Epidemiology of Transmitted Drug Resistance in Chronically HIV-Infected Patients in Germany: The RESINA Study 2001–2009

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Key Words

Primary drug resistance • Highly active antiretroviral therapy • Resistance prevalence

Abstract

Objectives: Transmitted HIV drug resistance may impair treatment efficacy of combination antiretroviral therapy (ART). This study describes the epidemiology of transmitted resistance in chronically infected patients. **Methods:** In a prospective multicenter trial in Nordrhein-Westfalen, Germany, transmitted drug resistance was determined by genotypic resistance testing in patients on initiation of first-line ART. **Results:** From 2001 to 2009, 2,078 patients were enrolled in the study. 79.9% were male, 81.2% were Caucasians, and a homosexual transmission mode was found in 51.3%. Of these patients, 41.5% were at the stage of AIDS, median CD4 cell count was 230/ μ l, and median viral load was 64.466 copies/ml. Transmitted drug resistance mutations were seen in 9.2% (95% CI, 7.9–10.4). Resistance in the nucleoside reverse transcriptase inhibitor class was found in 5.8% (4.8–6.8), in the nonnucleoside reverse transcriptase inhibitor class in 2.8% (2.1–3.6), and in the protease inhibitor class in 2.7% (2.0–3.4). After a continuous increase to a level above 10% in the years 2006 and 2007,

a decline of drug resistance prevalence followed in 2008 and 2009. **Conclusions:** Transmitted HIV drug resistance was found in around 10% of chronically infected patients in Germany who started their ART. We showed a moderate decline of the prevalence of mutant virus strains in recent years. Further surveillance of this phenomenon is mandatory.

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Introduction

After more than a decade of widespread application of resistance testing in guiding highly active antiretroviral therapy (HAART), it has been shown that this technique is very likely to reduce mortality in HIV-infected patients [1]. Resistance-associated mutations may not only be selected by nonsuppressive antiretroviral therapy (ART), but can be transmitted at primary infection. Drug resistance in these patients, though never exposed to antiretroviral drugs, is defined as primary or transmitted resistance.

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tance. This phenomenon has been addressed in a number of studies in recent years [2–11]. Different analyses showed reduced efficacy of ART in cases with primary drug resistance in bulk sequencing or as a minority strain [12–18]. Epidemiology of transmitted resistance varies according to the investigated population, the applied methods, and the geographic region.

In order to determine the role of the epidemiology of primary drug resistance and its implications in the efficacy of first-line HAART, the RESINA cohort has been carried out continuously since 2001 as a prospective multicenter study in Germany [19, 20]. Moreover, clinical and resistance data were used to develop the bioinformatics tool geno2pheno for the prediction of drug susceptibility in ART [21]. The aim of the present work is to give an update on the epidemiology of transmitted drug resistance in north-west Germany.

Methods

The RESINA Study is an ongoing prospective multicenter investigation carried out in Nordrhein-Westfalen, the largest federal state of Germany. A major goal of the project is to generate continuous surveillance of transmitted HIV drug resistance in chronically infected patients before administration of first-line HAART. Thirty-six out of 42 initially contacted centers providing specialized care for HIV-positive patients contributed to the study (see Appendix).

Inclusion criteria were documented HIV infection, eligibility for ART, and agreement of the patient and the treating physician on the start of HAART. Exclusion criteria were prior exposure to antiretroviral drugs and unwillingness to participate. Enrolled subjects gave written informed consent. All relevant institutional review boards issued positive approval.

Genotypic drug resistance testing, alignment and interpretation using geno2pheno were performed as previously described [19]. All physicians had resistance testing results available for the decision on therapy combinations. For determination of the prevalence of resistance, the latest guidelines on classification of significant mutations were used to avoid misinterpretation caused by naturally occurring polymorphisms [22].

