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Title: *IL7RA* polymorphisms are not associated with AIDS progression

Short title: *IL7RA* polymorphisms and AIDS progression

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ABSTRACT

Background: Our aim was to determine whether α-chain of the IL-7 Receptor (*IL7RA*) polymorphisms (rs10491434, rs6897932, and rs987106) are associated with the clinical pattern of AIDS progression in ART-naïve HIV-infected patients.

Methods: We carried out a cross-sectional study in 673 HIV-infected patients who were classified into three groups according to the clinical pattern of AIDS progression (188 long-term non-progressors (LTNPs), 334 moderate progressors (MPs), and 151 rapid progressors (RPs)). Additionally, 134 healthy blood donors participated as a Control-group. We selected three *IL7RA* polymorphisms located at three regulatory regions [rs6897932 (exon 6), rs987106 (intronic region), and rs10491434 (3′UTR)]. DNA genotyping was performed using Sequenom's MassARRAY platform.

Results: The Control-group and all HIV-infected patients had similar age and percentage of males. LTNP-group was older at HIV diagnosis and at the inclusion in the study, and had higher percentage of intravenous drug users (IDU) ($p<0.001$). Besides, LTNP-group had lower proportion of male patients and homosexual HIV transmission than MP and RP groups ($p<0.001$). Moreover, similar values of allelic, genotypic, and haplotype frequencies for *IL7RA* polymorphisms were found between healthy-controls and HIV-infected patients ($p>0.05$), and among different subgroups of HIV patients according to AIDS progression (LTNPs, MPs, and RPs) ($p>0.05$). The adjusted logistic regression did not show any significant association between *IL7RA* polymorphisms and AIDS progression.

Conclusions: *IL7RA* polymorphisms (rs6897932, rs987106 and rs10491434) were not associated with AIDS progression in Spanish population. Therefore, *IL7RA* polymorphisms do not seem to help us to understand HIV pathogenesis in untreated HIV–infected patients with different clinical evolution.

Keywords: Single nucleotide polymorphisms; *IL7RA*; LTNPs; AIDS; progression

INTRODUCTION

The CD4+ T cell is the major cellular target of human immunodeficiency virus (HIV). During HIV-infection, the continuous loss of CD4+ T cells leads to immunodeficiency, opportunistic diseases, and death [1, 2]. However, there is a substantial inter-individual variability in the rate and extent of progression to acquired immunodeficiency syndrome (AIDS) in antiretroviral treatment (ART)-naïve HIV-infected patients [3].

Interleukin-7 (IL-7) and IL-7 receptor (IL-7R) are critical factors for immune response by promoting thymic output, development, survival, and proliferation of CD4+ T cells [4]. Besides, IL-7 induces cytotoxic T-lymphocyte response against viruses [5]. IL-7R is an heterodimer which consists of the common cytokine receptor gamma chain (CD132) and the α-chain of the IL-7R (IL7RA or CD127) [4]. The progression of HIV infection is associated with a complex dysregulation of IL-7/IL7R pathway [6]. During HIV-1 infection, high levels of IL7 are inversely correlated with levels of CD4+ T cells expressing CD127; but following antiretroviral therapy, the IL7 levels decrease while the CD4+ T cell restoration occurs [6].

The host genetic heterogeneity may confer differential susceptibility to HIV infection and rates of progression to AIDS [7]. In this regard, *IL7RA* polymorphisms have been related to CD4+ T cell declining in untreated HIV-infected Europeans [8] and mortality in ART-naïve HIV-infected Africans [9]. Besides, *IL7RA* polymorphisms have been associated with CD4+ T cell recovery in HIV-infected patients on combination antiretroviral treatment (cART) [10-12]. Although *IL7RA* SNPs have been extensively studied in HIV infected patients, there is only one previous study in Caucasian population that associated *IL7R* polymorphisms with clinical AIDS progression [8]. Therefore, it is necessary to confirm or refute these previously findings reported about AIDS progression pattern.

OBJECTIVES

The aim of our study was to determine whether *IL7RA* polymorphisms (rs10491434, rs6897932, and rs987106) are associated with the clinical pattern of AIDS progression in ART-naïve HIV infected patients of two Spanish large cohorts [Cohort of the Spanish HIV Research Network (CoRIS) and Cohort of the Long Term Non-Progressors (LTNPs)].

