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Reduced community viral load does not coincide with a reduction in the rate of new HIV diagnoses and recent infections: data from a region of southern Italy

L Monno,[1] A Saracino,[1] L Scudeller,[2] C Santoro,[1] G Brindicci,[1] G Punzi,[1] A Lagioia,[1] S Lo Caputo[1] and G Angarano[1]
[1]*Clinic of Infectious Diseases, University of Bari, Bari, Italy and* [2]*Scientific Direction, Clinical Epidemiology Unit, IRCCS San Matteo Foundation, Pavia, Italy*

Objectives
We assessed whether changes in community viral load (CVL) over time were associated with the rate of new HIV diagnoses (NDs).

Methods
HIV-1-positive individuals referred to our institute and permanently residing in our province were considered for inclusion in the study. A total of 861 HIV-infected adults with at least one HIV RNA measurement (12 530 measurements in total) between 2008 and 2014 were included. Viraemia copy-years were calculated from all HIV RNA values for each patient using the trapezoidal rule; multiple CVL indicators were considered. Total NDs and recent infections (< 1 year) were analysed separately. The association between NDs and CVL was tested by means of mixed Poisson models, with CVL as a fixed effect and year as a random effect.

Results
The incidence of NDs was 2.28 per 100 000 residents in 2008 and 2.52 per 100 000 residents in 2014. Total numbers of NDs and recent infections did not vary significantly over time (P for trend 0.879 and 0.39, respectively). Mean HIV RNA decreased from 31 095.8 HIV-1 RNA copies/mL in 2008 to 21 231.5 copies/mL in 2014 ($P < 0.001$); a downward trend was always observed regardless of the CVL indicator considered. Depending on the indicator, there were some differences in CVL by patient characteristics. The most substantial contributors to CVL appeared to be male individuals, men who have sex with men (MSM), non-Italians, and untreated subjects (all $P < 0.05$). The relative risk of ND increased among Italians and MSM with an increasing proportion of subjects having an undetectable HIV RNA, and decreased in the same population with increasing levels of CVL.

Conclusions
In our setting, CVL represented a good marker of access to care and treatment; however, reduced CVL did not coincide with a reduction in the rate of NDs.

Keywords: community viral load, HIV-1, new diagnoses, prevention, transmission

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Introduction

High plasma HIV RNA levels are a major risk factor for HIV transmission [1]; thus, effective antiretroviral therapy (ART) which lowers viraemia in HIV-1-infected subjects should also reduce the risk of transmission to their sexual partners [2,3]. In fact, Quinn *et al.* [2] reported no heterosexual HIV transmission when the plasma viral load was < 1500 HIV-1 RNA copies/mL, and the most numerous transmission events for viral loads > 30 000 copies/mL. The crucial role of ART in preventing HIV transmission has been highlighted in ecological studies of communities represented by aggregations of men who have sex with men (MSM) and injecting drug users (IDUs) [4,5]. In these studies, community viral load (CVL) [6] was used to estimate the burden and infectivity of

Correspondence: Dr Laura Monno, Clinic of Infectious Diseases, University of Bari, P.zza G. Cesare 11, Bari 70124, Italy. Tel: +39 080 5592135; fax: +39 0805478333; e-mail: laura.monno@uniba.it

individuals in these particular populations. Das and coworkers [4] defined CVL as the mean of the most recent viral loads of all reported HIV-positive individuals included in their study, and found that CVL reductions coincided with decreased rates of HIV infection. Similarly, utilizing the highest viral load value for each subject in a specific year, Montaner *et al.* [5] reported a strong association between increased ART coverage, reduced CVL, and decreased number of new HIV diagnoses per year; moreover, this result appeared to be independent of high-risk behaviours [7]. Conversely, Castel *et al.* [8] observed that the number of new HIV diagnoses remained stable in spite of significant CVL reductions over time and increased proportions of subjects with undetectable viral loads.

How these conflicting observations can be interpreted in the context of treatment as prevention (TasP) strategies [9] is particularly intriguing. TasP is a method for reducing the spread of HIV infection based on the assumption that, if the number of people receiving earlier and more effective HIV treatment increases, new HIV infections are expected to decrease.

In Italy, the surveillance of new HIV diagnoses, providing information on individuals newly diagnosed with HIV infection, became mandatory in 2008 (G.U., number 175; 28 July 2008). Data are reported to the Centro Operativo AIDS (COA) of the National Institute of Health [Istituto Superiore di Sanità (ISS)] from regions and provinces. According to the COA, the Italian incidence of new HIV-1 diagnoses was 7.1 per 100 000 population in 2007 [10]; thereafter, only a minor decrease in the incidence of new cases was observed [11]. Therefore, although HIV-infected patients are exempted from payment for both clinical examinations and ART, and 91.9% of the 100 049 patients living with HIV-1 were on ART in 2014 [11], the HIV epidemic in Italy has shown only limited signs of slowing down.

The aims of our study were to evaluate the changes in CVL over time and to determine whether CVL was associated with the rate of new HIV diagnoses in a region of southern Italy.

Methods

Data sources

The Metropolitan City of Bari (previously the Province of Bari) is the capital of Apulia in southern Italy and includes 41 municipalities within an area of 5138 km^2 with a total population of 1 260 000 people (in 2014).

Between January 2008 and December 2013, 791 individuals with a new HIV-1 diagnosis were reported to the COA from Apulia. Notifications for 337 (42.6%) of these newly diagnosed patients were made by the Clinic of Infectious Diseases, University of Bari, and the principal characteristics of these patients did not differ from those of the other Apulian patients reported to the ISS. Moreover, from the beginning of the HIV-1 epidemic in Apulia (at the end of 1985) to 2013, of 2411 patients with AIDS for whom notifications were made to the COA, 33% were from our clinic where, during the same time period, more than 1200 HIV-positive patients were being followed. Therefore, the HIV-infected population referred to our institute can be considered representative of the entire Apulian region.

