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RESEARCH ARTICLE

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Anal and oropharyngeal HPV distribution in HIV-negative multipartner MSM using self-sampling kits for HIV and sexually transmitted infection screening

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Abstract

Men who have sex with men (MSM) are at high risk of sexually transmitted infections, among which HPV infections are particularly prominent. We took advantage of the MémoDépistages study to evaluate HPV distribution at anal and oropharyngeal sites in HIV-negative multipartner MSM. HPV DNA was detected in 82% (n = 344) of anal and 11% (n = 45) of oropharyngeal self-collected samples taken from 421 participants. Multiple HPV types were detected in 70% of anal samples, and single HPV types in 91% of oropharyngeal samples. HPV16 was the most frequent type detected in the anus, followed by HPV6, HPV51, and HPV52. HPV6, HPV16, and HPV11 were the most prevalent types in the oropharynx. HPV targeted by the nonavalent vaccine was detected in 71% and 50% of HPV-positive anal and oropharyngeal samples, respectively. The main risk factor associated with HPV detection was frequenting gay meeting places, living in large cities, and having an anal *Chlamydia trachomatis*/*Neisseria gonorrhoeae* infection. In this cohort of highly sexually active MSM, HPV detection was highly frequent and rendered them at high risk of precancerous and cancerous lesions. Universal vaccination against HPV before sexual debut is an important public health strategy to prevent HPV-associated cancers in this highly vulnerable population of HIV-negative MSM.

KEYWORDS
anus, HPV distribution, multipartner MSM, oropharynx

1 | INTRODUCTION

Persistent infection by a high-risk HPV (hrHPV) is the leading cause of cervical and anal cancers and variable fractions of cancers of the vulva, vagina, penis, and head and neck.[1] While HPV infection is a necessary cause for some cancers, factors related to the virus (type), the host (immune/hormonal status), and the behavior (number of sexual partners, multiparity, intercurrent infections) favor cancer development.[2] Almost 400 different HPV types have been described[3] among which 12–14 are considered carcinogenic.[4]

Among behavioral factors, there is strong evidence that men who have sex with men (MSM) living with HIV and, to a lesser extent, HIV-negative MSM are at high risk of HPV infection.[5] Consequently, MSM belongs to a population at high risk of anal cancer compared to the general population, with an incidence increased by 19 in HIV-negative MSM and by 85 in HIV-positive MSM.[6] HPV16 represents the most prevalent hrHPV detected at the anal level. It is also

strongly associated with the severity of anal dysplasia, regardless of HIV status.[7,8] HPV16 is also the most frequently detected type in the oral cavity and in head and neck cancers.[9–13] In France, HPV vaccination has been recommended to MSM since 2016, but vaccine uptake is low (estimated at 15% in 2019).[14]

The MémoDepistages programme was set up by the French public health agency (Santé Publique France, SpFrance) to evaluate the feasibility of self-sampling for the screening of HIV and other sexually transmitted infections (STI) (*Chlamydia trachomatis* [CT], *Neisseria gonorrhoeae* [NG], Syphilis, HBV, HCV) in the high-risk population of HIV-negative multiple partner MSM.[15] To reach this population, an original strategy was adopted with the promotion of the study through gay social media and a dating app. The authors reported that this strategy made the study feasible and allowed the determination of the prevalence and risk factors for each studied STI.[16] To gain further insight into HPV infection at the anus and oropharynx levels among highly sexually active HIV-negative MSM, HPV distribution was studied in residual self-samples taken during the MémoDepistages programme.[15,16]

2 | MATERIALS AND METHODS

2.1 | Study population

The study population was recruited thanks to advertisements on gay social media and a dating application as described elsewhere.[15] The population was composed of MSM originating from Auvergne-Rhône-Alpes, Ile-de-France, Occitanie Est, and Provence-Alpes-Côte d'Azur. Participants had to be aged 18 years and over, declaring more than one sexual partner in the past year, reporting a seronegative or unknown HIV status, and not taking pre-exposure prophylaxis (PrEP). After their consent to participate, participants filled out an online sociodemographic and behavior questionnaire. Self-sampling kits (SSK) for blood, anal, and oropharyngeal samples were then sent to the participants. The detailed composition of the SSK has been previously described.[16] HIV/STI screening was offered to 1948 MSM at inclusion (April–August 2018) and then to 444 MSM who participated in the second period of the study 12 months later (April/June 2019). Anal and oropharyngeal self-sampling was initially done using the "Cobas R PCR Dual Swab Sample Kits" or the "Abbott R multi-Collect Specimen Collection Kit." Samples were stored at 4°C before CT/NG detection. One milliliter of aliquots was then sent to the French Papillomavirus National Reference Centre, where they were stored at 4°C up to 1 year before DNA extraction and HPV typing. The protocol was approved by local authorities under number ID RCB 2017-A00838-45 and by the ethics committee CPP-Ouest II Angers. Informed consent was obtained for all participants.

