Poster #: 689

Activation and Senescence Markers in HIV Patients with Chronic Kidney Disease

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BACKGROUND

- ✓ Prevalence of chronic kidney disease (CKD) varies between 2 to 30% according to observational studies among HIV-infected patients[1].
- ✓ ART-treated HIV-infected individuals remain at higher risk for CKD than HIV-uninfected individuals[2].
- ✓ Recent studies have described associations between T cell immune activation (IA) and -senescence (IS), and chronic kidney disease[3].
- ✓ However, the link between a combination of IA and IS markers and CKD have not been assessed yet.
- ✓ **OBJECTIVE:** We aimed to evaluate the association between a score combining IA and IS and CKD in patients included in the ANRS CO3 Aquitaine Cohort (CIADIS sub-study).

METHODS

✓ **Study design**: cross-sectional study nested in the open prospective ANRS CO3 Aquitaine cohort (CIADIS sub-study).

✓ **Study population: Inclusion criteria**
- Patient followed in participating hospitals (South Western France);
- Informed consent to contribute to the biobank of the cohort;
- A planned blood sampling within the framework of the biobank;
- Viral load <40 cop/mL for at least 2 years;
- eGFR measurement available at inclusion (D0);

✓ **Definition of Chronic Kidney Disease (CKD)**: Estimated Glomerular Filtration Rate (eGFR) using the Modification of Diet in Renal Disease formula (MDRD)

✓ **Definition of Early Kidney dysfunction**: Urine protein-creatinine ratio (uPCr) >30 mg/mmol was combined with eGFR≥90 to define early kidney dysfunction by the following combination:
- eGFR ≥ 90 mL/min/1.73m^2; uPCR >30

✓ **Definitions of other non-AIDS-related comorbidities**
- Diabetes: ICD codes, hypoglycemic drug use or use of insulin, two consecutive blood glucose results ≥7 mmol/L
- Dyslipidemia: ICD codes, statin or fibrate treatment
- Cardiovascular events: cardiovascular ICD codes, bypass surgery or angioplasty treatment, Central Nervous System (CNS) ICD codes, peripheral vascular ICD codes or endarterectomy treatment
- Degenerative CNS disorders: ICD codes
- Cancer: AIDS-related or non-AIDS-related cancer.
- Hypertension: two consecutive systolic blood pressure measurements (BP) ≥ 140 mm/Hg or diastolic BP ≥ 90 mm/Hg (within 2 years before D0) or with antihypertensive therapy.

✓ **Statistical analysis**
- **Main outcome variables:** The association between the CIADIS score and CKD was evaluated for two separate outcomes: i) eGFR <60 mL/min/1.73m^2 and ii) eGFR<90 mL/min/1.73m^2.
- **Main explicative variables:** The CIADIS score was determined with CD4 and CD8 activation (DR+), maturation (T naive, memory) and senescence (CD57+CD28-) markers[4]: positive values represent a phenotype with high IA and IS, negative values a profile with low IA/IS and high proportion of naive T cells.
- **Principal analysis:**
  - ➢ Analyses were performed for both eGFR <60 vs ≥60 and eGFR<90 vs ≥90.
  - ➢ Logistic regression models were used to evaluate the association between the CKD and the CIADIS score. Models were adjusted for age, sex, tenofovir (TDF) use and the number of non-HIV related comorbidities (≥2 among the followings: diabetes, cardiovascular events, dyslipidemias, hypertension and cancer) in addition to the CKD.
- **Subgroup analyses:** Comparison of the CIADIS score between patients having an eGFR ≥90 with uPCR >30 or uPCR≤30 using the non parametric Mann-Whitney test to study the association between early kidney dysfunction and IA and IS.