PATIENTS AND METHODS

Patients

We carried out a cross-sectional study in 673 HIV infected patients from CoRIS [13], Cohort of LTNPs, and its associated Spanish HIV HGM Biobank [14]. Additionally, 134 healthy blood donors (HIV, HCV, and HBV negative subjects) from the "Centro de Transfusión de la Comunidad de Madrid" participated as a Control-group. This study was approved by the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII) and the study was conducted in accordance with the Declaration of Helsinki.

HIV-infected patients were classified into three groups according to the clinical pattern of AIDS progression (long-term non-progressors (LTNPs), moderate progressors (MPs), and rapid progressors (RPs)) [15]. Firstly, 188 LTNPs, who were defined as subjects with asymptomatic HIV infection over 10 years after seroconversion, and always showing CD4+ ≥500 cells/mm^3 and RNA-viral load ≤10,000 copies/ml. Secondly, 334 typical or MPs, who

had at least 2 years of follow-up without ART, with an average decrease of 50-100 CD4+/mm^3 per year. Thirdly, 151 RPs, who had two or more CD4+ T-cell measurements below 350/mm^3 within 3 years after seroconversion without ART, and/or who had AIDS or AIDS-related death. These three definitions of clinical patterns of AIDS progression involved no antiretroviral treatment, although MP and RP patients could be subsequently treated. Thus, these three groups of patients may be considered as three different progression patterns for the natural history of HIV infection.

Samples and clinical data

The epidemiological and clinical data provided by patients were included in CoRIS and Cohort of LTNPs. Each participating patient signed an informed consent form. The programme was approved by the Institutional Review Boards of the participating hospitals and centers (the cohorts have been described in detail elsewhere) [13, 16].

Samples from patients were kindly provided by the Spanish HIV HGM BioBank integrated in the Spanish AIDS Research Network (RIS) [14]. Samples were processed following current procedures and were frozen at -80 ºC immediately after their reception. All patients participating in the study gave their informed consent and protocols were approved by institutional ethical committees [14].

DNA Genotyping

We selected three *IL7RA* polymorphisms located at three regulatory regions [rs6897932 (exon 6), rs987106 (intronic region), and rs10491434 (3′UTR)], which have been recently related to AIDS progression in ART-naïve patients and CD4+ T cell recovery in patients on cART [8-10, 12, 17].

Total DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were genotyped at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) by Sequenom's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system.

Statistical Analysis

Categorical data and proportions were analyzed by using the chi-squared test or Fisher's exact test. Mann-Whitney test was used to compare data among independent groups when the dependent variable was continuous. The genetic analysis model was evaluated according to the additive, recessive and dominant model. The association between *IL7RA* SNPs and the clinical pattern of AIDS progression was tested by binary logistic regression adjusted by age, gender, age at HIV diagnosis, and intravenous drug users (IDU). All statistical tests were performed with the Statistical Package for the Social Sciences (SPSS) 21.0 software (IBM Corp., Chicago, USA). All *p*-values were two-tailed and statistical significance was defined as p value <0.05.

In addition, Hardy-Weinberg equilibrium (HWE) analyses and pairwise linkage disequilibrium (LD) analysis were computed by Haploview 4.2 software, considering equilibrium when p>0.05. Haplotype frequencies were inferred with the Expectation-Maximization algorithm and the haplotype association was tested by binary logistic regression using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/). All p-values were two-tailed and statistical significance was defined as p<0.05.

RESULTS

Study population

Table 1 shows the epidemiological and clinical characteristics of 673 HIV infected patients (188 LTNPs, 334 MPs, and 151 RPs) and 134 healthy-controls (Control-group). The Control-group and all HIV-infected patients had similar age and percentage of males. However, when HIV-infected patients were classified according to clinical pattern of AIDS progression, LTNP-group was older at HIV diagnosis and at the inclusion in the study, and had higher percentage of intravenous drug users (IDU) ($p<0.001$). Besides, LTNP-group had lower proportion of male patients and homosexual HIV transmission than MP and RP groups ($p<0.001$).

