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CD4+ T lymphocyte recovery in the modern antiretroviral therapy era: Toward a new threshold for defining immunological non-responders

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Introduction: Despite the high level of efficacy of modern antiretroviral therapy (ART) in reducing HIV viremia and the control of viral replication, some people living with HIV (PLWH) do not recover their CD4+ T cell count.

Methods: To evaluate the frequency and predictive factors of discordant immune responses, we performed a retrospective cohort study of 324 antiretroviral-naïve PLWH who initiated first-line ART between 2008 and 2018 and maintained HIV RNA < 50 copies/ml during 36 months of follow-up. PLWH were defined as immunological non-responders (INRs) when CD4+ T cell count was < 20% compared with baseline ($INR_{20\%}$), or < 500 cells/mm^3 (INR_{500}) or < 200 cells/mm^3 (INR_{200}) at 36 months.

Results: The prevalence of $INR_{20\%}$, INR_{500}, and INR_{200} was 12.5%, 34.6%, and 1.5%, respectively. After adjustment for possible confounders, CD4 nadir showed a significant association with all INR definitions, with lower values predicting INR_{500} (aOR 0.98, 95% CI 0.98–0.99, $p < 0.001$) and INR_{200} (aOR 0.98, 95% CI 0.95–1.01, $p = 0.096$). Moreover, a higher baseline CD4/CD8 ratio was inversely related to the probability of being INR_{500} (OR 0.03, 95% CI 0.01–0.12, $p < 0.001$) and INR_{200} (OR 0.002, 95% CI 18^{-7}–67.72, $p = 0.255$). By contrast, $INR_{20\%}$ had a higher CD4 nadir and CD4/CD8 ratio than other INRs, suggesting the identification of an heterogenous population with such definition.

Discussion: The present study highlights how INR_{200} has become rare in the contemporary ART era, and about one-third of PLWH meet the criteria for

INR_{500}. Overcoming the threshold of 500 CD4/mm^3 could be an appropriate definition of immune response, in contrast with the older definitions of INR_{200} and $INR_{20\%}$. Early diagnosis and rapid treatment initiation, before CD4 counts and the CD4/CD8 ratio begin to decline, are critical for achieving an optimal immune response.

KEYWORDS

immunological non responders, HIV, immune recovery, discordant immune response, antiretrovirals, CD4+T-cell count

1 Introduction

Because of the availability of modern combined antiretroviral therapy (ART), people living with HIV (PLWH) have experienced a reduction in overall mortality and incidence of AIDS-defining conditions, improving survival and quality of life (1–3). The efficacy of ART is traditionally evaluated using two parameters: immunological recovery, namely the increase in CD4+ T-cell count (CD4), and the viral suppression, defined as an HIV RNA load of < 50 copies/ml in plasma. The extent of a CD4 increase, however, might have interpatient variations depending on age, ethnicity, test imprecision, intercurrent infection, and relative leukocyte count (4, 5), and the immunological response in the course of ART can be heterogeneous, based on individual, clinical, and genetic factors (6–8). Despite this variability, there is general agreement that CD4 count is the main marker of immunological staging (9). However, some PLWH fail to reconstitute CD4 count, even in the course of successful ART. These people are referred to as immunological non-responders (INRs). A consensus on the definition of INR has not been reached yet, owing to consistent interpatient variability of different factors, including age, HIV RNA load and CD4 count before starting ART, coinfections with HCV, HBV, and CMV, bone marrow and thymic dysfunction, genetic factors, and immune activation (10–12). INRs have been defined in the past based on CD4 count relative or absolute recovery. In studies that use relative CD4 count recovery from baseline, thresholds varied from less than 20% to 25% or 30% (13–15). Other studies consider the achievement of a predefined CD4 value, with thresholds that range from 200 to 500 cells/mm^3 (16–18).

Each classification has pros and cons because of variable sensitivity and specificity. The choice of > 500 CD4+ T cells/mm^3 to define full immune recovery could be justified in comparison to the general population, as it is the approximately the minimum expected CD4 count in a healthy, HIV-uninfected person. In addition, when counts are > 500 CD4+ T cells/mm^3 in PLWH, their mortality rates become similar to those of the general population (19) and both mortality from AIDS- and non-AIDS-defining causes decreases as CD4 counts increase (20). On the other hand, the threshold of > 200 CD4+ T cells/mm^3 might better represent the turning point between high and low risk of opportunistic diseases and AIDS-defining conditions (21). However, despite different definitions, most studies agree that INRs are at higher risk of disease progression toward AIDS-defining conditions, non-AIDS-defining conditions, and death (11, 14, 22). Because of the heterogenicity of definitions, the prevalence of INRs varies from 10% to 40% in different studies (13, 23); in the modern ART era INRs are expected to become rarer thanks to early diagnosis and immediate initiation of ART (24), and new antiretroviral agents characterized by high levels of efficacy and tolerability (25, 26).

