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Building up a collaborative network for the surveillance of HIV genetic diversity in Italy. A pilot study

Nunzia Sanarico, Stefania D'Amato, Orietta Picconi, Barbara Ensoli, Stefano Buttò and the Italian Network for HIV Characterization*

Centro Nazionale AIDS, Istituto Superiore di Sanità, Rome, Italy
**The members of the Italian Network for HIV Characterization are listed before the References*

Abstract

Introduction. Prevalence of infection with HIV-1 non-B subtypes in Italy has been reported to raise, due to increased migration flows and travels. HIV-1 variants show different biological and immunological properties that impact on disease progression rate, response to antiretroviral therapy (ART) and sensitivity of diagnostic tests with important implications for public health. Therefore, a constant surveillance of the dynamics of HIV variants in Italy should be a high public health priority. Organization of surveillance studies requires building up a platform constituted of a network of clinical centers, laboratories and institutional agencies, able to properly collect samples for the investigation of HIV subtypes heterogeneity and to provide a database with reliable demographic, clinical, immunological and virological data.

Aim. We here report our experience in building up such a platform, co-ordinated by the National AIDS Center of the Istituto Superiore di Sanità, taking advantage of a pilot study aimed at evaluating HIV subtypes diversity in populations of HIV-infected migrant people in Italy.

Materials and methods. Four hundred and thirty four HIV-infected migrants were enrolled in 9 Italian clinical centers located throughout the Italian territory. Standard Operating Procedures (SOPs) for sample collection were provided by the National AIDS Center to each clinical center. In addition, clinical centers were required to fill up a case report form (crf) for each patient, which included demographic, clinical, immunological and virological information.

Results. All centers properly collected and stored samples from each enrolled individual. Overall, the required information was correctly provided for more than 90% of the patients. However, some fields of the crf, particularly those including information on the last HIV-negative antibody test and presence of co-infections, were properly filled up in less than 80% of the enrolled migrants. Centers from Northern and Central Italy showed a better tendency to report correct information in the crf than centers from the South. These results provide evidence that procedures for establishing a platform for the surveillance of HIV subtype heterogeneity are affordable by all the components of the network and lay the ground for the organization of a broader HIV subtypes surveillance in Italy.

Key words
- HIV
- variability
- surveillance
- network

INTRODUCTION

The high HIV-1 genetic heterogeneity leads to the establishment of virus lineages that are increasing in number overtime. Of the two known types of HIV (HIV-1 and HIV-2), HIV-1 is responsible for the global pandemic and shows an extensive genetic diversity. HIV-1 quasispecies are organized into four groups, M (Main), O (Outlier), N (non-M/non-N) and P (pending the identification of further human cases) [1]. HIV-1 group M is further diversified into genetic subtypes (named A to D, F to H, J and K) and six sub-subtypes (A1-A4, F1, F2) (Los Alamos Sequence Database, http://hiv-web.lanl.gov/). HIV strains from different subtypes can also recombine giving origin to Circulating Recombinant Forms (CRFs). Currently, 70 CRFs are known (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html [last accessed June 5, 2015]). HIV group M strains are responsible for the global

Address for correspondence: Stefano Buttò, Centro Nazionale AIDS, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: stefano.butto@iss.it.

AIDS pandemic, with more than 90% of the total infections occurring in Africa. Among this group, HIV-1 subtype C is the most prevalent clade, globally, and is mainly found in the Southern African and Indian regions [2]. HIV-1 subtype B is most prevalent in North and South America, Western Europe (including Italy), Australia and Japan; subtypes A and D predominate in East Africa, with subtype A also being possibly responsible for much of the Russian epidemic [3, 4]; subtype G is prevalent in West and Central Africa [3]. Notably, prevalence of recombinant forms is increasing overtime, worldwide; in particular CRF02_AG is now the most represented genotype in most of the West and Central African countries [1, 3]. In Southeast Asia the most represented genotypes are CRF01-AE, subtypes A and subtype E, while in China, the recombinant forms CRF_07 BC and CRF 08_BC are prevalent together with subtype B [1].

