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**TITLE: Number, type and cost of microbiological tests during HIV Pre-Exposure Prophylaxis:
the experience of a French hospital**

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Running title: Microbiological tests during PrEP

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ABSTRACT

BACKGROUND: microbiological tests are required for individuals on HIV Pre-Exposure Prophylaxis (PrEP), but their real-life numbers, types and cost are poorly described.

METHODS: number, type, and results of microbiological tests performed in a Besançon Hospital-associated laboratory, France, from 2016 to 2019, in the setting of PrEP consultations were retrospectively collected. Costs were estimated by the current reimbursement rate set by the French national protection system.

RESULTS: 756 consultations for PrEP initiation or follow-up of 135 persons were performed over 4 years. Among 3,434 tests performed in the institution-associated laboratory, 1,083 and 2,351 were virological and bacteriological tests, respectively. Serology was predominant in virology (98% of virological tests), with HIV, HCV, and HBV screening as the 3 more frequent assays, whereas molecular biology was predominant in bacteriology (63.1% of bacteriological tests) with *N. gonorrhoeae* and *C. trachomatis* screening as leader assays. Agar-based culture accounted for 1% of bacterial tests. The global cost of microbiological tests was 45,983.20 euros, corresponding to a mean cost of 60.80 euros per consultation. Virological and bacteriological tests accounted for 37.7% and 62.3% of this budget, respectively. No seroconversion was observed for HIV or HCV. *N. gonorrhoeae* and *C. trachomatis* were detected at least once in 39.3% and 22.4% of individuals, respectively, with 15% of symptomatic episodes in both cases. Active syphilis infection was detected in 15.4% of individuals.

CONCLUSIONS: since numerous microbiological tests are required during PrEP, the availability of specific technical platforms should not be neglected by centers wishing to set up PrEP consultations.

KEYWORDS: PrEP; HIV; Microbiological assays, sexually transmitted diseases

INTRODUCTION

Pre-Exposure Prophylaxis (PrEP) has emerged as a highly effective strategy for preventing new HIV infections [1,2]. PrEP programs are nationally implemented and reimbursed by national health services in several Western European countries such as the Netherlands, Germany, Belgium, and France [3]. Cost-effectiveness of these programs has been demonstrated by studies in the US [4] and in Europe [5–9].

Beside regular HIV testing by serology, several microbiological assays are needed for individuals on PrEP, notably designed to screen sexually transmitted diseases (STD) or to check the immune protection against vaccine-preventable infections [10]. Some of these tests require highly specialized technical platforms and some results have to be obtained rapidly (e.g., molecular HIV testing in case of symptoms compatible with a HIV primary infection). The laboratory equipment required to perform microbiological tests for PrEP users and the cost of these tests have been poorly described.

This study sought to characterize the number, the type, the cost, and the positivity rate of microbiological assays performed for individuals on PrEP in our institution.

MATERIALS AND METHODS

Study population

Adults (≥ 18 years) who consulted in the Besançon Teaching Hospital (France), from March 2016 to December 2019, for PrEP initiation and follow-up were eligible. Analysis was

restricted to individuals for whom the microbiological assays were performed in the hospital-associated laboratory (Departments of Virology, Bacteriology and Parasitology-Myology). All consultations were performed by the same physician from 2016 to 2019. A second physician participated in the PrEP consultations in 2019. All assays were performed at the request of the physicians, in accordance with French guidelines [10], patients' symptoms and infectious disease exposure.

Clinical and biological data

Personal information including age, gender, and date of PrEP-associated consultations during the study period, were retrospectively obtained by interrogating the clinical database NADIS (ABL, Metz, France). Numbers and results of microbiological assays performed for each individual were retrospectively collected from the laboratory information management system (LABOserveur, INLOG, Limonest, France), including assays performed in the lab and outsourced assays (HAV genotyping, *Mycoplasma genitalium* molecular detection).

Type of assays

Virological assays

Serological assays for HIV and HBV (HBsAg, HBcAb, HBsAb), HCV, HAV (IgM, IgG), HTLV and EBV (VCA IgM, VCA IgG, EBNA Ab), screening were performed on Abbott ARCHITECT i2000SR (Abbott Diagnostics) following manufacturer's instructions. Most of these tests were performed under ISO 15189 accreditation and were monitored by using internal and external quality controls. Molecular assays for HIV, HBV, and HCV detection were performed on Abbott m2000rt/sp (Abbott Diagnostics), with the notable exception of urgent HIV viral load tests for which the GeneXpert® system (Cepheid) was used.