RESULTS

- ✓ Of 1010 patients included in the CIADIS sub-study, 849 were included in the current analysis (table 1), 35 patients were excluded for missing eGFR measurement and 126 patients for detectable viral load.
- ✓ We mainly present results from the analysis using a cut-off of 60 mL/min/1.73m^2

Table 1: Baseline characteristics

Characteristics	Total (N=849)	eGFR≥60 (N=819)	eGFR<60 (N=30)	P**
Male/Female, n (%)	626/223 (74/26)	609/210 (74/26)	17/13 (57/43)	**0.03**
Age (years)*	51 (45 ; 57)	50 (45 ; 57)	53 (50 ; 66)	**<0.01**
Contamination, n (%)				0,55
MSM	354 (42)	344 (42)	10 (33)	
Heterosexual	300 (35)	286 (35)	14 (47)	
Drug users	121 (14)	118 (14)	3 (10)	
Others	74 (9)	71 (9)	3 (10)	
Infection duration (years)*	17 (11 ; 22)	17 (10 ; 22)	18 (12 ; 23)	0.34
CDC classification (A/B/C), n (%)	423/233/193 (50/27/23)	412/227/180 (50/28/22)	11/6/13 (37/20/43)	**0.02**
Patients on HAART, n (%)				0.08
2NRTI + 1PI/r	354 (42)	341 (42)	13 (43)	
2NRTI + 1NNRTI	259 (31)	255 (31)	4 (13)	
Others	233 (27)	220 (27)	13 (44)	
History of Tenofovir use, n(%)	717 (85)	694 (85)	23 (77)	0.30
Time since first HAART (years)*	13 (8;16)	13 (8 ; 16)	16 (9 ; 17)	0.27
Viral suppression duration (years)*	5 (2 ; 9)	5 (2 ; 9)	7 (3 ; 9)	0.71
CD4+ nadir (cells/mm³)*	204 (101 ; 303)	210 (105 ; 303)	139 (59 ; 216)	**0.04**
Time from CD4+ nadir (years)*	8 (5 ; 15)	8 (5 ; 14)	10 (6 ; 16)	0.27
Last CD4+ (cells/mm³)*	578 (429 ; 760)	579 (431 ; 760)	526 (374 ; 733)	0.41
CD4/CD8 ratio*	0.9 (0.6 ; 1.2)	0.9 (0.6 ; 1.2)	0.8 (0.4 ; 1.4)	0.72
CMV, n(%)	614 (89)	591 (89)	23 (89)	0.22
HCV infection, n(%)	200 (24)	191 (24)	9 (32)	0.28
HBV infection, n(%)	62 (8)	57 (7)	5 (17)	0.06

* Values are medians (IQR) unless stated otherwise.
***Student's t-test or Mann-Whitney U test for quantitative variables / Chi-square test or Fisher's exact test for qualitative variables*

- ✓ Patients with eGFR<60 were older (53 vs. 50 years, p<0.01), were more frequently females (43% vs. 26%, p=0.03) and in CDC stage C (43% vs. 22%, p=0.02) and had a lower CD4+ nadir (P=0.04) compared to patients with eGFR ≥60. TDF use, CD4+ cell count or HCV infection were not different between patients presenting with eGFR ≥60 or <60. There was a trend of higher proportion of HBV co-infected patients in patients with eGFR <60 (p=0.06).
- ✓ Comparing patients with eGFR<90 to those with an eGFR ≥90, patients with an eGFR <90 were older (54 vs. 49 years, p<0.01), more likely to have an ongoing or previous TDF-containing regimen (p=0.01), and had lower CD4+ nadir (p=0.04). A trend for higher proportion for HCV co-infection was seen for patients with an eGFR <90 (p=0.06). None of the other variables tested were different between these groups. (Data not shown).

Table 2: Comorbidities

Comorbidities	Total (N=849)	eGFR ≥ 60 (N=819)	eGFR < 60 (N=30)	P*
Diabetes, n (%)	111 (13)	101 (12)	10 (33)	<0.01
Dyslipidemia, n (%)	307 (36)	289 (35)	18 (60)	<0.01
Cardiovascular events, n (%)	84 (10)	76 (9)	8 (27)	<0.01
Hypertension, n (%)	290 (34)	279 (34)	11 (37)	0.61
Degenerative CNS, n (%)	24 (3)	24 (3)	0 (0)	1.00
Cancer, n (%)	117 (14)	109 (13)	8 (27)	0.05
Nb of comorbidities ≥ 2, n (%)	256 (30)	239 (29)	17 (57)	<0.01

* *Chi-square test or Fisher's exact test for qualitative variables*

- ✓ The proportion of all comorbidities was higher among patients with an eGFR <60 (p<0.01), except for hypertension and degenerative CNS disorders. Patients with an eGFR <60 were also more likely to present two or more comorbidities (57% vs. 29%, p<0.01).
- ✓ Same results were found for the groups defined by eGFR ≥90 vs. <90 except for hypertension (32% vs. 46%, p<0.01) (Data not shown).