Characteristics of *IL7RA* polymorphisms

IL7RA polymorphisms were in HWE ($p>0.05$) for healthy-controls and HIV-infected patients. The minor allelic frequency (MAF) was greater than 5% and the genotyping call-rate success was over 95% for all the SNPs. Frequencies of *IL7RA* polymorphisms in our dataset (**Table 2)** were in accordance with data listed on the NCBI SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/). In addition, the frequencies of alleles, genotypes and haplotypes of *IL7RA* polymorphisms in healthy-controls were similar to the Iberian populations in Spain from 1000 genome project (http://www.1000genomes.org/1000-genomes-browsers, *data not shown*).
Moreover, LD was high for rs10491434 and rs6897932 (D'=1.00; r^2=0.131), rs10491434 and rs987106 (D'=0.952; r^2=0.337), and rs6897932 and rs987106 (D'=0.992; r^2=0.259). Although there was a high linkage disequilibrium between polymorphisms (D´ close to 1), the reduced r^2 statistic (which is often used in calculations of power to detect disease-susceptibility loci) means that each SNP is showing us different information.

IL7RA polymorphisms and AIDS progression

Similar values of allelic and genotypic frequencies for *IL7RA* polymorphisms (rs10491434, rs6897932, and rs987106) were found between healthy-controls and HIV-infected patients (**Table 2)**, and among different subgroups of HIV patients according to AIDS progression (LTNP, MP, and RP) (**Table 2**). The adjusted logistic regression did not show any significant association between *IL7RA* polymorphisms (rs6897932, rs987106 and rs10491434) and AIDS progression (**Table 3**). Moreover, we also analyzed *IL7RA* haplotypes (rs6897932, rs987106 and rs10491434) and their distribution in each of the groups of patients, but we did not find any significant differences in haplotype frequencies (rs6897932, rs987106 and rs10491434) between healthy-controls and HIV-infected patients (**Table 4**), and among the different subgroups of HIV patients according to AIDS progression (LTNPs, MPs, and RPs) (**Table 5**).

Table 1. Clinical and epidemiological characteristics of HIV infected patients.

	Controls vs. all HIV patients			**HIV groups of patients**			
Characteristics	**Control**	**All HIV**	**p-value [(a)]**	**LTNP-group**	**MP-group**	**RP-group**	***p*-value[(b)]**
No.	134	673		188	334	151	
Male	106 (79.1%)	545 (80.9%)	0.524	119 (63.2%)	283 (84.7%)	143 (94.7%)	**<0.001**
Age (years)	42.0 (36.0; 49.0)	41.4 (34.9; 48.4)	0.533	48.6 (40.1; 51.7)	38.7 (33.1; 45.3)	38.3 (33.1; 50.1)	**<0.001**
Age (years) of HIV diagnosis	-	34.3 (29.0; 40.5)	-	39.7 (34.1; 43.6)	31.8 (27.0; 38.4)	34.0 (29.6; 38.5)	**<0.001**
HIV acquired							
IDU	-	169 (25.1%)	-	132 (70.2%)	29 (8.7%)	8 (5.3%)	**<0.001**
Homosexual transmission	-	360 (53.5%)	-	14 (7.4%)	220 (65.8%)	126 (83.4%)	**<0.001**
Heterosexual transmission	-	120 (17.8%)	-	29 (15.4%)	76 (22.7%)	15 (9.9%)	**<0.001**
Others	-	24 (3.6%)	-	13 (6.9%)	9 (2.7%)	2 (1.3%)	**<0.001**

Values were expressed as absolute number (percentage) and median (percentile 25; percentile 75). Statistically significant differences are shown in bold. P-values were calculated by Chi-square and Mann-Whitney tests: (a). differences between control group and all HIV infected patients; (b). differences among HIV groups.

Abbreviations: IDU, intravenous drug users; HIV, Human immunodeficiency virus; LTNPs, Long Term Non Progressors; MPs, Moderate Progressors; RPs, Rapid progressors.**Table 2.** Frequencies of alleles and genotypes for *IL7RA* (rs6897932, rs987106, rs10491434) polymorphisms in HIV infected patients and healthy controls.

Table 2. Frequencies of alleles and genotypes for *IL7RA* (rs6897932, rs987106, rs10491434) polymorphisms in HIV infected patients and healthy controls.