In Italy, as well as in many other countries in the world, after 2008 new antiretroviral agents were introduced in clinical practice that completed the therapeutic armamentarium, and integrase strand transfer inhibitors (INSTIs) have begun to enter first-line therapy in PLWH (27, 28). With the aim of assessing the phenomenon of INR in an era of modern ART, we used three different definitions of immunological reconstitution, i.e., a CD4 level increase of > 20% from baseline, a CD4 level of > 200 cells/mm^3 or a CD4 level of > 500 cells/mm^3, and we evaluated the prevalence of INR according to these definitions in recent years in our single-center experience.

2 Material and methods

2.1 Study design, subject, and inclusion criteria

We conducted a retrospective observational study in a cohort of people diagnosed with HIV from 2008 to 2018 in our center (Policlinico IRCCS San Martino University Hospital).

The inclusion criteria were a confirmed HIV infection, being ART-naïve at the beginning of the study, being aged ≥ 18 years,

and achieving and maintaining viral suppression (i.e., HIV RNA < 50 copies/ml) throughout the follow-up period of 36 months after ART initiation.

Exclusion criteria were at least one HIV RNA of > 50 copies/ml fter achieving viral suppression, being aged ≥ 18 years, a lack of virological and immunological data during 36 months after the study baseline, and death before 36 months from the study initiation.

2.2 Data collection and study definitions

Data for all PLWH included in the study were extracted from the Liguria HIV Network Database (RLH-DB). The Liguria HIV Network is a locally developed online platform with a direct connection between medical and laboratory records of PLWH through the automatic and prospective transfer of anonymous data (29, 30). Each person has an identification code, which is registered in the RLH-DB at initial engagement (i.e., at the first ambulatory visit for outpatients or first day of hospitalization for inpatients). Safety and precision are granted by the approved use of hospital anonymized codes. The use of the RLH-DB was approved by the Ligurian Ethics Committee. All people registered in the RHL-DB signed informed consent forms to be included in the study. The study has been performed in accordance with the ethics standards of the Declaration of Helsinki and with Italian national laws.

For the classification of the INR we used the following definitions: (a) ART-treated PLWH who failed to demonstrate a 20% recovery in their CD4 levels, compared with their baseline CD4 count, at 36 months after their first HIV RNA of < 50 copies/ml (who were defined as $INR_{20\%}$) (31–33); (b) ART-treated PLWH with a total CD4 count of < 500 cells/mm^3 at 36 months after their first HIV RNA of < 50 copies/ml (who were defined as INR_{500}) (34–38); and (c) ART-treated PLWH with a total CD4 count of < 200 cells/mm^3 at 36 months after their first HIV RNA of < 50 copies/ml (who were defined as INR_{200}) (11, 15, 17, 33, 39–42).

Plasma viral load, measured as HIV RNA copies/ml, was quantified by using the K-PCR-HIV1 (Siemens Health Care, Erlangen, Germany) kit for samples collected between 2008 and 2010; by the Nucleosens HIV (bioMerieux, Marcy-l'Etoile, France) kit between 2011 and 2018; and by Aptima HIV-1 Quant Dx Assay from 2019 onward.

CD4+ T cell counts were assessed in EDTA blood samples, which were analyzed using a BD FACSCanto flow cytometer (BD Biosciences). The following monoclonal antibodies (MABs) were used to analyze T-cell subsets: CD3FITC/CD8 PE/CD45 PercCP-Cy5.5/CD4 APC (BD Multitest).

HCV co-infection was defined as positive anti-HCV antibody test and detectable HCV RNA. HBV co-infection was defined as positive hepatitis B surface antigen (HBsAg) test. The time of HIV RNA viral decay was calculated as the difference in days between ART initiation and achievement of first HIV RNA load of < 50 copies/ml.

Clinical events were classified as AIDS defining based on CDC's classification (43).

2.3 Study objective

The primary objective of the study was to evaluate the frequency of $INR_{20\%}$, INR_{500}, and INR_{200} at 36 months after starting ART in the period 2008–2018.

The secondary objective of the study was to evaluate the factors associated with a poor immune response in $INR_{20\%}$, INR_{500}, and INR_{200}.

2.4 Statistical analysis

Data were described using mean and standard deviation (SD) for normally distributed continuous variables, median and interquartile range (IQR) for not normally distributed continuous variables, and frequency (%) for categorical and ordinal variables. Continuous variables were compared by using the *t*-test or Mann–Whitney *U*-test, and categorical variables were compared using chi-squared or Fisher's exact test, as appropriate. Factors associated with INR were evaluated using a binomial logistic regression model with the INR as the dependent variable; factors with a p-value ≤ 0.1 at univariate analysis were included in the multivariable model. For INR_{500} and INR_{200} analysis, only PLWH with a CD4 nadir of < 500 and <200 cells/mm^3, respectively, were included in the logistic model. Since both CD4 and CD8 absolute numbers and the CD4/CD8 ratio reached significance in univariate analyses of different INR groups, a sensitivity analysis was performed to investigate the relationship between CD4/CD8 ratio, instead of absolute lymphocyte numbers, and INR. CD4 nadir was dichotomized according to the best threshold obtained from the receiver operating characteristic (ROC) curve analysis to identify the cut-off value predictive of becoming INR_{500} or INR_{200} in the study cohort. The significance level was defined as a p-value < 0.05.