The statistical analysis was performed with SPSS, release 18.0. Univariate comparisons were carried out using Wilcoxon rank sum test or Fisher's exact test where appropriate; p values <0.05 were considered significant. No adjustment for multiple testing was applied.

Results

Between January 2001 and December 2009, 2,078 individuals were enrolled in the RESINA Study. Of the initially screened population, 150 patients did not enter the

Fig. 1. Time trend of resistance prevalence. The median and 95% CI are demonstrated; the numbers of patients at the different years are shown in the bottom line.

database because of withdrawal of consent, a history of ART before enrollment, or unsuccessful resistance testing. Patients' characteristics are listed in table 1. Participants were predominantly middle-aged male Germans of Caucasian ethnicity. HIV diagnosis had been made recently, and the patients were moderately progressed in the course of disease with a relevant proportion of AIDS cases. The most important transmission mode was men who have sex with men (MSM); subtype B was the most prevalent HIV-1 subtype.

Primary drug resistance was detected in 9.2% (95% CI, 7.9–10.4) of the population, as shown in table 2. The majority of mutations were found in the substance class of nucleoside reverse transcriptase inhibitors (NRTI), prevalence of resistance in the nonnucleoside reverse transcriptase inhibitors (NNRTI) class was lower, and lowest for the protease inhibitors (PI) (table 2). The time trend of resistance epidemiology is visualized in figure 1. After an increase of prevalence until 2007, a decline of prevalence followed in the last 2 years. To determine patient groups with an increased presence of primary resistance, several subgroups were compared according to mutational status. The following groups significantly more often turned out to present with transmitted resistance: males, Caucasians, MSM transmission mode, and patients harboring HIV subtype B (table 3). All other parameters were not found to have a significantly different distribution between the subgroups.

Table 1. RESINA Study: patient characteristics

	Number
Complete population	2,078 (100)
Sex	
Male	1,661 (79.9)
Female	417 (20.1)
Age ^a , years	38.0 (18–80)
National origin	
Germany	1,501 (72.2)
Turkey	44 (2.1)
Thailand	41 (2.0)
Cameroon	40 (1.9)
Kenya	39 (1.9)
Other	379 (18.3)
Missing data	34 (1.6)
Ethnic origin	
Caucasian	1,688 (81.2)
African	268 (12.9)
Asian	67 (3.2)
Other	33 (1.6)
Missing data	22 (1.1)
Duration of ART since HIV diagnosis ^a months	1 (0–268)
HIV transmission route	
MSM	1,067 (51.3)
Heterosexual	379 (18.2)
Endemic region	282 (13.6)
Intravenous drug abuse	153 (7.4)
Bisexual	20 (1.0)
Other	13 (0.6)
Unknown/missing data	164 (7.9)
CDC stage of disease	
A	332 (41.5)
B	204 (25.5)
C	264 (33.0)
Missing data	1,278
CD4 cell count/ μ l ^a	230 (1–1,362)
Viral load ^a , copies/ml	64,466 (48–10,030,000)
HIV-1 group M subtype	
B	1,489 (71.7)
A1	141 (6.8)
CRF 02_AG	133 (6.4)
A (01_AE)	89 (4.3)
C	71 (3.4)
G	42 (2.0)
D	37 (1.8)
Other	74 (3.5)
Missing data	2 (0.1)

Unless otherwise indicated values in parentheses represent percentages.

^a Values represent median with the range in parentheses.