SNP		Control	All HIV	p-value [(a)]	LTNPs	MPs	RPs	p-value [(b)]
rs6897932								
No.		134	673		188	334	151	
HWE (p-value)		0.647	0.664		0.407	0.352	0.121	
Alleles	C	75.4%	76.9%	0.878	77.1%	77.1%	76.2%	0.942
	T	24.6%	23.1%		22.9%	22.9%	23.8%	
Genotypes	CC	57.5%	59.4%	0.974	60.6%	58.4%	60.3%	0.379
	CT	35.8%	34.9%		33.0%	37.4%	31.8%	
	TT	6.7%	5.6%		6.4%	4.2%	7.9%	
rs987106								
No.		134	671		187	334	150	
HWE (p-value)		0.487	0.642		0.657	1.00	0.623	
Alleles	T	43.2%	53.2%	0.172	56.4%	52.2%	50.7%	0.225
	A	56.7%	46.8%		43.6%	47.8%	49.3%	
Genotypes	TT	20.1%	28.9%	0.396	32.6%	27.2%	26.7%	0.574
	TA	46.3%	49.4%		47.6%	50.0%	48.0%	
	AA	33.6%	22.5%		19.8%	22.8%	25.3%	
rs10491434								
No.		133	672		187	334	151	
HWE (p-value)		0.693	0.079		0.321	0.285	0.537	
Alleles	A	67.7%	70.5%	0.142	66.8%	71.3%	73.2%	0.163
	G	32.3%	29.5%		33.2%	28.7%	26.8%	
Genotypes	AA	46.3%	48.2%	0.324	42.8%	49.4%	52.3%	0.419
	AG	41.8%	44.5%		48.1%	43.7%	41.7%	
	GG	11.2%	7.3%		9.1%	6.9%	6.0%	

P-values were calculated by Chi-squared test: (a). differences between control group and all HIV infected patients; (b). differences among HIV groups.

Abbreviations: HWE, Hardy-Weinberg equilibrium; IL7RA , α-chain of the interleukin 7 receptor.; LTNPs, Long Term Non Progressors; MPs, Moderate Progressor; RPs, Rapid progressor.

Table 3. Association between *IL7RA* polymorphisms and AIDS progression in HIV infected patients.

	LTNPs vs RPs		LTNPs vs MPs		MPs vs RPs	
***IL7RA* SNPs**	**aOR (95%CI)**	**P-value(*)**	**aOR (95%CI)**	**P-value(*)**	**aOR (95%CI)**	***P*-value(*)**
rs6897932						
Dominant (CT/TT)	0.99 (0.41; 2.43)	0.991	1.36 (0.78; 2.36)	0.273	1.01 (0.64; 1.59)	0.970
Recessive (TT)	2.48 (0.28; 21.36)	0.408	0.57 (0.17; 1.88)	0.357	2.05 (0.79; 5.34)	0.139
Additive (nT)	1.17 (0.53; 2.34)	0.769	1.14 (0.72; 1.80)	0.566	1.12 (0.77; 1.62)	0.554
rs987106						
Dominant (AT/AA)	0.74 (0.29; 1.90)	0.538	1.13 (0.63; 2.04)	0.683	1.01 (0.60; 1.67)	0.991
Recessive (AA)	1.87 (0.65; 5.43)	0.245	1.07 (0.55; 2.05)	0.842	1.11 (0.62; 1.86)	0.693
Additive (nA)	1.08 (0.59; 1.96)	0.787	1.07 (0.74; 1.56)	0.706	1.04 (0.76; 1.42)	0.806
rs10491434						
Dominant (AG/GG)	0.77 (0.32; 1.88)	0.577	0.82 (0.48; 1.41)	0.491	0.92 (0.59; 1.44)	0.732
Recessive (GG)	0.74 (0.18; 2.99)	0.675	0.46 (0.17; 1.28)	0.140	0.66 (0.26; 1.70)	0.396
Additive (nG)	0.81 (0.42; 1.57)	0.538	0.77 (0.50; 1.19)	0.241	0.89 (0.62; 1.28)	0.538

(*) *P*-values were calculated by binary logistic regressions adjusting for age, gender, age at HIV diagnosis, and intravenous drug users (IDU).