3 Results

During the study period, 452 PLWH were newly diagnosed in our center. Among them, 128 were excluded from the study because they were lost to follow-up (N = 98), died (N = 20), or did not meet virological criteria for study inclusion (N = 10) within 36 months of starting ART.

The remaining 324 PLWH were included in the study. Among them, 91 out of 324 (28%) were female and 240 out of 324 (74%) were Italian. The median age of study participants was

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TABLE 1 Clinical and demographic characteristics of the study population.

Demographic variables		
Age in years, mean (SD)	42 (±12)	
Male gender, N (%)	233/324	(72)
Italian, N (%)*	240/324	(74)
Risk factor for HIV infection, N (%)		
Heterosexual	183/324	(56)
MSM	95/324	(29)
IVDU	29/324	(9)
Unknown	17/324	(5)
CDC classification at time of diagnosis, N (%)		
Stage A	121/324	(37)
Stage B	131/324	(40)
Stage C	71/324	(22)
Unknown	1/324	(0)
Co-infections, N (%)		
HCV Ab positivity	33/324	(10)
HBsAg positivity	15/324	(5)
CMV IgG positivity	272/324	(84)
Other, N (%)		
Chemotherapy	8/324	(2)
Immunovirological data		
CD4 nadir (n/mmc)	276	(222)
HIV RNA zenith, log_{10} (copies/ml)	5.6	(6.2)
Time to HIV RNA ≤ 50 copies/ml (months)	4	(3)

*Overall, 259 out of 324 (80%) study participants were from Europe, 39 out of 324 (12%) were from South America, 18 out of 324 (6%) were from Africa, 4 out of 324 (1.2%) were from Asia, and 1 out 324 (0.3%) was from North America.
HBsAg, hepatitis B surface antigen; IQR, interquartile range; IVDU intravenous drug users; MSM, men who have sex with men; SD, standard deviation.

41 years (IQR 18–80 years). The risk factors for HIV acquisition were as follows: unprotected intercourse, 278 PLWH (heterosexual, 183 [56%]; men who had sex with men [MSM], 95 [29%]); intravenous drug use (IVDU), 29 (9%) PLWH; and other/unknown, 17 (5%) PLWH.

The mean CD4 nadir of study participants before they received ART was 276 (± 222) cells/mm^3, and the mean CD4 count after 36 months of ART was 664 (± 326) cells/mm^3.

According to the CDC classification, 121 (37%), 131 (40%), and 71 (22%) PLWH were on stage A, B, and C, respectively, at time of diagnosis (Table 1).

3.1 Prevalence of suboptimal CD4+ T-cell recovery and factors associated with INR

3.1.1 $INR_{20\%}$

Overall, 41 out of 324 (12.6%) study participants met the criteria for $INR_{20\%}$; 32 were male (78%) and the mean age was 42 years (±14.08 years). $INR_{20\%}$ were less frequently in CDC stage C (7.3% vs. 24%, $p = 0.02$), had a lower CD4 nadir (254 cells/mm^3 in $INR_{20\%}$ vs. 420 cells/mm^3 in full responders, $p < 0.001$), and a lower HIV RNA zenith (4.44 log_{10} in $INR_{20\%}$ vs. 4.96 log_{10} in full responders, $p = 0.01$). The mean CD8+T cell counts were similar in $INR_{20\%}$ and full responders at baseline ($p = 0.41$), but were lower in $INR_{20\%}$ at 36 months (731 vs. 916 cells/mm^3, $p = 0.006$).

Age ($p = 0.07$), sex ($p = 0.35$), risk factor for HIV infection ($p = 0.23$), country of origin ($p = 0.31$), and prevalence of HCV RNA, HBsAg, and CMV-IgG positivity were similar between $INR_{20\%}$ and full responders (p =0.91, $p = 0.38$, and $p = 0.50$, respectively). In addition, the dynamics of HIV RNA decay during follow-up was similar in the two groups ($p = 0.38$), and no difference was found in time of exposure to different ART regimens in $INR_{20\%}$ and full responders. The mortality rate was similar in the two groups ($p = 0.39$; Table 2).

3.1.2 INR_{500}

A total of 112 out 324 (34.6%) PLWH met the criteria for INR_{500} at 36 months; 86 were male (77%) and the median age was 42 years (±12.06 years). INR_{500} were more often non-Italian (40% vs. 22%, $p = 0.01$) and in CDC stage C at the time of HIV diagnosis (37% vs.14%, $p < 0.001$) compared with full responders. CD4 nadir (109 vs. 364 cells/mm^3, $p < 0.001$) and 36-month CD8+ T cell counts (780 vs. 949 cells/mm^3, p <0.001)

TABLE 2 Comparison of clinical and demographic characteristics of $INR_{20\%}$ and full responders (FR).