Study population/community

For purposes of this study, the "community" consisted of the HIV-1-positive population referred to our institute and permanently (according to self-reported residence) residing in our province. Patients had to have at least one HIV viral load test between January 2008 and December 2014. Epidemiological, demographic and virological data for the patients were recorded in a dedicated database. Newly diagnosed HIV-infected patients were adults $\geq$ 18 years of age, testing HIV positive for the first time during the study period; although new HIV diagnoses may represent newly diagnosed but chronically infected individuals, new HIV diagnoses have been used as a proxy for HIV incidence [12,13]. However, in our study, patients with a new HIV diagnosis were subsequently classified according to the duration of HIV infection estimated by calculating the proportion of ambiguous nucleotides of polymerase (pol) [protease (PR) and reverse transcriptase (RT)] sequences in the first available plasma sample after HIV diagnosis: $\leq$ 0.2% ambiguity signified a recent infection ($\leq$ 1 year), versus older infections [14]. Pol sequences were also analysed for HIV subtype by phylogenetic analysis; for the purpose of this study, patients were classified as infected with either B or non-B HIV-1 variants.

On the basis of self-reported risk factors, patients were characterized as injecting drug users (IUDs), blood recipients, men who have sex with men (MSM), and individuals who acquired their infection through heterosexual contacts (including non-drug-addicted commercial sex workers and individuals with a history of unprotected sex with one or more unknown partners).

Statistical analyses

Descriptive statistics were produced for patient demographic, clinical and laboratory characteristics. According

to the data distribution, groups were compared using parametric or nonparametric tests for continuous variables, and Pearson's chi-square test (or Fisher's exact test where appropriate) for categorical variables. Correlation between continuous variables was assessed by means of Pearson's or Spearman's coefficient according to the data distribution. The trend over time (study years) for demographic and viroimmunological parameters at first diagnosis was assessed by means of the chi-square for trend. In all cases, two-tailed tests were used. The *P*-value significance cut-off was 0.05.

CVL was defined in several ways [15], utilizing measured viral loads (MVLs), that is, all available viral load measurements of all patients followed at our institute in the study period. In more detail, for each study year, the following CVL indicators were used: sum, mean, sum $\log_{10}$, mean $\log_{10}$, geometric mean, proportion of undetectable (< 25 HIV-1 RNA copies/mL) and high viral load (> 100 000 copies/mL) samples, and proportion of suppressed (< 200 copies/mL) and unsuppressed (> 200 copies/mL) samples.

Unlike the methods of other authors, the CVL used for all calculations was expressed as the viraemia copy-years (VCY) for each individual patient from one visit (t) to the following visit ($t + 1$), and estimated by means of the trapezoidal approximation $[(t + 1 - t) \times (VL_{(t+1)} + VL_{(t)})/2]$ in order to better reflect the continuous exposure to HIV.

For undetectable viral loads, half of the lower limit of detection (LLD) of the assay used for quantifying HIV viral load was considered [15]. Of note, the LLD was < 50 copies/mL in 2008–2009 and thereafter < 25 copies/mL; for consistency, we assumed 25 copies/mL.

For patients with a single HIV RNA measurement, this value was used without calculation of copy-years. For consecutive samples over two calendar years, the VCY was distributed between the two years according to the proportion of time spent within each year, following linear interpolation. Each indicator was calculated for the entire sample, and in a number of subgroups according to gender, age (< 18, 18–25, 25–35, 35–45, 45–55, 55–65 or > 65 years old), risk factor for HIV acquisition, nationality (Italian or other), duration of HIV infection (< 1 year or > 1 year), HIV subtype (B or non-B) and ART (on ART or not on ART).

To assess the significance of trend over time for each indicator, multilevel generalized mixed models (linear for continuous CVLs and logistic for binomial CVLs) were fitted on all available measurements, with linear trend for study year as a fixed effect (and with study year also as a random effect). The interaction of study year and subgroup was also explored, to assess whether differences between groups changed over time.

The association between the rate of new diagnoses (all new diagnoses and recent infections only) and CVL indicators was investigated by means of mixed Poisson models, with CVL measure as a fixed effect and year as a random effect. Models were fitted to data for the whole group, for Italians only, and for those with "heterosexual" and "MSM" risk factors only, to take into account differences in the dynamics of HIV transmission.

For each year, the HIV incidence was calculated as the number of new HIV diagnoses per 100 000 inhabitants and plotted on a geographical map of the Apulia region according to province and year. Newly diagnosed HIV-1-infected patients residing within the Bari Province were also plotted according to municipality of residence against HIV incidence.

Ethics

The study did not require approval from the ethics committee, according to Italian law, as it was performed in the context of normal clinical routine (art.1, leg. decree 211/2003). However, all patients referred to our institute provided consent for the use of their data for research purposes. Data were previously anonymized, according to the requirements set out by Italian Data Protection Code (leg. decree 196/2003).

Results

Incidence of new HIV-1 diagnoses

In Figure 1, the incidence (number of new cases per 100 000 residents) of new diagnoses of HIV-1 infection in Apulia is shown by province and year of study. The HIV incidence was 2.28 per 100 000 residents in 2008 and 2.52 per 100 000 residents in 2014. In the Bari Province, the total number of new diagnoses and recent infections only did not differ significantly over time (*P*-value for trend 0.879 and 0.39, respectively), with the highest number of cases found in the City of Bari.