The geographic distribution of HIV-1 lineages is a dynamic process that is continuously evolving overtime, due to increased travelling and globalization. To this regard, data from the Centro Operativo AIDS (COA) of the Istituto Superiore di Sanità indicate that the proportion of foreign people with HIV infection in Italy has been increasing from 11% of total infected individuals in 1992 to 24.0% in 2013 [5]. Most migrants are native of geographic regions where many subtypes and CRFs are present. These HIV forms can be introduced into the Italian native population, where subtype B strains are largely predominant. Indeed, frequency of infections by HIV-1 non-B subtypes in the general population of HIV-infected individuals in Italy has been increasing, from 2.6% in 1985-1992 to 18.9% in 1993-2008 [6].

The broad genetic diversity of HIV can have important implications for public health since subtypes and CRFs can show different properties that can affect their fitness, transmissibility, and response to therapy [7, 8]. Furthermore, even if antiretroviral therapy has been shown to be effective, although with different potency, against all subtypes/CRFs, there is growing evidence that different subtypes and CRFs can carry clade-specific mutations that can confer resistance to ART, thus accelerating the emergence and the spreading of drug-resistant variants in the populations [7]. In addition, HIV diversity can impact on sensitivity and specificity of diagnostic laboratory assays [9]. Therefore, a continuous surveillance of the dynamics of subtypes and CRFs in both general population and selected groups of HIV-infected individuals in Italy is strongly needed in order to effectively control HIV infection spreading and, in particular, to evaluate the epidemiological trends resulting from social changes and increased migration in Italy.

To this regard, the World Health Organization (WHO) has created a database to monitor the dynamics of HIV variants circulating worldwide and has recommended to create local networks with the aim to closely monitor HIV variants dynamics in each distinct country [10]. In agreement with these suggestions, many countries in Europe have established surveillance of the circulating HIV subtypes and CRFs in order to monitor spreading of new variants and the efficacy of antiretroviral therapy [11-14] (http://dare.uva.nl/document/59097).

Surveillance of HIV diversity requires building up a collaborating platform constituted of a network that should comprehend clinical centers for collection of samples and clinical data from individuals of selected populations, and laboratories able to process the samples and to provide sequences of the isolated HIV variants for HIV subtyping and for identification of mutations conferring resistance to ART.

We here describe our experience, including limits, pitfalls and obstacles in building up such a network in Italy, co-ordinated by the National AIDS Center of the Istituto Superiore di Sanità, taking advantage of an already in place study aimed at investigating HIV subtypes diversity in populations of migrants resident in the Italian territory.

METHODS

The platform was composed of 9 clinical centers in Italy located in the cities of (from North to South) Brescia, Prato, Florence, Latina, Naples, Bari, Cosenza, Catanzaro and Lamezia Terme and was coordinated by the National AIDS Center of the Istituto Superiore di Sanità. Such a platform was constituted to allow studies aimed at monitoring the distribution of HIV subtypes and presence of variants with ART resistance mutations in populations of migrants on the Italian territory. These studies were approved by local ethical committees of each center. Figure 1 reports the location of the involved centers in the country.

The National AIDS Center provided each clinical center with Standard Operating Procedures (SOPs) for collection and storage of plasma samples from HIV-infected migrant individuals in full anonymity, until their shipping to the National AIDS Center for HIV subtyping and detection of ART-resistance mutations. Plasma samples of 434 HIV-infected migrant individuals, referring to the clinical centers during the years 2009-2013, were obtained from whole blood collected in EDTA or Na-Citrate. Plasma was aliquoted and stored preferentially at -80 °C until shipping in dry ice, however, storing at -20 °C was permitted if -80 °C freezers were not available at the center. Inclusion of already stored plasma samples was allowed if samples were previously collected and properly stored, according to the SOPs.

Distribution of participant migrants varied according to the geographical areas. Namely, 263 participants were from the North, 125 from the Centre and 46 from the South of Italy.

The number of the enrolled migrants was in agreement with the distribution of migrant people in Italy, according to national statistics, with 86.5% of foreigners living in the North and Centre of the country and just 13.5% in the South in 2011 (data from the Italian National Institute of Statistics at http://www.istat.it/it/archivio/39726).