Variable	$FR_{20\%}$	$INR_{20\%}$	*p*-value
Demographic variables			
Age in years, mean (SD)	41 (±12.07)	42 (±14.08)	0.073
Male (%)	201/283 (71%)	32/41 (78%)	0.350

(Continued)

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TABLE 2 Continued

Variable	$FR_{20\%}$	$INR_{20\%}$	*p*-value
Risk factor for HIV			
Heterosexuals (%)	162/283 (57%)	21/41 (51.3%)	0.236
MSM (%)	79/283 (28%)	16/41 (39%)	
IVDU (%)	26/283 (9%)	3/41 (7,3%)	
Unknown (%)	16/283 (6%)	1/41 (2,4%)	
Nationality			
Italian (%)	207/283 (73%)	33/41 (80.5%)	0.316
CDC stage at HIV diagnosis			
A (%)	100/283 (35%)	21/41 (51.3%)	0.029
B (%)	115/283 (41%)	16/41 (39%)	
C (%)	68/283 (24%)	3/41 (7.3%)	
Unknown (%)	0 (0%)	1/41 (2.4%)	
Co-infections			
Ab anti-HCV positivity at baseline	29/280 (10.4%)	4/41 (9.8%)	0.906
HBsAg positivity at baseline	12/283 (4%)	3/41 (7%)	0.381
CMV IgG positivity at baseline	235/283 (83%)	37/41 (90.2%)	0.502
Other			
Chemotherapy	8/283 (3%)	0 (0%)	0.276
Deaths	5/283 (2%)	0 (0%)	0.391
Immunovirological data			
CD4 nadir (n/mmc) mean (SD)	420 (±235)	254 (±212)	< 0.001
CD8+ at baseline, mean (SD)	918 (±592)	1067 (±491)	0.419
CD8+ at 36 months, mean (SD)	916 (±391)	731 (±450)	0.006
CD4/CD8 ratio at baseline, median (IQR)	0.28 (0.14–0.52)	0.63 (0.41–0.86)	<0.001
CD4/CD8 ratio at 36 months median (IQR)	0.76 (0.47–1.06)	0.92 (0.62–1.28)	0.022
HIV-RNA zenith, log_{10} (copies/ml), mean (SD)	4.82 (±1.02)	4.46 (±1.04)	0.036
Time to HIV-RNA ≤ 50 copies/ml (months), mean (SD)	4.4 (±0.21)	3.8 (±0.43)	0.389
Therapy			
Months of INI exposure, mean (SD)	13 (±0.97)	14 (±2.73)	0.788
Months of PI exposure, mean (SD)	10 (±0.91)	8 (±2.24)	0.161
Months of NNRTI exposure, mean (SD)	12.7 (±0.96)	16.7 (±2.73)	0.319
Months of ABC/3TC exposure, mean (SD)	4 (±0.6)	2 (±1.33)	0.117
Months of TDF/FTC exposure, mean (SD)	28 (±0.78)	26 (±2.19)	0.287

ABC/3TC, abacavir+ lamivudine; $FR_{20\%}$: full responders, defined as people who recovered at least 20%CD4+T cell after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; HBsAg hepatitis B surface antigen; $INR_{20\%}$ people who did not recover at least 20%CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; IQR, interquartile range; INI, integrase inhibitors; IVDU intravenous drug users; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside or nucleotide reverse transcriptase inhibitors; PI, protease inhibitors; SD, standard deviation; TDF/FTC tenofovir disoproxil fumarate/emtricitabine.

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were lower in INR_{500}, and, at baseline, CD8+ T cell counts were higher in INR_{500} (1030 vs.768 cells/mm^3, $p = 0.038$). According to the ROC curve, a CD4 nadir ≤ 200 cells/mm^3 showed 83.93% specificity and 71.43% sensitivity in predicting becoming INR_{500} [area under the curve (AUC) 0.839;143 people had CD4 nadir ≤ 200 cells/mm^3 in the study cohort] in this cohort. The CD4/CD8 ratio was lower in INR_{500} at both baseline and 36 months' evaluation (0.14 vs.0.43, $p < 0.001$ and 0.48 vs. 0.92, $p < 0.001$, respectively).

No difference was found in time to achievement of HIV RNA of < 50 copies/ml ($p = 0.71$) or in the zenith of HIV RNA ($p = 0.31$). No differences were found in time of exposure to different ART classes or in the other patient characteristics that were evaluated. The mortality rate was similar in the two groups ($p = 0.22$; Table 3).

3.1.3 INR_{200}

In the study population, 5 out of 324 (1.5%) PLWH did not recover at least 200 CD4/mm^3 at 36 months after ART initiation and were thus classified as INR_{200}. Among them, four were male (80%) and the median age was 51 years (±12.06 years). They had a lower CD4 nadir (33 vs. 279 cells/mm^3, $p < 0.001$) and a lower CD4/CD8 ratio at both baseline and 36 months' follow-up than full responders (0.14 vs. 0.42, $p = 0.012$, and 0.17 vs. 0.78, $p < 0.001$, respectively). According to the ROC curve, a CD4 nadir ≤ 65 cells/mm^3 showed 56.5% specificity and 100%

TABLE 3 Comparison of clinical and demographic characteristics of INR_{500} and full responders (FR).