Bacteriological assays

Serological status for *Treponema pallidum* (enzyme immunoassay) were determined on Abbott ARCHITECT i2000SR, completed with the non-treponemic Rapid Plasma Reagin / VDRL test (RPR Test kits, Biorad) to diagnose active infections. Until April 2018, a non-treponemic test was systematically associated with the treponemic screening test, following French recommendations at that time. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were exclusively tested by PCR. Bacterial DNA from *N. gonorrhoeae* and *C. trachomatis* was extracted using the MagNa Pure Compact® (Roche) and amplified using the S-DiaCTNG® kit (Diagenode) following manufacturer's instructions. Bacteriological urine tests were performed by traditional agar-based culture. *Mycoplasma genitalium* tests were outsourced (Laboratoire Cerba, Saint-Ouen-l'Aumône, France).

Determination of costs

Costs were estimated on the basis of the reimbursement rate for assays included in a reference list by the French social protection system (Nomenclature des Actes de Biologie Médicale, version 56 [11]), or in a complementary list designed for more innovative tests [12].

Ethics

This study was conducted in accordance with the amended Declaration of Helsinki. Informed consents were obtained from the PrEP users.

RESULTS

Study population

From March 2016 to December 2019, 135 individuals had consulted for PrEP initiation or follow-up in our institution, leading to a total of 756 consultations (Figure 1). The number of consultations for PrEP increased from 14 in 2016 to 337 in 2019. Among individuals on PrEP, 110 had benefited from at least 1 microbiological assay in our institution. In this group, the median age was 36.2 and 99.1 % (n = 109) were male.

Microbiological assay numbers and costs

The global number of microbiological assays performed in our institution for the cohort of 110 persons on PrEP was 3,434 including 1,083 virological assays and 2,351 bacteriological assays. The mean number of assays by person was 33.7, ranging from 6 to 84. When considering all consultations (PrEP initiation and follow up), 678 (89.7%) resulted in the prescription of at least one microbiological assay. The mean number of prescriptions for microbiological assays (whatever the number of tests in each prescription) per individual was 4.36 per year, corresponding to an average interval of 2.75 months between two prescriptions.

Virological assays

Virological assays are detailed on Table 1. Regarding the techniques involved, 98% of samples were tested by serological assays and 2% by molecular assays. The most frequent prescriptions were for the serological screening of HIV (589 tests), HCV (199 tests) and HBV (111 tests). Before PrEP initiation, 48.5% (n=32/66) and 14.3% (n=10/70) of individuals had an appropriate immunization against HBV and HAV, respectively. None of the tested persons seroconverted for HIV during the study period but one person was weakly seropositive for

HCV (4 tested samples for this person with fluctuations in or just above the method's grey zone). One subject was diagnosed with acute icteric hepatitis A (genotype 1a). Among 17 HIV RNA testing performed, 8 were carried out in ER for individuals experiencing symptoms compatible with a primary infection (expected result in this context within 4 hours in our institution). The global cost of virological tests was 17,334.80 euros (including 16,402 and 932.80 euros for serological and molecular tests, respectively). The 3 leading expenses were for HIV (41.3% of the budget dedicated to virological tests), HBV (25.8%), and HCV (14.9%) serology tests.

Bacteriological assays

Bacteriological assays are detailed in Table 2. Molecular assays were predominant (63.1%), followed by serological (35.9%) and agar-based culture (1%) assays. The most frequent pathogens screened were *N. gonorrhoeae* and *C. trachomatis* (1,415 molecular tests). *N. gonorrhoeae* and *C. trachomatis* were screened by sampling 3 anatomical sites (urine, throat, anus) or by sampling only throat and urine in 53.2 % (n=310/583) and 32.2% (n=189/583) of cases, respectively. *N. gonorrhoeae* DNA was detected in 7% of anal samples, 6.7% of throat samples and 1.5% of urine samples, whereas *C. trachomatis* DNA was detected in 7.0%, 1.1%, and 2.0% of anal, throat and urine samples, respectively. Urethral pus, rarely collected (n=6), was positive for *N. gonorrhoeae* in 33.3% of cases. No bacteria was detectable by traditional agar-based urine culture.