Table 2. Prevalence of resistance mutations in participants of the RESINA Study

	n (%)	95% CI
Altogether	191 (9.2)	7.9–10.4
NRTI-associated mutations		
Total	121 (5.8)	4.8–6.8
Revertant variants	84 (4.0)	3.2–4.9
NNRTI-associated mutations	59 (2.8)	2.1–3.6
PI-associated mutations	57 (2.7)	2.0–3.4
Mutations in ≥ 2 substance classes	40 (1.9)	1.3–2.5

Table 3. Risk factors for primary drug resistance

Parameter	Strata values according to primary resistance	p value
Sex, %		
Male	10.1	0.003
Female	5.5	
Median age, years		
Resistance detected	39	0.36
No resistance	38	
Ethnic origin, %		
Caucasian	10.1	0.002
Others	5.1	
Median duration of HIV infection, months		
Resistance detected	1	0.44
No resistance	1	
CDC stage, %		
AIDS	8.0	0.46
Others	9.6	
Median CD4 cell count, / μ l		
Resistance detected	232	0.65
No resistance	230	
Median viral load, copies/ml		
Resistance detected	93,668	0.06
No resistance	61,918	
HIV subtype, %		
B	10.9	<0.001
Others	4.8	
Transmission route, %		
MSM	11.0	0.005
Others	7.3	

Percentages indicate prevalence of resistance.

Discussion

In this large prospective study we sought to monitor transmitted drug resistance in HIV-infected cases. Due to the collaboration of the majority of HIV treatment centers of the largest federal state of Germany, the patient sample provided by the RESINA Study might be regarded as representative for the HIV-infected population in the geographic region in middle Europe. Participants were predominantly middle-aged male German Caucasians. Almost 20% of the cases had other ethnicities, and, corresponding to this, almost 30% of patients showed an HIV-1 subtype other than B. HIV infection was diagnosed shortly before inclusion in the majority of individuals, but the stage of disease showed moderate progression, as determined by the proportion of AIDS cases and mean CD4 cell count as well as viral load. In concordance with inclusion criteria, the study presents data of chronically infected patients. The most important transmission mode was MSM.

Primary drug resistance was detected in 9.2% individuals (95% CI, 7.9–10.4). This number corresponds with a number of other surveys in Europe [3, 5, 6, 9, 11, 12, 23], but several studies from Europe [4, 8, 24] and Northern America [7, 10, 25] report different frequencies. The majority of mutations were found in the substance class of NRTI. A substantial part of these cases showed virus strains with mutation at position 215 of the reverse transcriptase. These mutations indicate prior exposure to NRTI treatment and may lead to impaired efficacy of combination ART [17]. Prevalence of resistance in the NNRTI class was lower, and lowest for protease inhibitors, as demonstrated in table 2. These results are consistent with the discussed studies from Europe. After a continuous increase of prevalence in the first years of the last decade, a plateau at a level well above 10% followed in the years 2006 and 2007. In the last 2 years of the survey, a consistent decline of prevalence followed. Thus, the prevalence of transmitted drug resistance seems to stabilize or even decrease. This was also found in other countries [3, 4, 8, 11, 26]. The most likely explanation for this trend is that the overall efficacy of HAART promotes reduced prevalence of resistance in treated populations and thus also leads to a reduced risk of transmission of mutant virus [26–29].

In contrast to the majority of other studies, we enrolled patients at the time when ART was initiated. This means that almost the whole study population had carried the HIV infection for a number of years, as therapy is normally initiated at a moderately progressed stage of

disease before the development of clinical symptoms of AIDS. As outlined by the baseline characteristics, this was the case in the presented data pool. From the epidemiologist's perspective, this may underestimate the true prevalence of transmitted resistance, as mutations may disappear over time without treatment [30]. However, a number of trials showed that transmitted resistance may remain at a stable level for years in affected persons [15, 31, 32]. This is due to the fact that primary infection is caused by a single mutated strain, when wild-type virus is not recruitable from a latent reservoir – as in patients with a large variety of HIV quasiespecies. Therefore, the rate of resistance is expected not to be much different in seroconverters and chronically infected patients, as demonstrated by comparison of the RESINA data with a large German study on acutely infected patients, predominantly in Berlin [3]. The reason for choosing patients with established infection in the investigation presented was to provide evidence for physicians in the clinical situation when information on resistance is most wanted: the initiation of treatment. Thus, our results present valuable tools for effective first-line HAART.