Variable	FR_{500}	INR_{500}	*p*-value
Demographic variables			
Age in years, mean (SD)	43 (±12.07)	41 (±12.6)	0.347
Male (%)	147/212 (70%)	86/112 (77%)	0.156
Risk factor for HIV			
Heterosexuals (%)	120/212 (56.6%)	63/112 (56.8%)	0.843
MSM (%)	64/212 (30.2%)	31/112 (27.9%)	
IVDU (%)	17/212 (8%)	12/112 (10.8%)	
Unknown (%)	11/212 (5.2%)	5/112 (4.5%)	
Nationality			
Italian (%)	166/212 (78.3%)	74/112 (66.1%)	0.017
CDC stage at HIV diagnosis			
A (%)	95/212 (44.8%)	26/112 (23%)	<0.001
B (%)	87/212 (41%)	44/112 (39%)	
C (%)	30/212 (14.2%)	41/112 (37%)	
Unknown	0 (0%)	1/112 (1%)	
Co-infections			
Ab anti-HCV positivity at baseline	18/212 (8.5%)	15/112 (14%)	0.153
HBsAg positivity at baseline	8/212 (3.8%)	7/112 (6.3%)	0.313
CMV IgG positivity at baseline	175/212 (82.5%)	97/112 (86.6%)	0.221
Other			
Chemotherapy	3/212 (1.4%)	5/112 (4.5%)	0.093
Deaths	2/212 (1%)	3/112 (3%)	0.228
Immunovirological data			
CD4 nadir (cells/mm^3), mean (SD)	363.6 (±220.3)	109.5 (±94.5)	< 0.001
CD8+ at baseline, mean (SD)	768 (±441)	1030 (±628)	0.038

(Continued)

TABLE 3 Continued

Variable	FR_{500}	INR_{500}	*p*-value
CD8+ at 36 months, mean (SD)	949 (±434)	780 (±309)	< 0.001
CD4/CD8 ratio at baseline, median (IQR)	0.43 (0.24–0.73)	0.14 (0.08–0.27)	< 0.001
CD4/CD8 ratio at 36 months median (IQR)	0.92 (0.67–1.21)	0.48 (0.32–0.70)	< 0.001
HIV-RNA zenith, log_{10} (copies/ml) mean, (SD)	4.74 (1.02)	4.81 (1.06)	0.615
Time to HIV-RNA ≤ 50 copies/ml (months), mean (SD)	4.3 (±0.24)	4.3 (±0.29)	0.713
Therapy			
Months of INI exposure, mean (SD)	12.8 (±1.12)	14.3 (±1.58)	0.567
Months of PI exposure, mean (SD)	9.8 (±1.04)	11.4 (±1.44)	0.123
Months of NNRTI exposure, mean (SD)	14.6 (±1.17)	10.7 (±1.41)	0.103
Months of ABC/3TC exposure, mean (SD)	4.4(±0.73)	2.6 (±0.76)	0.507
Months of TDF/FTC exposure, mean (SD)	27 (±0.95)	29 (±1.16)	0.518

ABC/3TC, abacavir+ lamivudine; FR_{500}: full responders, defined as people who recovered at least 500 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; HBsAg hepatitis B surface antigen; INR_{500} people who did not recover at least 500 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; IQR, interquartile range; INI, integrase inhibitors; IVDU intravenous drug users; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside or nucleotide reverse transcriptase inhibitors; PI, protease inhibitors; SD, standard deviation; TDF/FTC tenofovir disoproxil fumarate/emtricitabine.

sensitivity in predicting becoming INR_{200} (AUC 0.765) in this cohort (65 people had a CD4 nadir ≤ 65 cells/mm^3 in the study cohort). The CDC stage at diagnosis was not significantly different in INR_{200} than in full responders (p = 0.559). The crude mortality was higher in this group because of one death out of five INR_{200} (Table 4). No other significant differences were found in the other analyzed characteristics and drug exposure in the study population.

3.2 Comparison of factors associated with $INR_{20\%}$, INR_{500}, and INR_{200} at multivariable analysis

Univariate analysis of factors associated with $INR_{20\%}$, INR_{500}, and INR_{200} are shown in Supplementary Tables 1, 2, and 3. After adjustment for possible confounders, CD4 nadir remained the only factor that maintained a significant

TABLE 4 Comparison of clinical and demographic characteristics of INR_{200} and full responders.