Characteristics of individuals with a new HIV-1 diagnosis

Among the "community" of 861 individuals, a total of 283 individuals were identified as having a new HIV diagnosis (as previously defined) between January 2008 and December 2014. Patients were mainly male (79.5%), of Italian origin (79.5%), and between the ages of 25 and 45 years (61.8%) at the time of HIV diagnosis, and included 40.7% MSM and 37.8% individuals who acquired their infection through heterosexual contacts

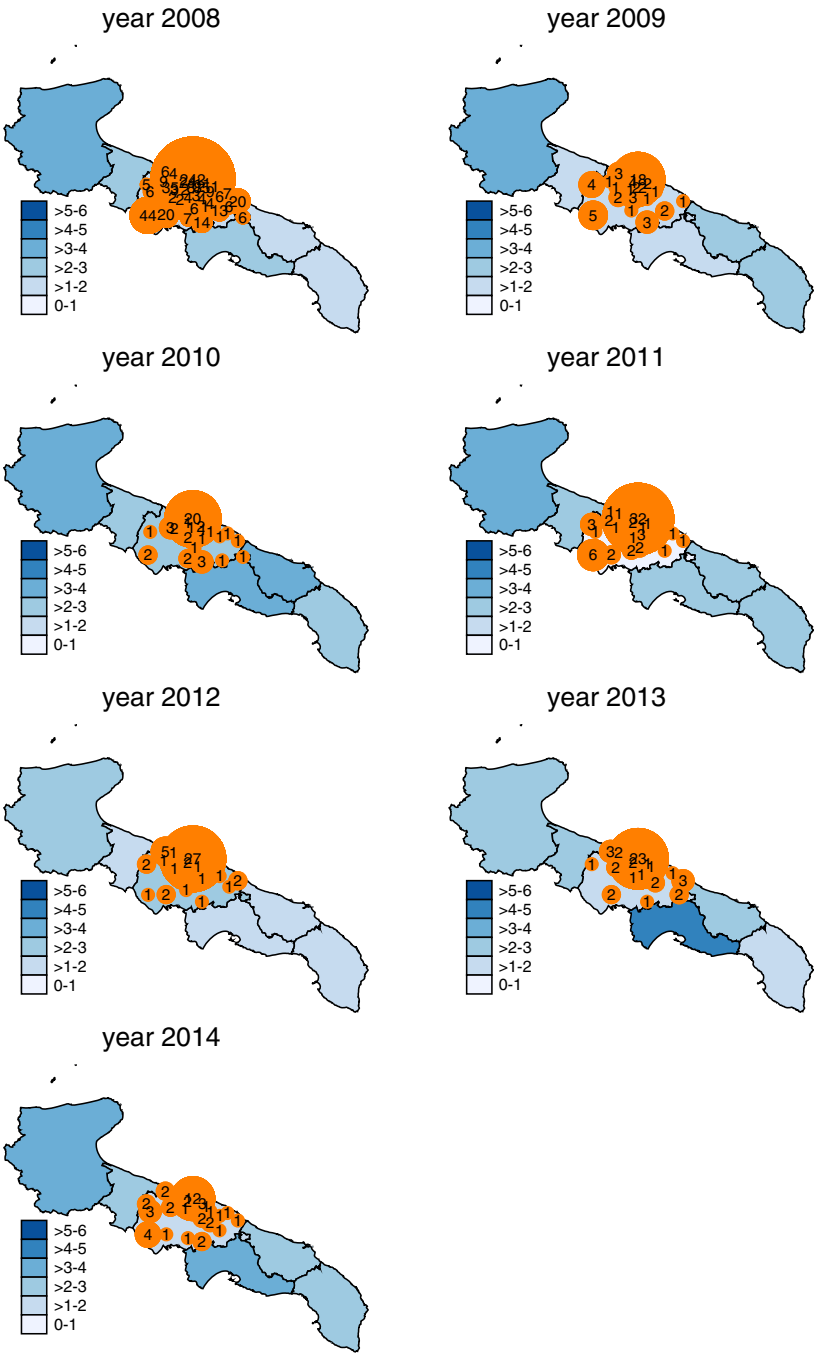

Fig. 1 The number of newly diagnosed HIV-1-infected patients referred to our institute and residing in Bari Province (shown inside circles) plotted by municipality and by year (2008–2014) superimposed on HIV incidence (number of new cases per 100 000 residents) in each Apulia province, indicated by the intensity of shading.

(Table 1). Pol sequences were available for 267 (94.3%) of these 283 individuals; 155 (58.1%) patients were assigned to subtype B and 112 (41.9%) to HIV-1 non-B variants. According to the proportion of ambiguous nucleotides within the PR/RT sequences, HIV-1 infection was estimated as recent in 40.6% and older in 53.7% of patients, respectively. At the time of presentation, the mean HIV RNA and CD4 cell count were 393 694 copies/mL and

363.7 cells/μL, respectively. The characteristics of newly diagnosed patients did not differ over time except for the proportion of individuals with non-B infection (P for trend = 0.003) (Table 1). When the subgroup of 115 patients with a recent new infection was considered, the proportion of non-B infections was the only variable with a statistically significant trend over time ($P = 0.032$). Moreover, even after excluding patients of non-Italian nationality, non-B infections increased from 10.3% in 2008 to 40.7% in 2014 (P for trend = 0.004), as a result of the social and epidemiological changes that have occurred in southern Italy [16].

Patients with chronic HIV-1 infection

A total of 578 patients with a known chronic HIV-1 infection, regardless of current treatment status, were included in the community as they had one or more viral load measurements between 2008 and 2014. In total, 67.3% of patients were male, 93.6% were Italian, and the risk factor was intravenous drug abuse in 40.6% of patients, heterosexual contacts in 33.4% and homosexuality in 15.7%, while 7.4% of patients denied any risk factor for HIV infection. Overall, 457 (79.1%) patients received ART, with the remaining subjects either interrupting (14.2%) or never initiating (6.7%) ART because of toxicity or personal beliefs.