To characterize individuals with increased prevalence of primary resistance, additional analyses were applied according to mutational status. The following subgroups turned out to present transmitted resistance significantly more often: males, Caucasians, MSM transmission mode, and patients harboring HIV subtype B, which is the largest group of the cohort. This finding is in line with some other data sets [4, 5, 26] and represents the widespread application of nonsuppressive treatment regimens in early years of ART in Europe. During this time, access to ART was limited in developing countries. Accordingly, patient groups with lower prevalence of transmitted resistance in our study were non-Caucasians with other transmission modes than MSM, harboring HIV subtypes other than B: patients from high-prevalence countries with a heterosexual mode of HIV transmission. Therefore, a rise in prevalence of primary resistance should be expected in countries with increasing distribution of antiretroviral medication without availability of drug resistance testing. In conclusion, resistance testing in populations with increased risk of drug resistance is of special importance.

The following limitations have to be taken into account: the definition of resistance [22] was applied in a retrospective manner (publ. in 2009, fitted to a data set starting in 2001) in order to provide results that are comparable with other investigations in terms of the definition of transmitted resistance. Moreover, enroll-

ment of patients was carried out in clinical routine in a variety of centers offering specialized care for HIV-infected patients: general practitioners, hospital-based units and university clinics worked together in this trial. Thus, selection bias may have influenced the results. Finally, the decision of treatment initiation was left to the local physician. Therefore, patient characteristics may differ according to preferences of patient management in the various centers. However, national guidelines in Germany have presumably been well adopted by the study centers having substantial expertise in the treatment of HIV infection. Generally, limitations of this study are considered as small due to the large sample size, the constant cooperation of the centers over the years and the near-complete covering of a defined geographic area of Germany. Thus, results are representative for the population in question and valid in the context of the study aims.

In summary, transmitted HIV drug resistance may be found in around 10% of chronically infected patients in the RESINA cohort. In this large prospective study, we showed a moderate decline of prevalence of mutant virus strains in recent years. Risk factors for the presence of resistance were male gender, Caucasian ethnicity, MSM transmission mode, and HIV-1 subtype B. Further surveillance of primary resistance is mandatory.

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Appendix

Cooperating Centers

Peter Arbter, Krefeld; Robert Baumann, Neuss; Ingulf Becker-Boost, Duisburg; Akos-Sigmund Bihari, Wilfried Stücker, Cologne; Stefan Esser, Essen; Beate Gantke, Düsseldorf; Horst Carls, Frank Huber, Düsseldorf; Stefan Christensen, Münster; Gerd Fätkenheuer, Cologne; Rüdiger Gippert, Peter Hartmann, Hildegard Quaing, Münster; Ingo Greiffendorf, Krefeld; Ute Grüneberg, Münster; Dieter Häussinger, Stefan Reuter, Düsseldorf; Petra Hegener, Stefan Mauss, Günther Schmutz, Düsseldorf; Martin Hower, Dortmund; Konrad Isernhagen, Nazifa Qurishi, Katja Römer, Cologne; Petra Juretzko, Jürgen Stechel, Cologne; Heribert Knechten, Aachen; Wolfgang Köthemann, Anton Neuwirth, Cologne; Friedhelm Kwirant, Duisburg; Sabine Mauruschat, Wuppertal; Vladimir Miasnikov, Düsseldorf; Antonius Mutz, Osnabrück; Mark Oette, Cologne; Michael Paffenholz, Cologne; Daniela Petry, Anette Strehlow, Düsseldorf; Michael Radecki, Cologne; Martin Reith, Düsseldorf; Jürgen Rockstroh, Bonn; Erhardt Schäfer, Bielefeld; Stefan Scholten, Cologne; Theo Scholten, Hagen; Stefan Schoelzel, Troisdorf; Sarah Schons, Düsseldorf; Albert Theisen, Werner Wiesel, Esther Voigt, and Michael Wichmann, Cologne.

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