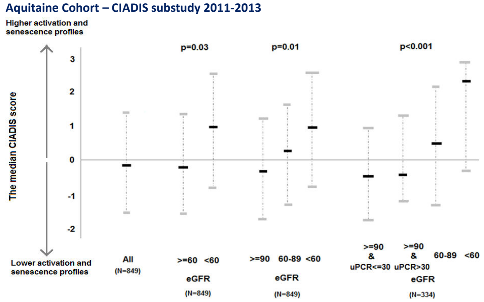
Table 3: Immune markers in the CIADIS score

Immune markers	Total (N=849)	eGFR ≥ 60 (N=819)	eGFR < 60 (N=30)	P**
Lymphocytes T CD4*				
CD4+ DR+ (%)	14 (10;19)	14 (10 ; 19)	16 (12;22)	0.03
CD4+CD57+CD28- (%)	3 (1;7)	3 (1 ; 7)	5 (1;11)	0.13
CD4+TN (%)	40 (28;51)	40 (29 ; 51)	34 (22;46)	0.08
CD4+TEMRA (%)	1 (0;3)	1 (0 ; 4)	2 (1;3)	0.29
Lymphocytes T CD8*				
CD8+ DR+ (%)	35 (26;46)	35 (25 ; 46)	40 (30;51)	0.10
CD8+CD57+CD28- (%)	27 (17;36)	26 (17 ; 36)	30 (20;42)	0.14
CD8+TN (%)	38 (29;49)	39 (30 ; 49)	30 (22;46)	0.03
CD8+TEMRA (%)	25 (16;36)	25 (16 ; 36)	23 (15;43)	0.57

* Values are medians (IQR) unless stated otherwise / ***Student's t-test or Mann-Whitney U test for quantitative variables*

- ✓ Looking at the different immune markers used to construct the CIADIS score separately, patients with an eGFR <60 compared to those with and eGFR >=60 had higher percentages of CD4 T cells expressing CD4+ DR (16% vs. 14%, p=0.03) and lower percentages of CD8+ naïve T cells (30% vs. 39%, p=0.03), respectively.
- ✓ Patients with an eGFR <90 compared to those with an eGFR >=90 had higher percentages of CD4 T cells expressing CD4+ CD57+CD28- (4% vs. 2%, p<0.01), CD8 T cells expressing CD8+ CD57+CD28- (28% vs. 26%, p<0.01) and lower percentages of CD8+ naïve T cells (37% vs. 40%, p<0.01), respectively (Data not shown).
- ✓ The distribution of the CIADIS score (combining the above described markers) according to eGFR stages is depicted in Figure 1: the lower the eGFR, the higher the CIADIS score.

Figure 1: Description of median CIADIS score between eGFR groups in the ANRS CO3 Aquitaine Cohort – CIADIS substudy 2011-2013



Legend: eGFR, estimated Glomerular Filtration Rate. P/C, Ratio proteinuria on creatininuria. Black lines show the median values of CIADIS score, dotted lines between grey lines represent the interquartile range. The Mann–Whitney test was used to compare the score between groups defined by eGFR.
A median value of CIADIS score above 0 represents an immune phenotype with higher T cell activation, expression of terminally differentiation and senescence markers whereas negative values of CIADIS score represent a less activated and less senescent profile.

- ✓ The median CIADIS score was -0.1 (IQR -1.5;1.5) and was significantly higher in patients with reduced kidney function. The median CIADIS score was higher when eGFR<60 (0.99[IQR -0.74;2.61]) compared to eGFR >=60 (-0.19 [IQR -1.57;1.43]) (p=0.03). Same results were found for the groups defined by eGFR ≥ 90 vs. <90 (Data not shown).
- ✓ Among 334 patients with available eGFR and uPCR, 221 patients had an eGFR ≥90. 9% of them had an early kidney dysfunction. There was no difference in the CIADIS score between those having a uPCr ≤30 or >30 when eGFR ≥90 (*P*=0.46).