Treponemic tests (enzyme immunoassay in association with a TPHA test if necessary until April 2018) for *T. pallidum* infections were performed on 480 samples and were positive for 125 (26.0%) of them (Table 2). Non treponemic RPR/VDRL tests were positive for 40 of the 262 tested samples (15.3%). The global cost of the bacteriological tests was 28,648.40 euros

(including 23,426.60, 4,833 and 388.80 euros for molecular, serological and agar-based culture tests, respectively), with the leading expenses for *N. gonorrhoeae* and *C. trachomatis* (65.0% of the budget dedicated to bacteriological tests), syphilis (16.9%), and *M. genitalium* (16.0%).

Sexually transmitted bacterial infections

Cumulative prevalence of *N. gonorrhoeae* and *C. trachomatis* among the 107 tested individuals during the study period were 39.3% (n=42) and 22.4% (n=24), respectively (Table 3). Multiple episodes (≥ 2) of infection by each bacteria occurred in 6.5% (n=7) of individuals. When considering all episodes of *N. gonorrhoeae* and *C. trachomatis* infections, 15.7% (n=8) and 14.3% (n=5) were symptomatic, respectively, although the accurate attribution of symptoms to a specific pathogen was difficult in the cases of coinfections. Concomitant or successive infections by the 2 pathogens were observed in 15% of persons (n= 16). As no agar plate was obtained for *N. gonorrhoeae*, no antibiogram could be performed.

Seropositive status for syphilis was observed in 36 of the 110 tested individuals (32.7%), including 17 cases of active infections (15.4% of tested individuals) determined by a positive non treponemic RPR/VDRL test. Symptoms compatible with syphilis, such as rash and chancre were observed in 7 patients.

Interestingly, at least one active STD due to *N. gonorrhoeae*, *C. trachomatis* or *T. pallidum* (positive RPR / VDRL assay) was observed for 40.6% (n=43) of the 106 individuals tested for these 3 pathogens during their PrEP initiation or follow up.

Only one person was tested positive for *M. genitalium* and was resistant to azithromycin.

DISCUSSION

In this study, we planned to describe the type, the number and the cost of microbiological tests performed in a French laboratory collecting PrEP consultation-associated samples. Since PrEP programs are associated with regular recommended microbiological tests [10], and since PrEP users are frequently exposed to STDs [13–15], the availability of technical platforms for laboratories associated with a PrEP consultation should not be neglected. We observed that predominant laboratory techniques in this setting were serology (98% and 35.9% of virological and bacteriological tests, respectively) and molecular biology (63.1% of bacteriological tests), and that culture-based assays, which are easily available in numerous private or public laboratories, were poorly relevant (1% of bacteriological tests, performed here for classic urine culture). Although rarely used in our study (8 tests performed), the availability of an emergency technique for HIV RNA detection is required in case of symptoms compatible with a primary infection, since the clinical sensitivity of HIV serological assays in this context is imperfect [16].

Economic analyses that compared the budget of PrEP and of medical care for people living with HIV mainly took into account the costs of antiretroviral drugs, HIV-related healthcare and immuno-virological monitoring (visit and hospital admission, counseling, viral load, CD4 count, resistance testing), PrEP monitoring, and STD treatments [4,7,8]. The cost of HIV testing was often included to estimate the budget of PrEP [4,7] but the cost of other laboratory tests was not always considered or reported. We report here the costs of microbiological assays performed in our institution since the initiation of PrEP consultations. The cost of a laboratory test may correspond to the reimbursement price set by the national protection system (in France, these prices are listed in the Nomenclature des Actes de Biologie Médicale) or to the “real” cost that takes into account the cost of laboratory

reagents, controls, equipment, and medical or technical staff. Since the “real” costs significantly vary between laboratories, we chose to consider only the French reimbursement price set in the last version of the Nomenclature des Actes de Biologie Médicale (version 56 [11]). With this method, we estimated that the global cost of microbiological tests administered to individuals on PrEP in our institution for a total of 756 consultations (PrEP initiation and follow-up) reached 45,983.20 euros. Approximately one third of this budget (17,334.80 euros) was dedicated to virological tests and two thirds (28,648.40 euros) to bacteriological tests. Consequently, the mean cost of microbiological tests per consultation can be estimated at 60.80 euros, with a higher cost at the first consultation (104.40 euros) than at the others (56.60 euros/consultation).