Plasma samples and CVL

Among the 861 participants in the community, there were 12 530 plasma HIV RNA assessments with a median of 16 [interquartile range (IQR) 8–20; minimum–maximum range 1–34] measurements per person (Table 2).

For plasma samples with HIV RNA assessment, the contribution of non-Italian patients increased from 5.4% in 2008 to 10.3% in 2014 (P for trend < 0.001). Over time, the proportion of plasma HIV RNA measurements during ART increased from 83.7% to 87.9% ($P = 0.006$); consequently, the proportion of samples with detectable plasma viral load diminished from 30.7% in 2008 to 24.3% in 2014 ($P < 0.001$). The overall mean plasma HIV RNA was 27 566 copies/mL (Table 2), and decreased from a mean of 31 095.8 copies/mL in 2008 to 21 231.5 copies/mL in 2014 ($P < 0.001$), and from 2.144 to 1.562 $\log_{10}$ copies/mL, respectively ($P < 0.001$).

In Table 3 and Figure 2, all CVL indicators are reported. In particular, the CVL is reported for the entire study period for the total population in Table 3, with a breakdown according to risk factor (only heterosexuals and MSM are shown), gender, nationality and ART. As shown, the mean CVL was significantly higher for heterosexuals than for MSM ($P = 0.0078$), and for patients not on ART compared with those on ART ($P < 0.001$). Mean $\log_{10}$ and geometric mean CVLs were higher for MSM ($P < 0.001$ and $P = 0.0004$, respectively), male patients ($P = 0.008$ and $P = 0.13$, respectively), non-Italians ($P = 0.002$ and $P = 0.009$, respectively) and patients not receiving ART (both $P < 0.001$). The most numerous contributors to the overall CVL, in terms of the proportion of patients with either high (> 100 000 copies/mL) or unsuppressed viral load (> 200 copies/mL), were MSM ($P = 0.00014$ and $P < 0.001$, respectively), male patients (both $P < 0.001$), non-Italians (both $P < 0.001$) and, obviously, patients not receiving ART (both $P < 0.001$).

In Figure 2, the trend over time for different CVL indicators is depicted together with the number of new HIV diagnoses, and their incidence according to calendar year. As reported above, the number and incidence of new diagnoses did not vary significantly over time. Conversely, a downward trend was always observed regardless of the CVL indicator considered, with a reduction over time for the proportion of patients with either unsuppressed (> 200 copies/mL) or high (> 100 000 copies/mL) HIV RNA, which reached statistical significance (P value for CVL trend = 0.009 and $P = 0.021$, respectively). A similar trend was observed when, for the calculation of CVL, we used the mean viral load measurement for each patient, the most recent or the highest value during a given calendar year (data not shown).

Association between number of new HIV diagnoses and CVL

In Table 4a,b, we respectively report the associations of the total number of new HIV diagnoses and the number of recent infections only with CVL indicators. The incidence rate ratio (IRR) of new HIV diagnoses overall was significantly reduced (all $P < 0.05$) among individuals of Italian origin and MSM with increasing CVL, expressed as the sum and mean of CVL, $\log_{10}$ or geometric mean of CVL. The IRR significantly ($P < 0.05$) increased in Italians and MSM with an increasing proportion of individuals with undetectable viral load, and also in heterosexual patients with an increasing proportion of individuals with detectable viral load. The results were very similar when we considered only new HIV diagnoses with a recent infection (Table 4b).

Discussion

The widespread use of combination ART (cART) among HIV-positive individuals has led to a significant reduction in the HIV transmission rate, thus contributing to better control of the HIV epidemic [17]. In fact, serum HIV RNA