Abbreviations: aOR, adjusted odds ratio; 95% CI, 95% confidence interval; HIV, human immunodeficiency virus; LTNPs, Long Term Non-Progressors; MPs, typical or moderate progressors; RPs, rapid progressors; IL7RA, α-chain of the interleukin 7 receptor.

Table 4. Distribution of *IL7RA* haplotypes (rs6897932, rs987106, rs10491434) in healthy control and HIV infected patients.

Haplotypes	Frequencies		Healthy control vs. HIV patients	
	Healthy control	HIV patients	aOR (95CI) (*)	P-value
CAA	42.9%	46.4%	1.16 (0.86; 1.50)	0.289
CTG	31.7%	29.2%	0.88 (0.65; 1.17)	0.363
TTA	24.6%	23.1%	0.92 (0.68; 1.3)	0.598

Abbreviations: aOR, adjusted odds ratio; 95 CI, 95 of confidence interval; HIV, human immunodeficiency virus; IL7RA , α-chain of the interleukin 7 receptor.

(*). Binary logistic regressions were adjusted by age and gender.

Table 5. Distribution of *IL7RA* haplotypes (rs6897932, rs987106, rs10491434) in HIV infected patients.

Haplotypes	Frequencies			LTNPs vs RPs		LTNPs vs MPs		MPs vs PRs	
	LTNP	MP	RP	aOR (95CI) (*)	P-value	aOR (95CI) (*)	P-value	aOR (95CI) (*)	P-value
CAA	43.6%	47.1%	48.3%	1.14 (0.63; 2.06)	0.666	1.07 (0.73; 1.57)	0.736	1.04 (0.76; 1.43)	0.794
CTG	33.2%	28.4%	25.8%	0.76 (0.39; 1.48)	0.432	0.75 (0.49; 1.17)	0.208	0.84 (0.58; 1.21)	0.354
TTA	22.9%	22.8%	23.8%	1.08 (0.52; 2.24)	0.823	1.12 (0.71; 1.77)	0.618	1.11 (0.76; 1.61)	0.572

Abbreviations: aOR, adjusted odds ratio; 95 CI, 95 of confidence interval; HIV, human immunodeficiency virus; IL7RA , α-chain of the interleukin 7 receptor.; LTNPs, Long Term Non Progressors; MPs, Moderate Progressor; RPs, Rapid progressor.

(*). Binary logistic regressions were adjusted by age, gender, age at HIV diagnosis and intravenous drug users (IDU).

DISCUSSION

This study was performed to analyze the relevance of *IL7RA* polymorphisms on host natural resistance/sensitivity against AIDS progression in subjects who never received ART. However, we found *IL7RA* polymorphisms (rs6897932, rs987106 and rs10491434) were not associated with rapid AIDS progression in two Spanish large cohorts (CoRIS and LTNPs cohorts). Therefore, unlikely previous studies, this work weakens the strength of *IL7RA* polymorphisms as a potential predictor to classify HIV-naïve patients according to risk of AIDS progression.

Apparently, our results of *IL7RA* polymorphisms appear to be discordant with previous studies, since these *IL7RA* SNPs have been previously related to AIDS progression in naïve patients and CD4+ T cell recovery in patients on cART [8-10, 12, 17]. Besides, *IL7RA* polymorphisms (rs6897932, rs987106 and rs10491434) have been related to plasma levels of soluble isoform of IL7RA (sCD127) [17-19]. Rajasuriar *et al.* hypothesized that unfavorable *IL7RA* alleles could lead to an increased risk of AIDS progression due to regulation of sCD127 levels by decreasing CD4+ cells count.

Limou *et al.* published one of the studies that previously associated *IL7RA* polymorphisms with AIDS progression pattern according to rapid decline of CD4+ T cell count during HIV infection, which was performed in a French large cohort [8]. They only found significant differences among HIV groups (LTNPs and RPs) and healthy-controls, but they did not compare among HIV groups (LTNPs and RPs). In our study, we did not find any significant differences between all HIV patients versus healthy-controls, but we also did not find any significant difference of HIV groups separately (LTNPs, MPs, and RPs) versus healthy-controls (data not shown), and among different groups of HIV patients.

