresponsiveness [6-9].

Since the development of effective ART, obstructive lung diseases, such as asthma and chronic obstructive pulmonary disease, are becoming a growing concern in this population [9-12]. Recent studies demonstrated that 31 to 64% of HIV-infected adults have respiratory symptoms with up to 20% of airway obstruction determined by spirometry [13,14], and 20% of doctor-diagnosed asthma [15]. Likewise, a greater incidence of asthma was reported in HIV-infected versus non-infected children [16,17]. Foster et al. reported an association between HAART treatment and asthma-medication use in HIV-infected children [18]. However, we did not find any data regarding pulmonary function testing in the HAART era in HIV-infected children in the literature.

Methods

We conducted a cross-sectional study of pulmonary function tests (PFT) in HIV-1 vertically infected children followed at the Nice University Hospital. Inclusion criteria were: materno-foetal transmission, age 10 to 18 years old, no smoking, no acute or chronic lung disease and informed parental consent and child assent. Respiratory symptoms were assessed by questioning the patient and his parents regarding the prevalence of chronic cough, wheezing, shortness of breath at rest, dyspnea on exertion, use of any inhaled medication, and recurrent phlegm production.

Peripheral blood T-lymphocytes and subclass assays (CD4, CD8, CD19, CD16/56) were conducted by flow cytometry. Real-time plasma HIV RNA values were determined using the Cobas Ampliprep®-Cobas TaqMan® HIV Monitor assay (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. Undetectable viral load was defined as PCR-RNA below 1.60 $\log_{10}$ or 40 copies/mm³.

PFT evaluation included total lung capacity (TLC), slow vital capacity (VC), Forced expiratory flows (forced viral capacity: FVC,

forced expiratory volume in one second: FEV_1 and forced expiratory flow during the middle half of FVC: FEF_{25-75}) and carbon monoxide diffusing capacity (TL_{CO}) using the single breath-hold method. Measurements were performed with a water-sealed spirometer Sensormedics 2400-Viasys (Cardinal Health Inc., Dublin, OH, USA) in accordance with international criteria [19-21]. Results were expressed as raw values and percentages of predicted values according to the anthropometric data of each patient. PFT measurements were compared to a cohort of age, height, weight and gender-matched healthy children. Comparisons of qualitative parameters were done with the Fisher's exact test. Correlations between quantitative values used regression model. Adjusted R squared values (adjusted R^2) are furnished. Quantitative results between sub-groups of patients were compared with the Mann-Whitney-U-test. All statistical analyses were performed using Statview® software 5.0 for Windows (SAS Institute, Cary, NC, USA). *p* values below 0.05 are considered to indicate statistical significance.

Results

Seventeen HIV-1 chronically infected children were included: 12 girls and 5 boys, with a mean age of 14.9 ± 2.4 years (range: 10.8-18.3) (Table 1). Nine were HIV stage N/A, 3 were stage B and 5 suffered from AIDS definition illness. None of the children suffered from acute or chronic respiratory disease or had any respiratory symptoms determined by respiratory questionnaire and clinical examination. Mean CD4 T-cell count at inclusion was 820 ± 301 cells/mm³ (range: 313-1,254) or $35 \pm 10\%$ (range: 16-50). Mean viral load was 1.79 ± 0.85 $\log_{10}$ copies/mm³ (range: 1.3-4.0). Twelve children had undetectable viral load. Each child had been exposed to an average of 6 ± 3 (range: 2-11) antiretroviral drugs since birth. At the time of enrolment, 4 patients received HAART with two nucleoside reverse transcriptase

Parameters	HIV-1 infected children	Matched healthy controls	p values
Male/female ratio	5/12	11/6	0.08
Mean age (years)	14.9 ± 2.4	13.6 ± 1.7	0.06
Mean weight (kg)	54.8 ± 14.4	51.7 ± 10.3	0.78
Mean Height (cm)	160.4 ± 8.9	131.1 ± 13.2	0.94

p values: Fisher's exact test for qualitatives comparison, Mann-Whitney for quantitatives comparison.

Table 1: Patients and healthy controls' characteristics.