Table 1 Characteristics of the 283 patients newly diagnosed with HIV-1 infection

	Total	2008	2009	2010	2011	2012	2013	2014	*P*-value for trend
n (%)	283	48 (16.9)	39 (13.7)	32 (11.3)	51 (18.0)	37 (13.0)	40 (14.1)	36 (12.7)	
Sex [*n* (%)]									
Male	225 (79.5)	40 (83.3)	31 (79.5)	25 (78.1)	35 (68.6)	30 (81.1)	33 (82.5)	31 (86.1)	
Female	58 (20.5)	8 (16.7)	8 (20.5)	7 (21.9)	16 (31.4)	7 (18.9)	7 (17.5)	5 (13.9)	0.711
Age [*n* (%)]									
< 18 years	2 (0.7)	0	0	0	0	2 (5.4)	0	0	
18–25 years	40 (14.1)	4 (8.3)	7 (18.0)	7 (21.9)	9 (17.7)	4 (10.8)	7 (17.5)	2 (5.6)	
25–35 years	99 (34.9)	16 (33.3)	18 (46.1)	7 (21.9)	21 (41.2)	15 (40.5)	13 (32.5)	9 (25.0)	
35–45 years	76 (26.9)	15 (31.3)	10 (25.6)	9 (28.1)	8 (15.7)	11 (29.7)	10 (25.0)	13 (36.1)	
45–55 years	35 (12.4)	6 (12.5)	2 (5.1)	2 (6.3)	9 (17.7)	5 (13.5)	5 (12.5)	6 (16.7)	
55–65 years	21 (7.4)	6 (12.5)	2 (5.1)	5 (15.6)	1 (2.0)	0	3 (7.5)	4 (11.1)	
> 65 years	10 (3.5)	1 (2.1)	0	2 (6.3)	3 (5.9)	0	2 (5.0)	2 (2.6)	0.404
Nationality [*n* (%)]									
Italian	225 (79.5)	42 (87.5)	33 (84.6)	28 (87.5)	34 (66.7)	28 (75.7)	31 (77.5)	29 (79.5)	
Non-Italian	58 (20.5)	6 (12.5)	6 (15.4)	4 (12.5)	17 (33.3)	9 (24.3)	9 (22.5)	7 (19.4)	0.215
Risk factor [*n* (%)]									
ND	52 (18.4)	3 (6.2)	2 (5.1)	8 (25.0)	14 (27.5)	11 (29.7)	4 (10.0)	10 (27.8)	
Heterosexual	107 (37.8)	19 (39.6)	17 (43.6)	14 (43.8)	22 (43.1)	9 (24.3)	17 (42.5)	9 (25.0)	
MSM	112 (40.7)	22 (45.8)	18 (46.1)	9 (28.1)	13 (25.5)	16 (43.2)	18 (45.0)	16 (44.4)	
IDU	12 (4.2)	4 (8.3)	2 (5.1)	1 (3.1)	2 (3.9)	1 (2.7)	1 (2.5)	1 (2.8)	0.10
Duration of HIV infection [*n* (%)]									
< 1 year	115 (40.6)	13 (27.1)	21 (53.8)	13 (40.6)	18 (35.3)	15 (40.5)	18 (45.0)	17 (47.2)	
> 1 year	152 (53.7)	32 (66.7)	17 (43.6)	16 (50.0)	29 (56.9)	21 (56.8)	21 (52.5)	16 (44.4)	
ND	16 (5.7)	3 (6.2)	1 (2.6)	3 (9.4)	4 (7.8)	1 (2.7)	1 (2.5)	3 (8.3)	0.273
HIV-1 subtype [*n* (%)]									
B	155 (58.1)	36 (80.0)	25 (65.8)	17 (58.6)	20 (42.5)	20 (55.6)	20 (51.3)	17 (51.5)	
Non-B	112 (41.9)	9 (20.0)	13 (34.2)	12 (41.4)	27 (57.5)	16 (44.4)	19 (48.7)	16 (48.5)	0.003
HIV RNA (copies/mL) [mean (SD)]	393 694 (1 210 124)	151 673 (400 312)	602 183 (1 959 563)	855 067 (1 822 396)	282 695 (638 571)	479 953 (1 675 126)	208 337 (395 809)	354 961 (10 158 861)	0.881
Absolute CD4 cell count (cells/μL) [mean (SD)]	363.7 (261)	311.9 (256)	406.2 (265)	380.1 (232)	424.9 (292)	387.5 (249)	328.3 (268)	300.5 (234)	0.555
CD4 percentage [mean (SD)]	19.1 (11)	16.0 (10.9)	21.4 (12.0)	17.8 (8.6)	20.4 (11.0)	21.0 (12.3)	17.9 (11.0)	20.2 (10.0)	0.326

ND, not determined; MSM, men who have sex with men; IDU, injecting drug use; SD, standard deviation.

Table 2 Samples contributing to the calculation of community viral load (CVL), overall and by study year

	Total	2008	2009	2010	2011	2012	2013	2014	*P*-value for trend
Number of samples	12 530	1825	1821	1764	1792	1686	1771	1871	
Nationality [*n* (%)]									
Italian	11 615 (92.7)	1726 (94.6)	1733 (95.2)	1669 (94.6)	1660 (92.6)	1552 (92.1)	1597 (90.2)	1678 (89.7)	< 0.001
Non-Italian	915 (7.3)	99 (5.4)	88 (4.8)	95 (5.4)	132 (7.4)	134 (7.9)	174 (9.8)	193 (10.3)	
Sex [*n* (%)]									
Male	8819 (70.4)	1270 (69.6)	1269 (69.7)	1267 (71.8)	1251 (69.8)	1180 (70.0)	1247 (70.4)	1335 (71.4)	0.372
Female	3711 (29.6)	555 (30.4)	552 (30.3)	497 (28.2)	541 (30.2)	506 (30.0)	524 (29.6)	536 (28.7)	
Age (years) [mean (SD)]	44.82 (10.2)	44.04 (8.94)	44.30 (9.17)	44.93 (9.44)	44.20 (10.3)	44.99 (10.9)	44.91 (11.2)	46.31 (10.2)	< 0.001
ART [*n* (%)]									
Not on ART	1857 (14.8)	298 (16.3)	244 (13.4)	276 (15.7)	315 (17.6)	242 (14.4)	255 (14.4)	227 (12.1)	0.006
On ART	10 673 (85.2)	1527 (83.7)	1577 (86.6)	1488 (84.4)	1477 (82.4)	1444 (85.7)	1516 (85.6)	1644 (87.9)	
Plasma viral load [*n* (%)]									
Undetectable	8520 (68)	1265 (69.3)	1295 (71.1)	1086 (61.6)	1119 (62.4)	1108 (65.7)	1231 (69.5)	1416 (75.7)	< 0.001
Detectable	4010 (32)	560 (30.7)	526 (28.9)	678 (38.4)	673 (37.6)	578 (34.3)	540 (30.5)	455 (24.3)	
HIV RNA									
Copies/mL [mean (IQR)]	27 566.14 (12.5–100)	31 095.8 (12.5–340)	29 725.7 (12.5–91)	49 396.1 (12.5–170)	27 022.2 (12.5–270)	22 161.8 (12.5–93)	12 257.3 (12.5–68)	21 231.5 (12.5–100)	< 0.001
Log_{10} copies/mL [mean (SD)]	1.869 (1.28)	2.144 (1.41)	1.981 (1.29)	1.939 (1.38)	1.949 (1.37)	1.774 (1.19)	1.731 (1.17)	1.562 (1.04)	< 0.001

ART, antiretroviral therapy; SD, standard deviation; IQR, interquartile range.

