

# 18F-FDG PET in HIV Infection: Correlation with Virological and Immunological Parameters in Different Clinical Stages of Disease

Miriam Cesari<sup>1</sup>, Arturo Chiti<sup>2</sup>, Katia Marzo<sup>2</sup>, Eva Orunesu<sup>3</sup>, Michela Pacei<sup>4</sup>, Giulia Bechi<sup>4</sup>, Fabio Franzetti<sup>1</sup>, Giovanni Lucignani<sup>3</sup>, Mario Clerici<sup>4</sup> and Andrea Gori<sup>5</sup>

<sup>1</sup> Department of Clinical Sciences, Infectious Diseases Section, "Luigi Sacco" Hospital, University of Milan, Italy; <sup>2</sup> Nuclear Medicine Division, Istituto Clinico Humanitas, IRCCS, Milan, Italy; <sup>3</sup> Institute of Radiological Sciences, University of Milan, Italy;

<sup>4</sup> Department of Preclinical Sciences, Chair of Immunology, LITA VIALBA, University of Milan, Italy; <sup>5</sup> Department of Internal Medicine, Chair of Infectious Diseases, "San Paolo" Hospital University Medical School, Milano, Italy

### ABSTRACT

**Background:** Antiretroviral therapy (ART) leads to suppression of HIV plasma viral load and to a degree of immunological recovery, but HIV lingers in lymphatic tissues. To evaluate tissutal HIV replication in different stages of disease progression we examined <sup>18</sup>F-fluoro-deoxy-glucose (FDG) uptake by Positron Emission Tomography (PET) in antiretroviral ART-treated and ART-naïve HIV infected patients. Immunologic parameters were evaluated as well.

**Methods:** Twenty-seven HIV outpatients were divided into 5 groups according to HIV plasma viremia, CD4+ counts, clinical diagnosis and presence/absence of ART. A visual score for degree and pattern of FDG uptake was adopted: A) no uptake; B) faint uptake; C) intense uptake. Maximum Standardized Uptake Value (SUVmax) in each identified lesion as well as SUVmax of spleen and oral lymphoid tissue was measured in all patients. Activation markers-expressing T lymphocytes and lytic/apoptotic proteins in HIV-specific CD4+ and CD8+ T lymphocytes were evaluated by flow cytometric analysis.

**Results:** FDG uptake was observed in nine patients, mostly ART-naïve or initiating ART after a diagnosis of AIDS. FDG uptake in spleen and oral lymphoid tissues was augmented in these same patients and in immunological non-responders. Activation markers-expressing CD4+ and CD8+ T lymphocytes were increased in FDG positive patients; gap-specific, perforin and granzyme positive CD8+ T lymphocytes were reduced in these same individuals. Initiation of ART in FDG positive patients was associated with disappearance of FDG uptake.

**Conclusions:** Absence of ART, a previous AIDS diagnosis, and the persistence of low CD4 counts correlate with increased FDG uptake. Immune activation and impaired synthesis of CD8 T lymphocyte lytic/apoptotic molecules is seen in FDG positive patients. FDG PET is an accurate tool for assessing lymphatic tissue activation, adding independent information to virologic and immunologic parameters.

### INTRODUCTION

PET studies with <sup>18</sup>F-fluoro-deoxy-glucose have been lately performed in HIV infection mostly to assess cerebral manifestation of AIDS and to differentiate lymphomas from opportunistic infections. PET has also been recently used to verify immune activation in animal models of AIDS and in HIV-infected patients in attempt to establish anatomical correlates of infection. The technique used in these pilot studies was whole body FDG PET, which is able to visualize sites of increased glucose metabolism after i.v. injection of a radiolabeled glucose analog. Activated lymphocytes perform high rates of glycolyses compared with resting cells and can therefore be visualized with FDG PET. HIV infection is characterized by immune activation and an increased rate of T lymphocytes metabolism, which are suggested to be negative prognostic markers. FDG PET could therefore be an optimal tool to visualize and classify disease progression. To evaluate possible correlations between immune parameters and FDG PET results, we performed in depth studies in ART-naïve and ART-treated patients in different stages of disease and with different response to therapy. ART can suppress HIV plasma viremia to virtually undetectable levels, nevertheless, current methodologies allow us only to measure the percentage of HIV that replicates in plasma. In attempt to visualize extra-plasmatic, tissutal sites of HIV replication in infected individuals, we performed FDG PET on a number of patients in different stages of HIV infection. Results shown herein suggest that diverse modes of FDG uptake can be recognized in patients with different clinical patterns of disease.