Variable	FR_{200}	INR_{200}	*p*-value
Demographic variables			
Age in years, mean (SD)	41 (±12.4)	51(±13.4)	0.827
Male (%)	229/319 (72%)	4/5 (80%)	0.568
Risk factor for HIV			
Heterosexual (%)	180/319 (56%)	3/5 (60%)	0.425
MSM (%)	95/319 (30%)	0/5 (0%)	
IVDU (%)	28/319 (9%)	1/5 (20%)	
Unknown (%)	16/319 (5%)	1/5 (20%)	
Nationality			
Italian (%)	236/319 (74%)	4/5 (80%)	0.613
CDC stage at the time of diagnosis			
Stage A (%)	120/319 (38%)	1/5 (20%)	0.559

(Continued)

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TABLE 4 Continued

Variable	FR_{200}	INR_{200}	*p*-value
Stage B (%)	129/319 (40.7%)	2/5 (40%)	
Stage C (%)	69/319 (21%)	2/5 (40%)	
Unknown	1/319 (0.3%)	0 (0%)	
Co-infections			
Ab anti-HCV positivity at baseline	31/319 (10%)	2/5 (40%)	0.084
HBsAg positivity at baseline	304/319 (95%)	5/5 (100%)	0.788
CMV-IgG positivity at baseline	267/319 (84%)	5/5 (100%)	0.615
Other			
Chemotherapy	8/319 (2%)	0 (0%)	0.882
Deaths	4/319 (2%)	1/5 (20%)	<0.001
Immunovirological data			
CD4 nadir (n/mmc) mean (SD)	279 (±222)	33 (±26)	0.014
CD8+ at baseline, median (SD)	942 (±584)	609 (±308)	0.365
CD8+ at 36 months, mean (SD)	893 (±404)	811(±367)	0.652
CD4/CD8 ratio at baseline, median (IQR)	0.32 (0.16–0.61)	0.14 (0.05–0.15)	0.012
CD4/CD8 ratio at 36 months median (IQR)	0.78 (0.49–1.11)	0.17 (0.13–0.25)	<0.001
HIV-RNA zenith, log_{10} (copies/ml), mean (SD)	4.77 (±1.04)	4.61 (±0.55)	0.719
Time to HIV-RNA ≤ 50 copies/ml (months), mean (SD)	4 (±0.19)	5 (±2.34)	0.970
Therapy			
Months of INI exposure, mean (SD)	13 (±0.92)	21 (±8.81)	0.322
Months of PI exposure, mean (SD)	10 (±0.85)	14 (±8.18)	0.690
Months of NNRTI exposure, mean (SD)	13 (±0.92)	7 (±7.22)	0.310
Months of ABC/3TC exposure, mean (SD)	4 (±0.55)	0	0.322
Months of TDF/FTC exposure, mean (SD)	28 (±0.74)	22 (±8.81)	0.447

ABC/3TC, abacavir+ lamivudine; HBsAg hepatitis B surface antigen; INR_{200} people who did not recover at least 200 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; IQR, interquartile range; INI, integrase inhibitors; IVDU intravenous drug users; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside or nucleotide reverse transcriptase inhibitors; PI, protease inhibitors; SD, standard deviation; TDF/FTC tenofovir disoproxil fumarate/emtricitabine.

association with all INR definitions, with higher values predicting $INR_{20\%}$ [adjusted odds ratio (aOR) 1.003, 95% CI 1.001–1.004, $p < 0.001$] and lower values predicting INR_{500} (aOR 0.984, 95% CI 0.980–0.988, $p < 0.001$) and INR_{200} (aOR 0.98, 95% CI 0.95–1.001, $p = 0.098$). Both $INR_{20\%}$ and INR_{500} were associated with lower CD8+ T cell counts after 36 months of suppressive ART (aOR 0.99, 95% CI 0.99–1.00, $p = 0.010$, and aOR 0.99, 95% CI 0.99–1.00, $p < 0.001$, respectively). The complete multivariable analysis is shown in Table 5.

In the sensitivity analysis including the CD4/CD8 ratio instead of the absolute numbers of CD4 and CD8+ T cells (Table 6), a higher baseline CD4/CD8 ratio was confirmed to be inversely related to the probability of poor immune recovery for INR_{500} (OR 0.03, 95% CI 0.01–0.12, $p < 0.001$) and INR_{200} (OR 0.002, 95% CI 18^{-7}–67.72, $p = 0.255$), and it was directly related to the odds of being $INR_{20\%}$ (OR 6.14, 95%CI 2.36–15.97, p<0.001).

At the 36-month evaluation, the relationship between CD4/CD8 ratio and INR was confirmed, with OR 0.05 (95% CI 0.02–0.13, $p < 0.001$) and 1.9^{-14} (95% CI 4.5^{-25}–7.88^{-4}, $p = 0.011$) of being INR_{500} and INR_{200}, respectively, and OR 1.30 (95% CI 0.72–2.33) of being $INR_{20\%}$ for each unit of increase of CD4/CD8 ratio (Table 7).

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TABLE 5 Multivariable analysis of factors associated to $INR_{20\%}$, INR_{500} and INR_{200}.