Table 3 Description of different indicators of community viral load (CVL), in the period 2008–2014, overall and with breakdown by risk behaviour, gender, nationality and antiretroviral therapy (ART)

		Risk factor			Gender			Nationality			ART use		
	Whole sample	Heterosexual	MSM	*P*- value	Male	Female	*P*-value	Italian	Non-Italian	*P*-value	Yes	No	*P*-value
Number of samples	12 530	4601 (37%)	2821 (22.5%)		8819 (70%)	3711 (30%)		11 615 (93%)	915 (7%)		10 673 (85%)	1857 (15%)	
CVL indicator													
Mean copy-years	1 626 701	2 346 323	1 787 822	0.0078	1 366 123	2 243 589	0.13	1 499 478	3 285 263	0.22	484 900	8 340 765	< 0.001
Mean log copy-years	3.7	3.6	3.9	< 0.001	3.7	3.6	0.008	3.7	4	0.002	3.3	5.6	< 0.001
Geometric mean copy-years	4827	4099	7441	0.00037	4925	4253	0.13	4468	9500	0.009	2198	419 422	< 0.001
Proportion of samples with > 100 000 copies/mL (%)	3.8	3.7	5.1	0.00014	4.0	3.3	0.06	3.7	5.6	< 0.001	1.2	19.0	< 0.001
Proportion of samples with > 200 copies/mL (%)	22.0	17.7	27.1	< 0.001	23.0	20.0	< 0.001	21.0	35.0	< 0.001	10.0	91.0	< 0.001
Proportion of samples with < 25 copies/mL (%)	68.0	56.0	51.8	< 0.001	67.0	70.0	0.01	69.0	56.0	< 0.001	78.0	5.6	< 0.001

MSM, men who have sex with men.

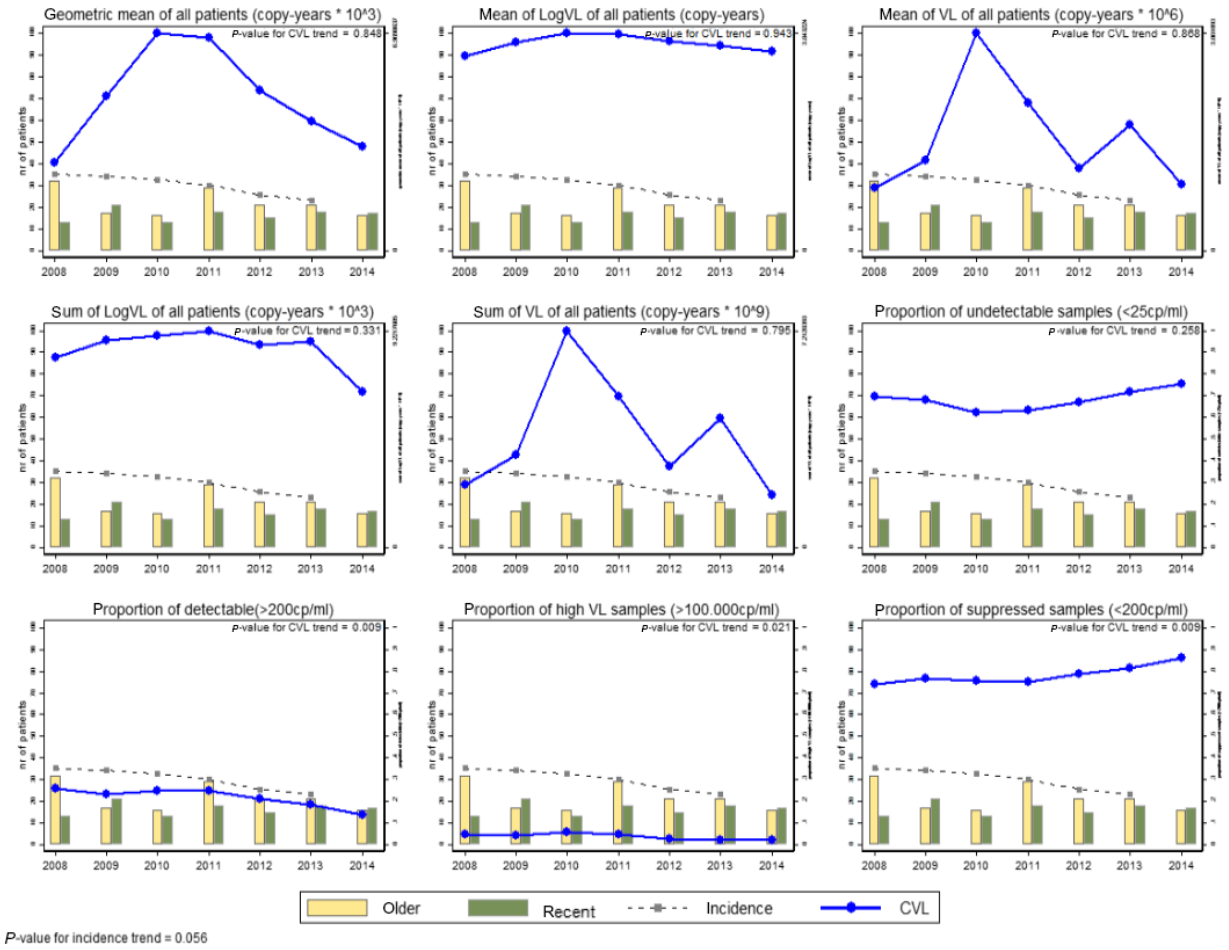

Figure 2 Trend over time (2008–2014) for different indicators of community viral load (CVL) plotted against the number of new HIV diagnoses (recent and older infections) and incidence of new diagnoses. VL, viral load.

concentration has been shown to be the main risk predictor for heterosexual HIV-1 transmission in serodiscordant couples [2,18]. Actually, secondary transmission was found to be rare or even absent for levels of HIV RNA < 1500 copies/mL and < 1100 copies/mL, respectively [2,18], whereas HIV transmission occurred for a plasma viral load > 35 000 copies/mL [2]. It is quite reasonable to assume that the beneficial effect of ART on HIV transmission is not restricted to the individual level, but can also influence the transmission of the infection at the community or population level [7,19].