Parameters	HIV-1 infected children	Matched healthy controls	p values
VC (L)	3.37 ± 0.72 (2.19-4.93)	3.69 ± 1.1 (2.51-6.58)	0.44
FEV1 (L)	2.90 ± 0.64 (2.05-4.59)	3.25 ± 1.0 (2.21-5.53)	0.34
FEF_{25-75} (L/s)	3.46 ± 0.96 (2.27-6.16)	4.08 ± 1.4 (2.55-7.40)	0.21
FEV1 / FVC (%)	$88 \pm 6\%$ (77-98)	$90 \pm 5\%$ (82-98)	0.43
TL_{CO} (ml/mn/Hg mm)	24.18 ± 9.3 (15.7-57.5)	NA	NA
Parameters (ppv)	HIV-1 infected children	Matched healthy controls	
VC	$101.4 \pm 14.7\%$	$103.5 \pm 15.5\%$	0.98
FEV-1	$92.6 \pm 12.5\%$	$100.2 \pm 13.9\%$	0.052
FEF_{25-75}	$98.1 \pm 21.2\%$	$112.1 \pm 24.6\%$	0.14
FEV1 / FVC	$91.9 \pm 7.7\%$	$97.1 \pm 6\%$	<0.0001
TL_{CO}	$102.9 \pm 23.8\%$	NA	

mean ± standard deviation and range for spirometric values. HIV-1 vertically infected children (n: 17) and healthy matched control (n: 17). Units: (L) liter, (L/s) liter per second, (ml/min/Hg mm) milliliter per minute per Hg millimeter. NA: non available, ppv: percentage of predicted value. Mean ± standard deviation for spirometric values expressed as percentages of predicted values according to the anthropometric data of each patient.

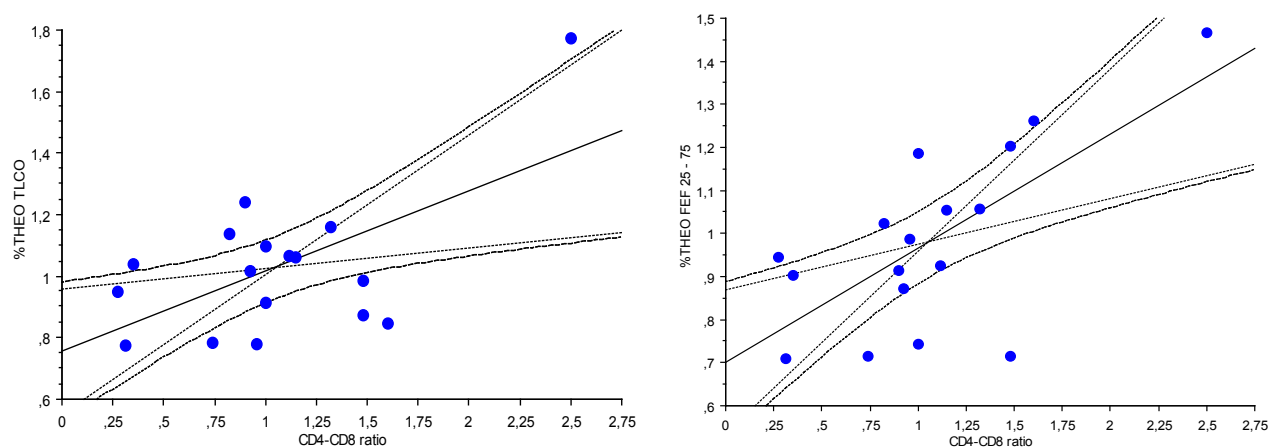p values: Mann-Whitney comparison

Table 2

inhibitors (NRTI) and 1 protease inhibitor (PI), 11 received 2 NRTIs + 1 non nucleoside reverse transcriptase inhibitor (NNRTI), 1 was on PI monotherapy, and 1 was receiving 1 drug of each class (NRTI + NNRTI + PI).