The *IL7RA* gene is located at 5p13.2 and expand 8 exons. The *IL7RA* rs6897932 polymorphism is located at the alternatively spliced exon 6, which is a missense variant that generates a threonine (C allele) by isoleucine (T allele) substitution. This change seems to influence the amount of sCD127 (soluble isoform) and CD127 (membrane-bound isoform) by putatively disrupting an exonic splicing motif [20]. On the other hand, the rs987106 A>T polymorphism, is located at intron 6. This genetic variant could be located at a regulatory region, as it has also been associated with higher levels of soluble IL7RA (sCD127) in HIV infected patients [19]. Finally, the rs10491434 polymorphism is located at the 3′UTR, and can be influencing methylation at nearby CpG sequences [17]. The rs10491434 C variant seems to be associated with higher methylation at CpG sites, which might decrease the CD127 expression; while the rs10491434 T allele seems to be associated with lower methylation at nearby CpG sites and, thus, it might increase the CD127 expression [17]. Moreover, these three polymorphisms are predicted to be involved in RNA binding protein-mediated post-transcriptional regulation, according to an *in silico* analysis by using rSNPBase software [16].

It is complicated to understand the lack of association between *IL7RA* polymorphisms and AIDS progression. It could be possible that the lack of significant association may be due to a limited sample size, which is critical when the effects of the studied variable is not large enough. Complex human diseases are under the control of many genes that usually contribute to the disease with modest individual effects. When dealing with small groups, only big effects would be detected, and would be necessary to study a higher population in order to observe a significant association. Another factor to consider is the study design, which was cross-sectional since we included patients from cohorts defined by the AIDS progression rate, thus we used these categories rather than a "time-to-event" analysis. We must also highlight the profound differences among HIV groups in our study regarding to epidemiological and clinical characteristics, which could introduce bias difficult to control. Additionally, we have to take

into account that the criteria used to define the phenotype of AIDS progression in previous and discordant studies were very different, which have to bear in mind for a correct interpretation of these data [21]. In our study, LTNPs are defined as subjects with asymptomatic HIV infection over 10 years after seroconversion and always CD4+ ≥500 cells/mm^3 and HIV viral load ≤10,000 copies/ml; while in the French cohort, LTNPs were defined as HIV-1 seropositive and asymptomatic individuals for more than 8 years with a CD4+ T cell count consistently above 500 cells/mm^3 in the absence of ART [8]. Besides, they did not define MP subgroup in their study and the CD4+ T cell count cut off in RP patients is 300/ mm^3 [8]. This heterogeneity can introduce inconsistencies in analyzed results, and make it difficult to identify biological mechanisms underlying these phenotypes.

In addition, there are other host genetic factors that could influence AIDS progression (e.g. known HLA types, *CCR5* mutations, etc.). Nevertheless, in our previous work we have explored the interaction of mtDNA haplogroups and polymorphisms at *ACSM4* and *PECI* genes in the same HIV infected patients (own data from two recent articles [15, 22]) with *IL7RA* polymorphisms, and we did not find any significant result.

In conclusion, *IL7RA* polymorphisms (rs6897932, rs987106 and rs10491434) were not associated with AIDS progression in Spanish population. Therefore, *IL7RA* polymorphisms do not seem to help us to understand HIV pathogenesis in untreated HIV–infected patients with different clinical evolution. This might be instrumental upon reaching clinical decisions and performing future immunological and pathogenic studies.

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COMPETING INTERESTS:

The authors do not have a commercial or other association that might pose a conflict of interest.

ETHICAL APPROVAL:

The study was approved by the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII). This study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study.

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AUTHORS' CONTRIBUTIONS:

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Appendix 2. Centers involved in Long Term Non-Progressors (LTNP) cohort:

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H. Arnau de Vilanova - Lleida
H. Asturias
H. Bellvitge - Barcelona
H. Castellón
H. Clínic - Barcelona
H. Donostia - San Sebastián
H. Elche - Alicante
H. Germans Trias i Pujol - Badalona
H. Gregorio Marañón - Madrid
H. Joan XXIII - Tarragona
H. La Fe - Valencia
H. La Paz/Carlos III - Madrid
H. La Princesa - Madrid
H. Navarra - Pamplona
H. Parc Taulí- Sabadell
H. Ramón y Cajal - Madrid
H. San Cecilio - Granada
H. San Pedro - Logroño
H. Son Dureta - Mallorca
H. Virgen del Rocío - Sevilla