As in other countries, in Italy after 2007 a number of changes were introduced into clinical practice and created the potential for ensuring viral suppression, even in patients with extensive drug resistance [20], thereby reducing HIV circulation. Moreover, the Italian government provides comprehensive nationwide, free-of-cost HIV treatment and care to all HIV-positive subjects. However, despite all this, the HIV epidemic in Italy has shown only limited signs of slowing down, at least in some communities [21,22].

Herein, we investigated whether CVL (as estimated from the MVL of HIV-positive residents referred to our institute) within our province did or did not vary between 2008 and 2014, and whether CVL was associated with the rate of new HIV diagnoses in our setting. A significant reduction ($P < 0.001$) of the mean HIV RNA (either absolute or after logarithm transformation) was found. In addition, a downward trend was always observed regardless of the CVL indicator considered. Of note, unlike other studies, we calculated CVL after transforming the individual measurements of plasma viral load into the VCY. For consecutive samples obtained from a single individual over two calendar years, we also distributed the VCY between the two periods based on the duration of time spent within each year. To our knowledge, for the

Table 4 (a) Association [incidence rate ratio (IRR)] between the number of new HIV diagnoses and individual community viral load (CVL) indicators, adjusting for year, overall and in specific subgroups; (b) association (IRR) between the number of new, recent only HIV diagnoses and CVL, adjusting for year

(a)

	Mean			Sum			Geometric mean		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	**p-value**
Entire population	0.91	0.795–1.036	0.15	0.96	0.91–1.017	0.176	0.97	0.881–1.065	0.506
Italian subset	0.88	0.804–0.966	0.007	0.95	0.915–0.984	0.005	0.93	0.886–0.985	0.012
Heterosexual subset	1.05	0.827–1.33	0.694	1.03	0.928–1.134	0.617	1.01	0.891–1.156	0.828
MSM subset	0.69	0.603–0.791	< 0.001	0.87	0.814–0.923	< 0.001	0.84	0.789–0.903	< 0.001

	Mean of log			Sum of log			Proportion with high VL (> 10^5 cp/mL)		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
Entire population	0.67	0.261–1.694	0.393	0.95	0.85–1.06	0.176	1.00	0.906–1.097	0.949
Italian subset	0.45	0.278–0.738	0.001	0.9	0.837–0.97	0.005	1.00	0.914–1.097	0.983
Heterosexual subset	1.04	0.25–4.33	0.957	1.14	0.975–1.324	0.617	1.11	0.954–1.287	0.18
MSM subset	0.17	0.086–0.326	< 0.001	0.87	0.723–1.038	< 0.001	0.95	0.807–1.131	0.595

	Proportion with undetectable VL (< 25 copies/mL)			Proportion with unsuppressed viraemia (> 200 copies/mL)			Proportion with suppressed viraemia (< 200 copies/mL)		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
Entire population	1.01	0.98–1.039	0.555	1.00	0.975–1.031	0.847	1.00	0.97–1.025	0.847
Italian subset	1.02	1.003–1.033	0.015	1.00	0.969–1.031	0.977	1.00	0.97–1.032	0.977
Heterosexual subset	0.99	0.951–1.025	0.504	1.04	1.006–1.073	0.02	0.96	0.932–0.994	0.02
MSM subset	1.04	1.004–1.075	0.027	1.00	0.948–1.047	0.878	1.00	0.955–1.055	0.878

(b)

	Mean			Sum			Geometric mean		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
Entire population	0.91	0.763–1.095	0.329	0.96	0.896–1.034	0.293	0.98	0.883–1.093	0.74
Italian subset	0.85	0.724–0.987	0.033	0.93	0.879–0.991	0.025	0.93	0.844–1.027	0.153
Heterosexual subset	1.29	0.871–1.904	0.206	1.11	0.937–1.306	0.232	1.24	1.013–1.506	0.036
MSM subset	0.74	0.621–0.872	< 0.001	0.89	0.825–0.951	0.001	0.85	0.779–0.92	< 0.001

	Mean of log			Sum of log			Proportion with high VL (> 10^5 copies/mL)		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
Entire population	0.91	0.274–3.018	0.876	0.93	0.821–1.043	0.293	0.93	0.857–1.016	0.111
Italian subset	0.54	0.168–1.758	0.308	0.9	0.806–1.009	0.025	0.92	0.836–1.002	0.055
Heterosexual subset	10.06	1.123–90.169	0.039	1.16	0.621–2.15	0.232	1.24	0.912–1.682	0.17
MSM subset	0.2	0.068–0.587	0.003	0.85	0.718–1.017	0.001	0.89	0.777–1.013	0.078

	Proportion with undetectable VL (< 25 copies/mL)			Proportion with unsuppressed viraemia (> 200 copies/mL)			Proportion with suppressed viraemia (< 200 copies/mL)		
CVL indicator	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**	**IRR**	**95% CI**	***P*-value**
Entire population	1.02	1.001–1.044	0.037	0.97	0.955–0.993	0.007	1.03	1.008–1.047	0.007
Italian subset	1.04	1.024–1.051	< 0.001	0.97	0.949–0.99	0.004	1.03	1.01–1.054	0.004
Heterosexual subset	0.95	0.864–1.048	0.313	1.03	0.915–1.164	0.604	0.97	0.859–1.093	0.604
MSM subset	1.06	1.023–1.092	0.001	0.97	0.928–1.005	0.089	1.03	0.995–1.077	0.089