Clinical centers were required to fill up a case report form (crf) for each individual, provided by the National AIDS Center that included: demographic data (age, gender, date of birth, country of origin), virological information (last negative and first positive HIV antibody

Figure 1
Cities where participating clinical centers were located.

tests, plasma HIV viral load), clinical parameters (AIDS clinical stage according to WHO criteria, current and previous antiretroviral therapy, opportunistic infections), immunological data (lymphocytes counts [absolute and percent], CD4+ T cell counts [absolute and percent], CD8+ T cell counts [absolute and percent], CD4/CD8 ratio) and information on co-infections (hepatitis B and C, tuberculosis). Information reported in the crf was required to be referred to the draw blood date of the shipped sample. The crf was provided to the centers as an Excell file and was structured in a way to limit the need to write long sentences by the operators at the centers. In most of the variables, the option "Not Determined" or "Data not known" was available in order to avoid unclear answers.

RESULTS

Each participating center properly collected and stored samples, following the provided SOPs. All samples arrived at the National AIDS Center properly frozen in dry ice. HIV RNA from samples with a detectable viral load was amplified and sequenced in order to subtype the infecting HIV virus, with a sensitivity similar to what reported in the literature (data not shown). Results of HIV subtyping are under evaluation and beyond the scope of the work here presented.

Regarding the information required in the crf, demographic information (*i.e.* age, country of origin and gender), draw blood date and viral load values, were always provided by all centers (100% of samples had this information), however, other information was not or properly given, for all the patients *(Table 1)*. Particularly, information regarding the last HIV-negative test and co-infections was given with a frequency < 80%, when considering all centers *(Table 1 and Figure 2, panel A)*. Sometimes, some fields in the crf, although filled in, reported a wrong or not clear information. In particular, while those regarding the demographic and immunological data reported a correct information for more than 80% of the patients, others were filled in with less accuracy. For example, the information regarding the date of the "First positive HIV test" (meaning the date

Table 1
Percent of reported information in the case report form

	Field	All centers (434 pts)	North (263 pts)	Centre (125 pts)	South (46 pts)
General/demographic	Age	100	100	100	100
	Geographical origin	100	100	100	100
	Gender	100	100	100	100
	Draw blood date	100	100	100	100
	Date of last HIV-negative test	74.2	97.7	34.4	47.8
	Date of first HIV-positive test	92.6	100	74.4	100
Clinical	Clinical stage	93.3	100	85.6	76.1
	Previous therapy	88.7	100	70.4	73.9
	Current therapy	95.8	99.2	96.0	76.1
	Co-infections	79.3	100	39.2	44.0
Immunological	Lymphocyte count (absolute n)	93.8	99.2	96.8	54.3
	Lymhocytes (%)	93.3	100	93.6	54.3
	CD4+ T-cell counts (absolute n)	96.5	100	96.8	76.1
	CD4+ T-cells (%)	94.2	100	96.8	54.3
	CD8+ T-cell counts (absolute n)	95.8	99.2	96.7	71.7
	CD8+ T-cells (%)	95.8	99.6	96.6	71.7
Virological	Viral load	100	100	100	100

Percent of reported information for each field is indicated. Variables filled in with a frequency < 80% are underlined.