	$INR_{20\%}$		INR_{500}		INR_{200}	
Variable	aOR (95%CI)	*p* value	aOR	*p* value	aOR	*p* value
Non-Italian			1.47 (0.68–3.18)	0.332		
CDC C stage at HIV diagnosis	0.54 (0.15–1.95)	0.346	0.55 (0.23–1.33)	0.185		
Deaths					9.07 (0.64–126.9)	0.101
Immunovirological data						
CD4 nadir (n/mmc)	1.003 (1.001–1.004)	<0.001	0.98 (0.98–0.99)	<0.001	0.98 (0.95–1.01)	0.096
CD8 at 36 months	0.99 (0.99–1.00)	0.010	0.99 (0.99–1.00)	<0.001		
HIV-RNA zenith, log_{10}	0.99 (0.99–1.00)	0.010				
Months of INI exposure			1.03 (1.01–1.05)	0.037		
Months of NNRTI exposure			1.03 (0.99–1.05)	0.076		

95%CI, 95% Confidence Interval; aOR, adjusted Odds Ratio; FR_{200}, full responders, defined as people who recover at least 200 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy $INR_{20\%}$ people who did not recover at least 20% CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{500} people who did not recover at least 500 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{200} people who did not recover at least 200 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy.

4 Discussion

In the present study we found a prevalence of INR ranging between 1.5% and 34.6%, exploring different definition of immunological non-response.

The most restrictive definition that we used, namely that of INR_{200}, revealed an INR_{200} rate of only 1.5%, which was lower than that reported in older studies that used the same threshold, which ranged between 15% and 26% (11, 42, 44), but which is consistent with the results of others (45). However, comparison among studies might be influenced by different study periods and antiretrovirals used in different settings and by heterogeneous duration of follow-up. The higher frequency of INR_{200} found in previous years might reflect the use of older drugs that are currently considered sub-optimal or less tolerable, possibly compromising overall ART efficacy and, consequently, also immunological recovery (11, 42, 44). On the contrary, newer drugs such as INSTIs (46, 47), and also PIs, when compared with older ART approaches (48), might favor a more effective immune recovery. However, in our study, we did not find a consistent correlation between INR rate and months of exposure to different classes of antiretrovirals. It is notable that the study was conducted in an era of modern ART; if different ART classes were used in first-line therapy, the usefulness of comparing newer and older drugs is limited by the absence of an historical control group. In addition, the observation about the role of a low CD4 nadir underlying the INR_{200} phenomenon underscores the very important role of

TABLE 6 Multivariable analysis of factors associated to $INR_{20\%}$, INR_{500} and INR_{200}, including CD4/CD8 ratio at baseline.

	$INR_{20\%}$		INR_{500}		INR_{200}	
Variable	aOR (95% CI)	*p*-value	aOR	*p*-value	aOR	*p*-value
Non-Italian			1.39 (0.76–2.56)	0.285		
CDC C stage at HIV diagnosis	0.46 (0.13–1.62)	0.227	1.36 (0.71–2.59)	0.348		
Deaths					10.24 (0.81–129.41)	0.072
Immunovirological data						
CD4/CD8 ratio at baseline	6.14 (2.36–15.97)	<0.001	0.03 (0.01–0.12)	<0.001	0.002 (18e-7–67.72)	0.255
HIV-RNA zenith, log_{10}	0.84 (0.59–1.19)	0.328				
Months of INI exposure			1.00 (0.99–1.03)	0.394		
Months of NNRTI exposure			0.99 (0.97–1.01)	0.592		

95%CI, 95% confidence interval; aOR, adjusted odds ratio; $INR_{20\%}$ people who did not recover at least 20% CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{500} people who did not recover at least 500 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{200} people who did not recover at least 200 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy.

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TABLE 7 Multivariable analysis of factors associated to $INR_{20\%}$, INR_{500} and INR_{200}, including CD4/CD8 ratio at 36 months evaluation.

	$INR_{20\%}$		INR_{500}		INR_{200}	
Variable	aOR (95% CI)	*p*-value	aOR	*p*-value	aOR	*p*-value
Age in years						
Non-Italian			1.42 (0.76–2.66)	0.271		
CDC C stage at HIV diagnosis	0.31 (0.09–1.07)	0.063	1.68 (0.88–3.23)	0.118		
Deaths					15.1 (4.59e-25–7.88e-4)	0.587
Immunovirological data						
CD4/CD8 ratio at 36 months	1.30 (0.72–2.33)	0.378	0.05 (0.02–0.13)	<0.001	1.9e-14 (4.5e-25–7.88e-4)	0.011
HIV-RNA zenith, log_{10}	0.81 (0.59–1.10)	0.170				
Months of INI exposure			1.00 (0.98–1.03)	0.512		
Months of NNRTI exposure			0.99 (0.97–1.02)	0.715		

95% CI, 95% confidence interval; aOR, adjusted odds ratio; $INR_{20\%}$ people who did not recover at least 20% CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{500} people who did not recover at least 500 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy; INR_{200} people who did not recover at least 200 CD4+T cells after 36 months of virological suppression (HIV RNA <50 copies/ml) on antiretroviral therapy.

early diagnosis in preventing the INR phenomenon; increased awareness of the risk of infection and improved timing of diagnosis could explain, at least in part, the reduction in the current number of INR_{200}.