CI, confidence interval; MSM, men who have sex with men; VL, viral load.
IRR is > 1 (< 1) if the risk of having more new HIV diagnoses is higher (lower) with higher values of the corresponding indicator of CVL in each year. The association with number of new diagnoses was tested by means of mixed Poisson models, with CVL measure and year as fixed effects, and year as a random effect.

calculation of CVL in particular communities, only one viral load measurement from each patient during a given calendar year has generally been used (usually either the most recent or the highest) [5,8], thereby not taking into account the possibility of fluctuating HIV RNA levels (such as those in patients partially adherent to therapy) or exaggerating a single short-lived increase of plasma viral load. Our choice of utilizing the VCY for the calculation of CVL indicators derived from the recognition that VCY actually reflects the individual's overall HIV burden [23]. Thus, VCY predicts important clinical outcomes, such as mortality, much better than plasma viral load and CD4 cell count [24]. Moreover, for the estimation of CVL, more than one categorical measure of viral load was also utilized, thereby obtaining additional information rather than just depending on means only [15]. In fact, the proportion of suppressed (< 200 copies/mL) or unsuppressed (> 200 copies/mL) patients better describes the clinical status of persons contributing to the community, and the proportions of individuals with high (> 10^5 copies/mL) and undetectable viral load better indicate the potential for ongoing HIV transmission [15].

The reduction of CVL over time was expected in view of the temporal increase in ART utilization and in the proportion of subjects with undetectable plasma viral load, and it describes an optimal landscape where successful linkage to and retention in care would predict a reduction of the number of patients with a new HIV diagnosis. However, this was not the case, and actually the total number of new diagnoses did not significantly change over time. As new HIV diagnoses do not necessarily coincide with new infections, we also confirmed nonsignificant variation over time in the number of individuals with recent HIV infection (< 1 year). This result is consistent with those of Krentz and Gill [25] but not with a previous observation of a clear cause–effect relationship between a reduced CVL and a decreased number of HIV infections [4]; however, in that study [4], it appears that new HIV diagnoses decreased starting from 2004, and that the decline of CVL actually started in 2006. Moreover, the authors did not distinguish between new/chronic and new/recent infections; therefore, patients diagnosed in 2006 might have become infected even earlier. According to previous observations [4,7], in our study the highest CVL values in 2010 would explain the greater number of HIV-positive individuals newly diagnosed in 2011; however, in reality, for the majority of individuals with a new HIV diagnosis in 2011, we estimated a duration of infection of > 1 year.

Depending on the numerical expression that we considered, there were some differences in CVL according to gender, risk factor for HIV acquisition, nationality and ART use of patients contributing to the totality of samples. Obviously, samples from persons on treatment had a significantly lower viraemia and therefore contributed only marginally to CVL. Although MSM contributed much less to CVL than heterosexuals when CVL was defined as a mean, their role appeared more prominent when the proportions of samples with unsuppressed/undetectable and high viral loads were considered. In addition, male patients appeared to make a greater contribution to CVL than female patients in all measurements, which inversely corresponds to an apparently greater contribution to CVL by non-Italians, who were mainly female. This might indicate that non-Italians have poorer access to care, and therefore higher overall exposure to HIV [26]

It is noteworthy that, in our study, the IRR of new HIV diagnoses (overall or recent infections only) decreased among Italians and MSM (MSM were almost entirely Italian) with increasing CVL, expressed as the sum and mean, and log or geometric mean. In contrast, the IRR of new diagnoses increased in the same population with an increasing proportion of subjects with an undetectable viral load. This result suggests that the control of viral replication following the introduction of ART encourages risky behaviours [27,28]. In fact, previous reports investigating the association between ART benefits and sexual risk behaviour in MSM provided evidence of a reduction in the number of individuals perceiving HIV to be a threat as a result of advances in HIV treatment [29]. This observation, in turn, may explain the continuous increase in HIV-1 incidence among MSM in countries such as Italy [21] where particular clusters of infection have been reported [16], thereby suggesting that MSM have not demonstrated a prevention benefit from widespread use of ART, and that ART cannot be considered as a substitute for other preventive measures for HIV transmission. If this is confirmed, the hypothesis that TasP can eliminate HIV transmission when used by individuals outside a stable relationship needs to be revised.

The strengths of our study include the extensive data collection spanning 6 calendar years, the availability of patient-level information which permitted the breakdown of measures of CVL into patient groups (e.g. risk behaviour groups), the distinction between recent and older HIV infections and the inclusion of all viral load measurements performed in the community.

We acknowledge that our study has certain limitations, other than those inherent in the methodology itself [15]. Firstly, the study had an ecological design, which cannot demonstrate (or exclude) a causal relationship between CVL and new HIV infections. This limitation is also evident when considering that the significance of the

associations varied greatly according to the CVL indicator used (for instance absolute copies/mL versus $\log_{10}$ copies/mL) and subgroup, for purely statistical (distributional) reasons. A more formal validation of specific CVL indicators merits a more dedicated research effort [25], to determine which is the most useful measure to depict the "true" HIV circulation within a community. Moreover, the use of new diagnoses as a proxy for new infections is arbitrary and misleading, in that infected individuals who do not seek testing are omitted [30], and newly diagnosed patients might have acquired HIV infection at some unknown earlier time. The bias caused by undiagnosed patients could not be corrected for; however, for almost all new diagnoses in our study, the approximate duration of HIV infection was calculated, thereby permitting a separate analysis of recent infections. Furthermore, the breakdown according to patient characteristics (either fixed, such as gender, or varying over time, such as ART) might also be misleading; as individuals might present more than one risk factor, subgroups are not closed populations, and some risk factors might change over time (e.g. sexual behaviour).

In conclusion, we agree that CVL provides a surrogate marker of access to care and treatment; however, we are reluctant to consider CVL effective for monitoring new diagnoses. In any case, we are convinced that more formal validation of specific indicators is necessary.

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