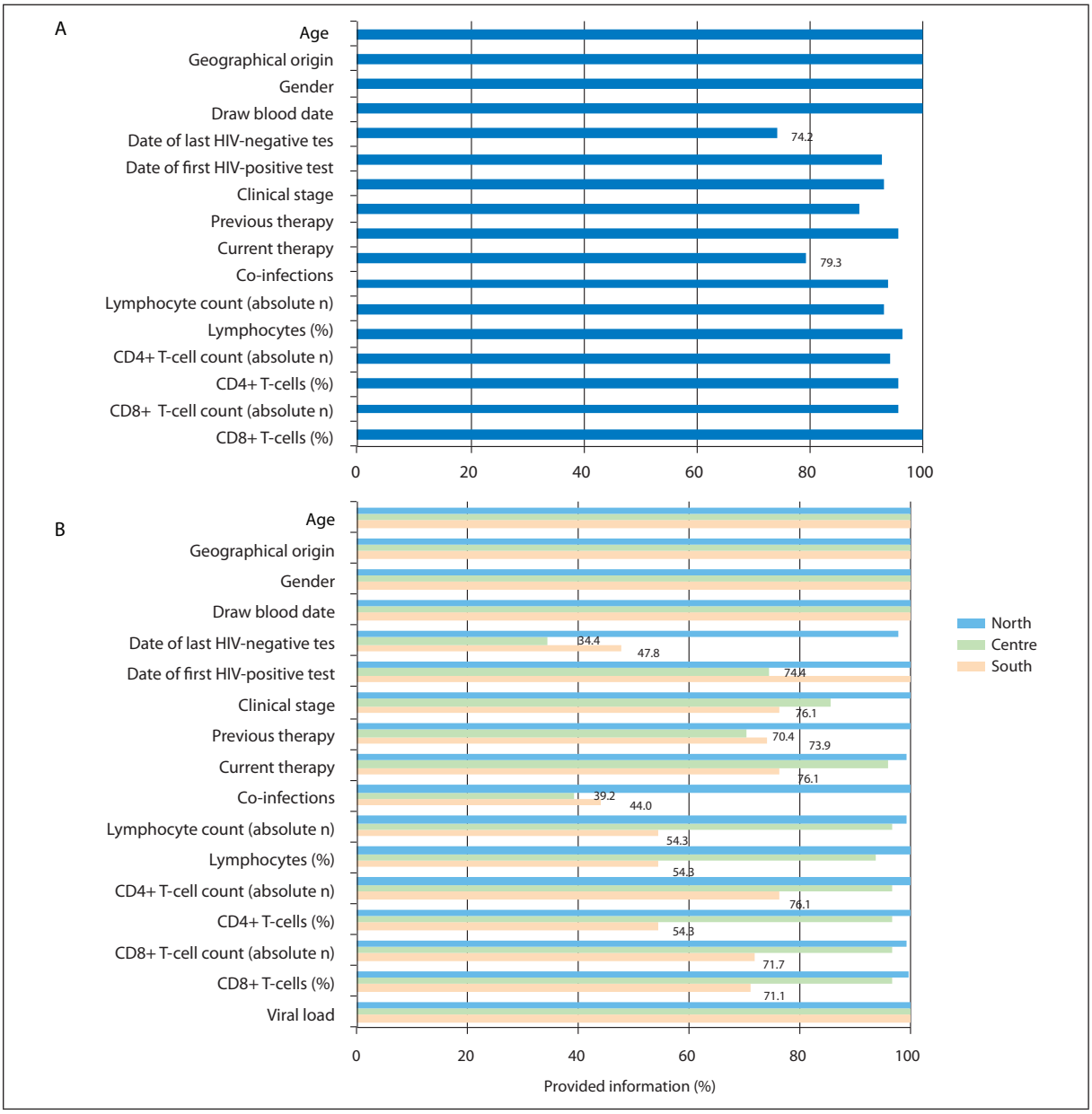

Figure2
Information on HIV+ migrants provided by clinical centers.
Panel A: percent of patients with the required information reported by all clinical centers (434 pts); Panel B: percent of patients with the required information reported by clinical centers from North (261 pts) (blue bar), Centre (125 pts) (light green bar) and South (46 pts) (orange bar) of the Country.
Percent values < 80% are indicated.

of first HIV-positive antibody test) was sometimes confused with the date of the first positive HIV viral load test that was carried out in that clinical center. Finally, the information on co-infections and therapy was sometimes incorrectly reported, since several centers just indicated the answer "yes", instead of reporting either the infecting agent, or the therapy regimen.

Looking at the contribution of centers according to their geographical distribution, a gradient North-to-South about the tendency to correctly fill in the crf was evident *(Table 1 and Figure 2, panel B)*. Overall, the center in the North provided a correct information with a frequency always greater than 97%. Clinical centers from Central and Southern Italy completely provided information concerning demographic data, co-infections and viral load, however, some information, such as the date of last HIV-negative test and the presence/absence of co-infections was present in less than 60% of the patients.

DISCUSSION AND CONCLUSIONS

As frequency and ease of travelling increases worldwide, transmission of infectious diseases among populations is becoming easier and more frequent. Regard-

ing HIV/AIDS, an increasing number overtime of subtypes and recombinant forms has been observed in most geographical areas, including Italy [9]. These variants can show different pathogenicity, diverse sensitivity to diagnostic tests and to antiretroviral drugs and carry mutations that confer resistance to ART that can be transmitted, thus posing growing problems for public health [10-12].