All of the children underwent successful PFT. Spirometric data were analyzed by 2 separate practitioners (CPB, MA). Overall, 11 of the 17 tests were normal. Four showed mild distal obstruction ($FEF_{25-75} < 70\%$ of the predicted value), with salbutamol reversibility. Two spirometric evaluations were considered restrictive (TLC < 80% of the predicted value). All TL_{CO} and FEV1/FVC ratios were in the normal ranges ($TL_{CO} > 80\%$ of the predicted value and FEV1/FVC>80%) (Table 2). We found a positive correlation between TL_{CO} and haemoglobin level (p=0.002), CD8 percentage (p=0.05), CD4/CD8 T-cell ratio (p=0.012 – R^2: 0.35 – Figure 1), viral load (p=0.05, R^2: 0.24) and FEF_{25-75} (p= 0.015, R^2: 0.33). FEF_{25-75} values were correlated with leucocytes count (p=0.05), haemoglobin level (p=0.03), percentage of CD4 (p=0.02) and CD8 (p=0.04) T-cells and CD4/CD8 ratio (Figure 1). We did not find any correlation between TL_{CO} or FEF_{25-75} and CD19⁺ B-lymphocytes, CD16/56⁺ NK cells, absolute CD4 or CD8 T-cells counts or duration of drug exposure.

When compared with matched healthy children, the only difference was the FEV1/FVC ratio that was significantly lower in the patients' group (91.9% of predicted value versus 97.1%, p<0.0001) (Tables 1 and 2). Furthermore, although FEF_{25-75} values were similar in the two groups, we observed that this parameter increased with age and weight in healthy children (p=0.006 and p=0.007 respectively), as was reported by Neukirch et al. [22], but not in infected patients (Figure 2).

Discussion

Our data shows that in vertically HIV-1 infected non-smoking adolescents, PFT values are in normal ranges, with a high prevalence of sub-clinical mild and reversible basal obstructive ventilatory impairment, as was observed in adult patients [9,10,14]. Similarly, in 1997 de Martino et al. published that children with perinatally acquired HIV-1 infection had higher airway resistance than matched controls, and that resistance decreased with age in the latter but not in infected patients [23]. We also observed that TL_{CO} analysis, which is a measure of gas exchange potential [24], demonstrated a strong correlation with the CD4/CD8 T-cell ratio and viral replication. In the mid-nineties, decreases in the TL_{CO} were described in patients with infectious lung disease, mainly *Pneumocystis* pneumonia [9-12]. In 1995, Rosen et al. published that advanced HIV infection, characterized by CD4 count < 200/mm³ or HIV-associated symptoms, were all associated with reductions in TL_{CO} measurements [9]. Conversely, Nieman et al. showed that patients with reduced TL_{CO} progressed more rapidly to AIDS [11]. TL_{CO} reduction may result from two main mechanisms: diffuse alveolar destruction (as observed in *Pneumocystis* pneumonia, pulmonary hypertension or fibrosis) or a decrease in alveolar expansion (as observed in neuromuscular disease) [24]. Patients with HIV infection receiving HAART may potentially suffer from each of these two mechanisms.

Recent data supported the fact that in adult patients, the use of HAART is independently associated with a decrease in FEV1/FVC after controlling for other independent risk factors such as age, cigarette smoking, intra venous drug use or previous pneumonia [14,15]. In our population, we also found a significantly lower FEV1/FVC ratio in our patients than in controls. Foster et al. suggested that the increased incidence of asthma in HAART-treated HIV-infected children may be driven by immunoreconstitution of CD4 T-cells [18]. They indeed

Figure 1: Correlation between TL_{CO} (%THEO TL_{CO} / left / p= 0.012), FEF_{25-75} (%THEO FEF_{25-75} / right / p= 0.03) and CD4/CD8+ T cell ratio, mean and slope. Full lines: slope; dotted lines: 95% confidence intervals
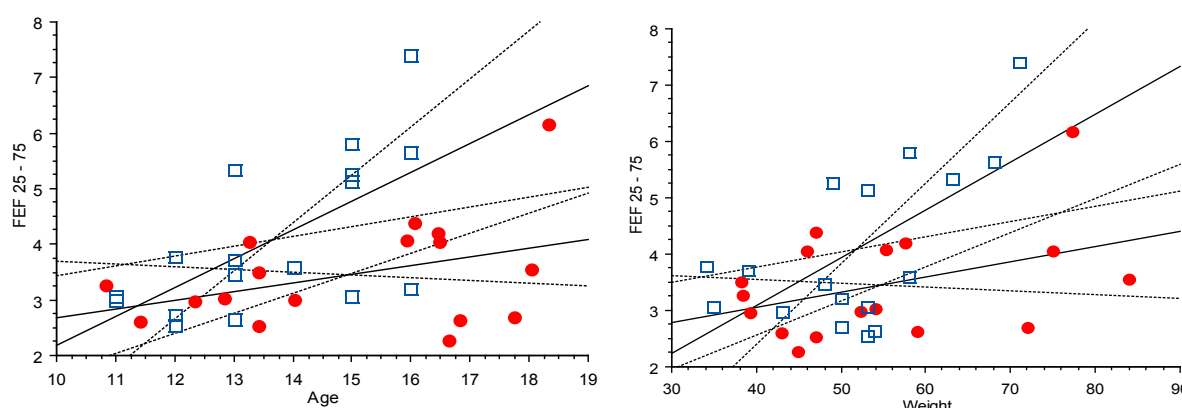