### METHODS

TABLE 1: PATIENT POPULATION

Twenty-five HIV-1 outpatients were consecutively enrolled and divided in 4 groups as follows. Two patients had one scan before starting the ART therapy and one after the treatment, so they appear in 2 groups and were counted twice.

	Group A (n=5)	Group B (n=6)	Group C (n=8)	Group D (n=6)	Group E (n=2)
<b>Current ART</b>	YES	YES	YES	NO	YES
<b>CD4+ count (cell/mL)</b>	<200	>500	>500	>300	>300
<b>HIV RNA (copies/mL)</b>	<50	<50	<50	ANY	>10,000
<b>Previous AIDS</b>	NO	YES	NO	NO	NO
<b>Age (years)</b>					
Median (IQ Range)	42 (41-43)	43 (42-43)	41 (37-45)	42 (36-47)	53 (50-56)
<b>Sex</b>					
Male	4	6	8	5	2
<b>Risk factors</b>					
Sexual	2	4	7	5	1
Injecting drug user	3	1	1	1	0
Unknown	0	1	0	0	1
<b>Nadir CD4+ T (cells/<math>\mu</math>l)</b>					
Median (IQ Range)	68 (28-77)	54 (25-65)	277 (233-329)	377 (295-565)	227 (203-251)
<b>Baseline CD4+ T (cells/<math>\mu</math>l)</b>					
Median (IQ Range)	131 (127-137)	675 (555-764)	598 (580-691)	536 (389-588)	456 (395-518)
<b>HIV-RNA (cp/mL)</b>					
Median (IQ Range)	<50	<50	<50	44627 (19723-109490)	123350 (106999-139701)
<b>Years of HIV infection</b>					
Median (IQ Range)	10 (7-18)	8 (7-8)	5 (3-11)	4 (2-7)	10 (9-10)
<b>Years on HAART</b>					
Median (IQ Range)	7 (4-10)	8 (2-11)	4 (1-7)	0	9 (8-10)

NOTE: IQ RANGE, interquartil range.

### FDG PET ACQUISITIONS AND ANALYSES

FDG was produced on site in the radiopharmacy of the Nuclear Medicine Department of Istituto Clinico Humanitas, following the European Pharmacopeia prescriptions.

Abnormal FDG uptake in nodal sites was defined as an activity greater than background in surrounding tissue and unrelated to sites of physiological tracer uptake or excretion. Interpretation criteria for positive lymph nodes were therefore derived from criteria used to define pathological lymph nodes in oncology studies.

Activity in the lymphoid tissue of the pharynx (tonsilla lingualis and tonsilla palatinae) and in the spleen was expressed as the maximum Standardized Uptake Value (SUVmax)

### IMMUNE ASSAYS

Whole blood was collected and used within 8 hours to evaluate: Activation markers expression by flow cytometric analysis (CD4+DR1 $\alpha$ , CD8 $\alpha$ /DR1 $\alpha$ , CD4+CD25+, CD8 $\alpha$ /CD25+, CD8 $\alpha$ /CD38+CD45RO+)

Gap-specific, IL-2 and IFN $\gamma$  expressing CD4+ T cells and IFN $\gamma$  as well as TNF $\alpha$  expressing CD8+ T lymphocytes

Perforin- and granzyme-expressing CD8 T lymphocytes upon gap-stimulation

### STATISTICAL ANALYSIS

Procedures were based on non parametric analyses (Mann-Whitney); comparisons between the different groups were made using a two-tailed T-test. Statistical analysis was performed using the SPSS statistical package (SPSS Inc. Chicago, Illinois, USA).