This cost might be underestimated, since a part of microbiological tests associated with PrEP consultations in our institution could have been performed by other laboratories, including private laboratories. Moreover, intermittent microbiological testing (e.g. for episodes of STD) may have been carried out in another setting than the PrEP consultations (e.g. prescriptions from general practitioners). However, since most sampling was directly performed at the time of the PrEP consultations, we consider that the number of samples collected in our lab accurately reflected the real number of microbiological tests performed by the individuals in the PrEP setting. The number of microbiological tests required for a cohort of PrEP users closely depends on local guidelines and on sexual practices or level of exposure to STDs (prevalence of STDs in each region, infection clusters ...). Consequently, the estimated number and cost of microbiological tests performed in our cohort may vary for other populations of PrEP users.

PrEP is a unique opportunity to (re-)enroll those highly exposed to sexually transmitted diseases (STD) in a prevention and care strategy. In particular, PrEP programs lead to regular

HIV testing, screening and treatment of STDs, completion of vaccination schedules, and reinforcement of prevention messages concerning at-risk behaviors [17]. The number of STDs diagnosed in the PrEP setting greatly varied in the reported cohorts. In our study population, we did not observe any seroconversion for HIV or HCV, although one person presented with acute hepatitis A (diagnosed during PrEP initiation in this unvaccinated person). By contrast, evidence of at least one STD due *N. gonorrhoeae*, *C. trachomatis*, or *T. pallidum* (positive non treponemic test) was observed in 40.6% of individuals tested for these 3 pathogens during the time course of PrEP. When restricting to *N. gonorrhoeae* and *C. trachomatis*, the prevalence of infections reached 39.3% (n=42/107) and 22.4% (n=24/107), respectively, including approximately 15% of symptomatic episodes. The real incidence of STDs in PrEP users greatly varies according to the specificities of the studied population and to the study design [14,15,18]. PrEP has been associated with an increased STD incidence (or a more efficient STD screening) in some cohorts [13,15], whereas other studies reported that STD incidence remained stable on PrEP [14]. In a meta-analysis including 20 studies, incidence rates for gonorrhea and chlamydia were estimated at 39.6 per 100 person-years (/100py) and 41.8/100py, respectively [14]. In a more recent Australian cohort of nearly 3,000 individuals on PrEP, 48% of participants were diagnosed with a bacterial STD (gonorrhea, chlamydia, syphilis), leading to an STD incidence of 98.4/100py [15]. Interestingly, a recent genomic analysis of *N. gonorrhoeae* transmissions between population groups in Australia reported that PrEP users were prone to have repeated gonorrhea infections or to be part of larger transmission clusters involving both HIV-positive and HIV-negative individuals [19]. Further molecular and epidemiological studies are needed to better identify possible transmission clusters in our cohort.

In summary, our study provides a ‘real life’ estimation of the number, the type, and the cost of microbiological tests performed for individuals on PrEP. We suggest that microbiological tests associated to PrEP predominantly required platforms of serology and molecular biology. Approximately two thirds of the budget were dedicated to bacteriological tests, which permit screening and diagnosing of frequent bacterial STDs in our study population.

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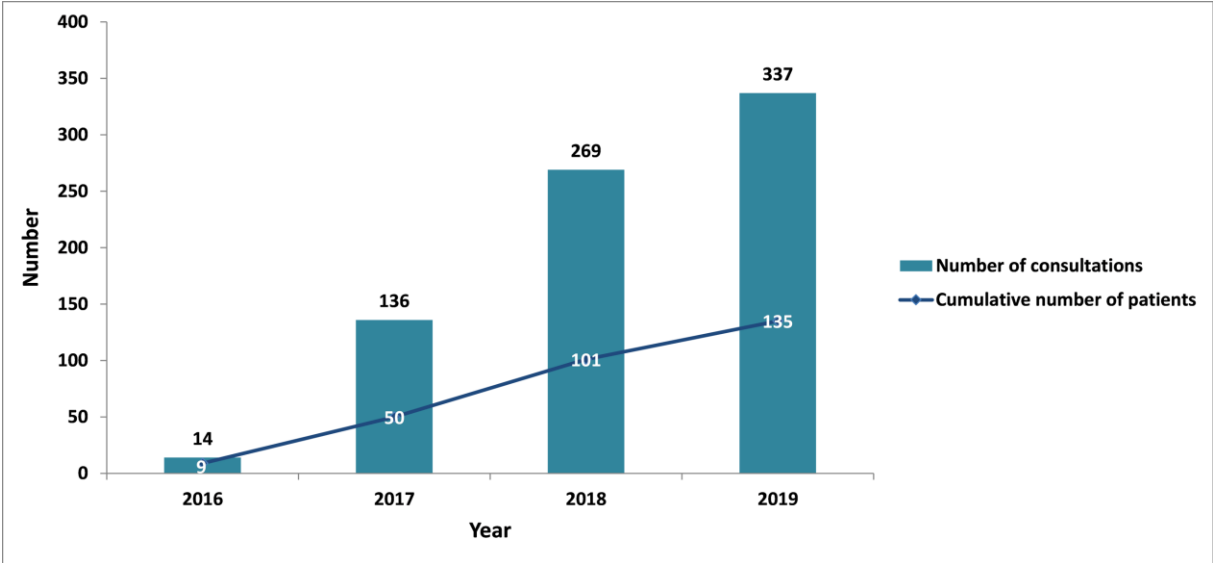
COMPETING INTERESTS: None declared