Figure 2: Logistic regressions of CKD (eGFR<60 vs. eGFR≥ 60) adjusted by the number of comorbidities

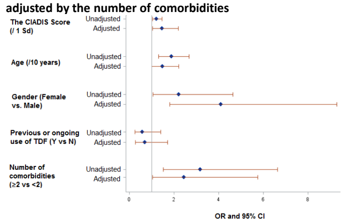


Figure 3: Logistic regressions of CKD (eGFR<60 vs. eGFR≥ 60) adjusted by diabetes

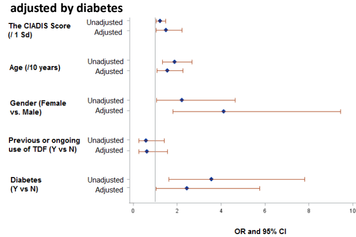
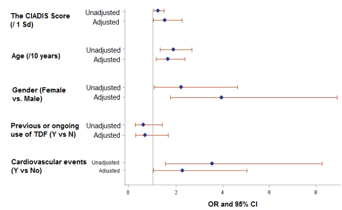


Figure 4: Logistic regressions of CKD (eGFR<60 vs. eGFR≥ 60) adjusted by cardiovascular events



✓**In unadjusted analysis**: an increase of the CIADIS score was significantly associated with an eGFR<60, (OR=1.2; 95% CI 1.0-1.5; *P*=0.04)(*Figure 2,3 and 4*) and eGFR <90 (OR=1.1; 95% CI 1.0-1.2; *P*<0.01) (*Data not shown*).

✓**In adjusted analysis:**
- The CIADIS score remained significantly associated with an eGFR <60 after adjustement for the number of comorbidities (OR=1.2; 95% CI 1.0-1.5; *P*=0.04) (*Figure 2*), diabetes (OR=1.5; 95% CI 1.0-2.2; *P*=0.04) (*Figure 3*) or for cardiovascular events (OR=1.5; 95% CI 1.0-2.3; *P*=0.04) (*Figure 4*) but not for the other comorbidities.
- Other variables that remained significantly associated to an eGFR <60 in adjusted analysis were age, gender, comorbidties (see figures 2-4) but not TDF to an eGFR <60.
- The CIADIS score was not associated with an eGFR <90 after adjusting for age, gender, TDF use and comorbidities (data not shown).
- The discriminative capacity of the CIADIS score alone was poor (area under the ROC curve (AUC)=0.62). The AUC of adjusted models without CIADIS score were 0.75 and with CIADIS score 0.76. Thus the discriminative capacity was fair in adjusted models but the CIADIS score did not improve much the predictive value of the model.

CONCLUSIONS

- ✓ A higher CIADIS score (Higher IA and IS levels) was independently associated with advanced CKD.
- ✓ Early kidney disease defined by eGFR ≥90 and uPCR >30 was not associated with CIADIS Score.
- ✓ The CIADIS score was only associated to an eGFR <60 independently of adjustment variables but not to an eGFR <90, thus the definition of CKD could have an important impact on study results, their interpretation and conclusions. Furthermore, comparisons between studies using different definitions may be hampered.
- ✓ Although other factors may contribute to kidney damage, persisting T-cells activation and senescence could induce inflammation and thus play a direct role even in successfully treated patients.
- ✓ **Limits and Perspectives:**
  - The interpretation of the association between IA and IS is limited due to the cross-sectional design. Furthermore, the low number of patients presenting the outcome limits the power of the study.
  - However, follow-up of patients included in this first study is ongoing allowing for longitudinal analysis. Furthermore, the integration of inflammation markers is planned, and may allow us to identify patients with high risk to CKD. These analyses may also help to better disentangle the interrelationships between IA, IS and inflammation and comorbidities directly associated with higher risk for CKD such as diabetes or cardiovascular diseases.

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