Figure 2: Correlation between FEF_{25-75} and age (years / left) and weight (kg / right) in HIV-1 vertically infected children (dot) and matched healthy controls (square). FEF_{25-75} is correlated with age and weight in healthy children but not in HIV-1 chronically infected children. FEF_{25-75}= 1.13+0.156*age; R^2= 0.149 - NS (HIV-1 infected children), FEF_{25-75}=-3.005+0.519*age; R^2=0.408 – p= 0.006 (matched healthy children). FEF_{25-75}= 1.985+0.27*weight; R^2=0.163 - NS (HIV-1 infected children), FEF_{25-75}=-0.317+0.085*weight; R^2=0.392 – p= 0.007 (matched healthy children). NS: non significant.

Full lines: slope; dotted lines: 95% confidence intervals.

showed that HIV+ HAART-treated children had the same cumulative incidence of asthma (around 30% by the age of 13) as non-infected children, whereas HIV-positive untreated children (who had lower CD4 and higher CD8 T cell percentages) had a thrice-lower incidence of asthma. This is indeed supported by the notion that CD4 T-cells are essential for the development of asthma in animal models [25,26]. Likewise, Gutin and Secord reported that the onset of asthma in HIV-infected children most frequently occurred within 3 years of beginning HAART [27]. Despite the fact that our patients were HAART-treated, none had developed asthma, and we did not find a significant relationship between FEV1 or FEV1/FVC and CD4+ or CD8+ T-cell counts or percentages in our patients. This could be explained by the relatively small sample size of our population.

We found however evidence of small-diameter bronchi obstruction (reduction in FEF_{25-75} in 4/17 of our patients), and a positive correlation between plasmatic CD4/CD8 ratio and FEF_{25-75}. It is possible that in pediatric patients, alterations of FEF_{25-75} may reflect or precede those observed in adult larger bronchi. Unfortunately none of our patients underwent broncho-alveolar liquid fluid (BALF) analysis or high resolution CT-scan. But even if the blood immuno-virological status does not reflect the exact events arising at the alveolar level, it is possible that our results reflect a particular response of the lungs to infection. In 1997 Lipman et al. compared BALF analysis (cells and cytokine levels) from HIV-1 infected patients with pneumonia and HIV-1 positive individuals with no respiratory disease to healthy controls and showed that alteration of the potential relevance to the pulmonary immune response are occurring in alveolar macrophages prior to the onset of respiratory disease [28]. Actual evidence for pulmonary tissue-level effects of HIV is conflicting. Alveolar macrophages and dendritic cells within the lung are targets for HIV infection and become increasingly infected as HIV infection progresses [3]. We recently showed that children's response to HAART is nowadays similar to that of adults with approximately 80% achieving an undetectable viral load and a good immunological response [29]. It is likely that with HAART, the plasmatic viral load decrease is associated with a reduction in the overall HIV lung concentration.

Altogether, the mechanistic link between HAART and airway obstruction is not precisely known. Potential explanations include: a) direct effects of HAART in the lung; b) chronic stimulation of inflammation induced by restoration of the immune system, with the release by CD8+ T-cells of inflammatory cytokines known to be implicated in airway inflammation [15]; c) the development of a hyperallergic state [16], as was shown by a higher sputum eosinophil count [15]; d) and/or the development of autoimmunity.

Although evidence of airway obstruction was small in our vertically HIV-infected HAART-treated pediatric population, these subjects face a lifetime of antiretroviral treatment and may be at risk of developing clinically significant disease as they age. Smoking avoidance, routine influenza and pneumococcal vaccinations and careful pulmonary follow-up must therefore be combined to decrease the risk of respiratory symptoms and airway obstruction in these patients.

Acknowledgements

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