On the other hand, when assessing frequency of INR_{500}, we still found about 35% of PLWH with a discordant viro-immunological response, in line with the frequency found in previous studies (34, 45, 46), suggesting that, even if modern therapies have contributed to make INR_{200} rarer, the complete viro-immunological response is still not guaranteed nor consistently improved compared with the past, when using the CD4 threshold of 500 cells/mm^3. Even if the highest risk of clinical events and death has been associated with INR_{200} (11, 42, 44), PLWH with a CD4 count in the range 200–499 cells/mm^3 still have higher mortality rates than those with a CD4 count > 500 cells/mm^3 (49). Therefore, the goal of ART should be to get patients over this threshold and reaching an immunological condition as close as possible to that of the general population. Moreover, INR_{500} have been shown to have higher percentages of activated CD4+ T cells, regulatory T cells (T_{reg}), effector T_{reg} and terminal effector T_{reg} suggesting, in this group of PLWH, a residual immune activation persisting after many years of ART (18, 35). In addition, immune responses against vaccines are still different among PWH with CD4+ T cell counts of < 200, 200–500, or > 500 cells/mm^3, and in those with counts of > 500 cells/mm^3 responses comparable to those in the HIV-uninfected population have been described (50). The persistence of a certain grade of inflammation might also be indirectly inferred from the CD4/CD8 ratio, a surrogate marker of T-cell compartment balance, reflecting both CD4 T-cell recovery and CD8 T-cell activation, expansion, and senescence (51, 8). Few studies have investigated the relationship between CD4/CD8 ratio and immune recovery, supporting an association between lower baseline levels and immunodiscordant response to ART (46, 52). In our study, we found not only that a higher CD4/CD8 ratio was protective toward becoming either INR_{200} or INR_{500} at time of ART initiation, but also that that CD4/CD8 ratio was more likely to be lower after 36 months of ART in INR_{200} and INR_{500} than in full responders, supporting the hypothesis of a possible persistent immune activation impairing reconstitution of the immune system and CD4 gains (53). Moreover, CD4 nadir was confirmed to be the stronger predictor of immunological non-response in INR_{200} and INR_{500}, in accordance with data from the literature (18, 34, 44, 45, 54), and all other epidemiological and clinical variables considered did not consistently correlate with INR. Instead, these data were not confirmed when using the definition of $INR_{20\%}$. In fact, in our study, the odds of being $INR_{20\%}$ was higher in PLWH with a higher CD4 nadir and higher CD4/CD8 ratio. Being $INR_{20\%}$ has been described in the past as a predictor of clinical progression (14); however, the definition of $INR_{20\%}$ is highly influenced by baseline CD4– T cell count and, in an era of modern ART, where therapy is initiated regardless of total CD4 count at HIV diagnosis, this definition may be misleading, as it may imply the inclusion of early-treated patients with unimpaired baseline CD4 counts. Consequently, these PLWH do not experience an increase in CD4 above the predefined threshold of 20% from baseline, but this should not be interpreted as a poor response. On the contrary, for PLWH with extremely low CD4 counts, a small CD4 gain could even exceed the threshold of 20% while remaining with a very low absolute number of lymphocytes and CD4/CD8 ratio. Therefore, even if the definition can still be applied to certain clinical situations in PLWH stratified based on baseline CD4 counts, or in certain research contexts, $INR_{20\%}$ might not be a suitable definition for identifying in general PLWH with poor responses in clinical practice.

The present study has several limitations. The retrospective design and relatively small sample size of the cohort limit the strength and generalizability of the findings. Moreover, data on biomarkers of immune activation, on viral reservoir, as well as data on herpetic viral co-infections, genetics, and behavioral or dietary factors were not available, limiting the possibility of investigation of further variables influencing immunological responses. In addition, the exclusion of people lost to follow-up or dead before the predefined time point of 36 months might have contributed to underestimation of INRs. Despite these limits, the study highlights how INR_{200} have become very rare in the contemporary ART era, and still about one-third of PLWH meet the criteria for INR_{500}. Overcoming the threshold of 500 CD4/mm^3 could be more appropriate to define full responders, in contrast with the older definitions of INR_{200} and $INR_{20\%}$. Although our results do not show a benefit from choosing different ART strategies to improve immune recovery, early diagnosis, and rapid treatment initiation before CD4 counts and CD4/CD8 ratio begin to decline are critical to achieving an optimal immune response.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ligurian Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LT, LL, FB, GB, SB, AB, and MB performed the research. SM, SB, and MG managed the database and checked the accuracy of the data. LT, LL, and AB designed the research study. LT analyzed the data. LT, LL, FB, and GB wrote the paper. MB and AB reviewed the final version of the paper and the scientific contents of the study. All authors have read and approved the final version of the paper.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fviro.2022.822153/full#supplementary-material

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