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Figure 1: Cumulative numbers of PrEP consultations and individuals on PrEP



1 **Table 1: Type, number, and cost of virological tests for individuals on PrEP**

	Tested samples	Positive samples (%)	Cost (Euros)	Part of the budget	Indications
HIV Serology	589	0 (0.0%)	7156.4	41.3%	Every 3 months or high risk exposure
HCV Serology	199	4 (2.0%)	2579	14.9%	Every year or high risk exposure
HBV Serology :	111	-	4465.8	25.8%	-
- HBs Ag	111	0 (0.0%)	-	-	Every year or high risk exposure
- HBs Ab	110	63 (57.3%)	-	-	Vaccination follow-up
HAV Serology (IgG)	82	13 (15.9%)	1107.0	6.4%	Vaccination follow-up
HAV Serology (IgM)	79	1 (1.3%)	1045.2	6.0 %	Every year or high risk exposure
HTLV Serology	1	0 (0.0%)	10.8	0.1%	-
EBV Serology*	1	1 (100%)	37.8	0.2%	-
HIV RNA	17	0 (0.0%)	803.2	4.6%	Suspicion of (primary) infection
HBV DNA	2	0 (0.0%)	75.6	0.4%	High risk exposure
HCV RNA	1	0 (0.0%)	54.0	0.3%	High risk exposure
HAV Genotyping	1	NA	NA	NA	Epidemiological
TOTAL	1083	-	17334.8		

2 * performed during a systematic investigation of elevated transaminases

3

4 **Table 2: Type, number, and cost of bacteriological tests for individuals on PrEP**

5

	Tested samples	Positive samples (%)	Cost (Euros)	Part of the budget	Indications
<i>C. trachomatis (CT)</i> and <i>N.gonorrhoeae (NG)</i> DNA	1415	CT = 40 (2.8%) NG = 69 (4.9%)	18634.1	65.0%	screening technique
<i>M. genitalium</i> DNA	68	3 (4.4%)	4590.0	16.0%	screening technique
<i>M. genitalium</i> resistance test	3	3	202.5	0.7%	
Syphilis EIA*	480	125 (26.0%)	2592.0	9.0%	treponemic test (screening)
Syphilis RPR/VDRL	262	40 (15.3%)	1414.8	4.9%	non treponemic test (confirmation)
Syphilis VDRL titration	102	21 (20.6%)	826.2	2.9%	active syphilis follow-up
Urine culture	24	0 (0.0%)	388.8	1.4%	
TOTAL	2351	-	28648.4		

6 *Enzyme ImmunoAssay

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12 **Table 3: Bacterial sexually transmitted diseases among individuals on PrEP**

13

	Tested patients	Positive patients (%)	Number of episodes*
<i>N. gonorrhoeae</i>	107	42 (39.3%)	51
- multiple episodes (n>1)		7 (6.5%)	9
- symptomatic		8	8
<i>C. trachomatis</i>	107	24 (22.4%)	35
- multiple episodes (n>1)		7 (6.5%)	11
- symptomatic		5	5
Syphilis EIA**	110	36 (32.7 %)	-
Syphilis RPR/ VDRL	79	17 (21.6 %)	17
- symptomatic		9	9
<i>M. genitalium</i>	47	1 (2.1%)	1

14 * including distinct episodes for the same patient; **Enzyme ImmunoAssay