To closely monitor HIV variants dynamics it is therefore mandatory to establish surveillance strategies. In an effort to lay the groundwork for a future surveillance strategy of HIV subtypes and recombinant forms dynamics in Italy, we took advantage of a pilot study, aimed at investigating HIV genotypes distribution in communities of migrants in Italy, to create a network of clinical centers in the Italian territory for surveillance of HIV subtypes dynamics in Italy. The paper describes our experience in creating and managing such a network, in order to assess possible problems and impediments to establish an efficacious HIV subtypes surveillance strategy in Italy.

Despite centers involved in the project were mainly from the South, patients were enrolled in greater numbers in the North (60.6% of all patients), followed by the Centre (28.8%) and the South (10.6%). This could be explained partly by the higher density of foreign individuals living in the North and partly by a different commitment of each center to the project ending up to different results in terms of accuracy and quality of the information.

Clinical centers were instructed to prepare plasma samples obtained from whole blood, according to SOPs provided by the National AIDS Center. No problems for preparing, handling, storing and shipping of samples were observed in any center, so that it was possible to correctly amplify HIV RNA and determine the infecting HIV subtype from these samples. This confirms that the required commitment to the centers did not hamper the normal work routine at each clinical center.

Centers were also required to provide demographic, clinical, virological and immunological information through a pre-prepared crf provided by the National AIDS Center. Although the crf was prepared in a way that reporting information did not require a time-wasting procedure, and the included options of choice were thought to avoid misinterpretations, some fields were not correctly filled in with the same high frequency as others. The accuracy to report the information showed a gradient North-to-South with the center from the North reporting a more accurate information. However, key information for patient care, like the one regarding immunological (T-CD4 and T-CD8 counts) and virological (HIV viral load) parameters was correctly provided by all centers. It is possible that some requested information that was provided with a lower frequency was considered by some centers not important for the purposes of the study. This poorer accuracy highlights the importance for the organising Center to provide a crf reporting questions that can be easily comprehended by all collaborating clinical centers, in order to establish a coherent data base. To this regard, more efforts should be made in order to let the collaborating centers understand the need of providing all the requested information, also providing them a crf in a file format that does not allow to save and send a document with missing information.

In summary, although the study was conducted as a small pilot, a relevant number of centers (9) participated to the study providing, overall, a large number of samples that were correctly collected and that, in general, reported correct demographic, immunological, virological and clinical data. This adherence to the project indicates that a sentinel, homogeneous and coordinated surveillance of HIV subtypes dynamics is strongly felt as important and should be considered as a high priority intervention for public health. These activities should be coordinated by a dedicated Center that is responsible for rigorous registering, managing and archiving of the data into a database and for storing samples into a proper bio banking. In addition, this reference Center should be responsible of organizing, normalizing and standardizing clinical samples collection and handling. Finally, the coordinating Center should be able to collect, from collaborating clinical centers, demographic, clinical, immunological and virological information that can be useful for the analysis of the dynamics of HIV genotypes and of variants carrying mutations that confer resistance to ART.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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**Contributing members of the Italian Network for HIV Characterization*: I. El Hamad and F. Zoboli (Division of Infectious Diseases, Spedali Civili General Hospital, Brescia, Italy); Azzurri A (Misericordia e Dolce Hospital, Prato); Marino N (Santa Maria Annunziata Hospital, Azienda Sanitaria di Firenze, Florence); Di Zeo P, Rovetto C, Salvi E (National AIDS Center, Istituto Superiore di Sanità, Rome); Lichtner M, (University Sapienza Polo Pontino Roma, Latina); Mazzella A, Romano N (ASL Napoli 1 Centro, Naples); Brindicci G (University of Bari, Bari); Cesario F, Luciani F (Annunziata Hospital, Cosenza); Surace L (Centro di Medicina del Viaggiatore e delle Migrazioni, ASP di Catanzaro Presidio Ospedaliero di Lamezia Terme, Lamezia Terme); Caroleo B (Unità Operativa di Malattie Cardiovascolari Geriatriche, Azienda Ospedaliera Mater Domini, Catanzaro); Berardelli G (Giovanni Paolo II Hospital, Lamezia Terme).

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