### RESULTS

FIGURE 1: FDG UPTAKE

Overall FDG uptake was augmented in ART-naïve compared to ART-treated patients. FDG uptake was classified as intense, faint or absent.

927 individuals had positive lymph nodes (see beside):

5 ART-treated patients with viral load < 50 copies/mL (1 immunological non-responder GROUP A, 4 full responders GROUP B and C).

3/5 ART-treated patients were characterized by a previous AIDS diagnosis (group B).

4 ART-naïve patients. The 2 ART-naïve patients with negative lymph nodes had increased uptake, respectively, in the oral mucosa and in the right colon.

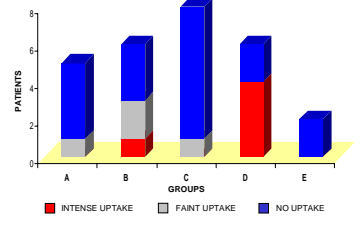


TABLE 2: FDG PET RESULTS IN LYMPH NODES AND IN THREE REGIONS OF INTEREST

PATIENT	PET	Ph SUV	Sp SUV	Th SUV
A1	No uptake	4.2	1.7	1.1
A2	No uptake	3.0	1.9	1.2
A3	No uptake	2.3	1.5	1.2
A4	Faint uptake	2.8	1.5	1.3
A5	No uptake	2.3	1.5	0.9
B1	Faint uptake	4.6	1.8	1.7
B2	Faint uptake	2.1	1.6	1.2
B3	No uptake	3.1	2.4	1.2
B4	No uptake	5.4	1.4	1.0
B5	Intense uptake	3.2	2.1	1.2
B6	No uptake	3.3	2.3	1.4
C1*	No uptake	2.6	2.4	1.2
C2**	No uptake	5.1	1.4	1.2
C3	No uptake	2.9	1.9	1.1
C4	No uptake	5.7	2.0	1.2
C5	No uptake	4.9	2.0	1.3
C6	No uptake	3.0	1.8	1.3
C7	No uptake	6.4	1.7	1.2
C8	Faint uptake	2.2	1.8	1.3
D1*	Intense uptake	3.6	2.5	1.2
D2**	Intense uptake	5.3	1.7	0.8
D3	No uptake	5.7	1.9	1.1
D4	Intense uptake	6.3	2.4	1.3
D5	Intense uptake	7.5	2.3	1.3
D6	No uptake	3.3	2.0	0.9
E1	No uptake	2.4	2.3	1.1
E2	No uptake	3.2	1.8	1.2

NOTE: PhSUV, SpSUV, ThSUV; SUVmax measured in pharyngeal lymphatic tissue, spleen and thymus.

Patient C1/C2\*\*: follow-up of patient D1/D2\*\* after ART introduction.

FIGURE 3: NEGATIVE SCANS

ART was initiated secondarily to a drop in CD4 count in two of the ART-naïve, FDG positive patients, shortly after the PET evaluation. A second PET was performed after 6 months of therapy and in both cases suppression of HIV viremia was associated with a negativization of the FDG-PET. Note FDG uptake in axillary nodes (Panel D) is completely cleared after therapy (E). FDG uptake was also negative for lymph nodes in two of the six ART-naïve patients. Interestingly, one patient was classified as a clinical long-term non-progressor and has very low level of viral replication.

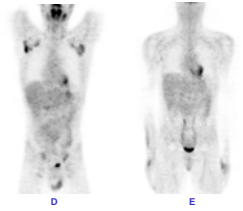
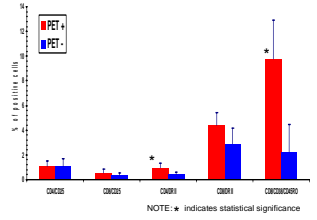


FIGURE 4: T LYMPHOCYTES IMMUNE ACTIVATION

CD4+DR1 $\alpha$  (p=0.008) and CD8 $\alpha$ /DR1 $\alpha$  (p=0.053) were augmented in FDG positive patients. No differences were observed between FDG positive, ART-naïve and ART-treated.



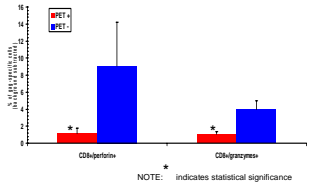
CD8 $\alpha$ /CD38+CD45RO+ T lymphocytes were reduced in ART-treated patients. These cells correlate with HIV plasma viral load and are predictor of poor response to therapy. They were significantly augmented (p<0.005) in FDG positive patients, independently from the therapy status. These cells were greatly augmented in the single FDG positive immunological non-responder.

FIGURE 5: EVALUATION OF LYTIC ACTIVITY OF HIV-SPECIFIC CD8+ T LYMPHOCYTES

Gap-specific perforin and granzyme positive CD8+ T lymphocytes

Both gap-specific CD8 $\alpha$ , perforin+ (p=0.042) and granzymes+ T lymphocytes were reduced in FDG positive patients (p=0.025) independently of the presence of ART.

Both CD8+ T cell populations were reduced in the FDG positive immunological non-responders patient compared to the immunological non-responder with a negative FDG result.



Gap-specific IL-2, IFN $\gamma$ , and TNF $\alpha$  positive T lymphocytes

IL-2 as well as IFN $\gamma$  expressing CD4+ and CD8+ T cells were augmented in FDG negative patients.

TNF $\alpha$  positive CD8+ T lymphocytes were reduced in these same individuals. These results, nevertheless, did not reach statistical significance.

### CONCLUSIONS

FDG PET could be useful to visualize extra-plasmatic, tissutal sites of HIV replication in HIV infected individuals. PET imaging in our study seems to confirm that actively replicating viruses are harbored not only in superficial lymph nodes but also in other lymphatic tissues.

Among patients under ART treatment a previous AIDS diagnosis was strictly associated with the persistence of PET positive scan, even late after the time of diagnosis, suggesting a direct correlation between AIDS and the persistence of reservoirs activation. Thus, these results have strong impact in the understanding of the poor immune reconstruction pattern characteristic of the advanced AIDS subjects.

FDG-PET positive patients were immunologically characterized by immune activation and a decrease of lytic activity, independently of HIV plasma viremia. These data indicate that immune activation in these patients is not directly caused by HIV replication in the peripheral circulation, but rather might be correlated with viral replication in the tissutal sites that have an increased FDG uptake.

On the other hand, initiation of ART in FDG positive patients, followed by suppression of HIV replication and CD4 count recovery, was associated with normalization of immunologic parameters and dampening of FDG uptake.

According to these immunologic findings, it is tempting to suggest that a FDG PET positive scan might be a direct, sensitive, and immediate tool to quantify the effectiveness of ART in suppressing HIV replication in extra-plasmatic compartments.

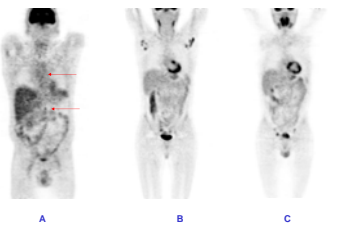


FIGURE 2: PATTERNS OF UPTAKE (examples)

Panel A: Coronal view of a patient with faint FDG uptake in mediastinal and perigastric lymph nodes (arrows).

Panel B and C: Intense and bilateral FDG uptake in cervical, axillary (B) and inguinal (C) lymph nodes. Note tracer uptake is clearly visible in right colon (B) and pharyngeal lymphatic tissue (C).