2.2 | DNA extraction

One hundred milliliters of anal and pharyngeal samples were centrifuged, and the cell pellets were resuspended in 100 µL of Specimen Transport Medium (Qiagen). DNA was then extracted using the QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. DNA was eluted in 80 µL Tris EDTA buffer (pH 9.0). An aliquot of 10 µL was used for PCR amplification with the INNO-LiPA® HPV Genotyping Extra II Assay (Fujirebio Europe).

2.3 | HPV typing

Thirty-two HPV belonging to the alpha genus were detected using the INNO-LiPA® HPV Genotyping Extra II Assay (Fujirebio Europe) according to the manufacturer's instructions. This test allows the identification of 13 hrHPV (HPV16, 18, 31,33, 35, 39, 45, 51, 52, 56, 58, 59, 68), 6 probably hrHPV (HPV 26, 53, 66, 70, 73, 82), and 13 low risk (lrHPV) or nonclassified HPV (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, 89). HPV amplimers that do not hybridize to any specific probe are considered uncharacterized (HPVX). "Any HPV" refers to the 32 specific HPV detected by the assay plus HPVX. Vaccine-type HPV refers to HPV types covered by the bivalent (2vHPV), quadrivalent (4vHPV), and nonavalent (9vHPV) vaccines. Our laboratory regularly participates in HPV DNA proficiency testing with this assay, especially the one organized by the International HPV Reference Laboratory. Our laboratory's success with this assay is 100%.

2.4 | Statistical analyses

Individual data were merged at SpFrance in a database combining sociodemographic data, results of CT/NG screening, and HPV typing results. Participants were included if they had a complete questionnaire and a result for both anal and oropharyngeal HPV tests. Statistical analysis was performed using Stata 14. We described the proportion of participants HPV positive in anal and oropharyngeal tests with 95% binomial confidence intervals. We compared this proportion according to participant characteristics collected at inclusion. Proportions were compared using chi-square or Fisher exact test.

3 | RESULTS

3.1 | Study population

Four hundred twenty-one MSM for whom both anal and oropharyngeal HPV typing data were available were included in analyses. The sociodemographic and behavioral characteristics of the study population are presented in Table 1 and were similar to baseline characteristics of the 1948 MSM enrolled in the MémoDépistages study (data not shown). The median age of the 421 participants was 34 years (interquartile range: 27–43 years). Half of the participants lived in large cities (>100 000 inhabitants), and the majority had a high level of education (78.4%), were employed (79.8%), and

TABLE 1 Sociodemographic and behavioral characteristics of the population ($n = 421$).

Characteristics	n	%
Age		
19–24 years	69	16.39
25–29 years	76	18.05
30–34 years	74	17.58
35–39 years	62	14.73
40–44 years	48	11.40
45–49 years	43	10.21
>50 years	49	11.64
Place of birth		
France	389	92.40
Overseas	32	7.60
Place of residence (inhabitants)		
<2000	18	4.28
2000–20 000	65	15.44
20 000–100 000	125	29.69
≥100 000	213	50.59
Level of education		
High school or less	91	21.62
College or more	330	78.38
Professional situation		
Employed	304	72.21
Self-employed	32	7.60
Unemployed	20	4.75
Student	51	12.11
Other	14	3.33
Perceived financial situation		
Good	227	53.92
Average	148	35.15
Bad	46	10.93
Self-identified sexual orientation		
Homosexual	359	85.27
Bisexual	38	9.03
Other	24	5.70
Frequenting gay meeting places		
Often	114	27.08
Sometimes	234	55.58
Never	73	17.34

TABLE 2 HPV detection in the anus and oropharynx of multipartner MSM.

	Anus		Oropharynx	
	N	% (95% CI)	N	% (95% CI)
Any HPV[a]	344	81.7% (77.7–85.3)	45	10.7% (7.9–14.0)
hrHPV	276	65.6% (60.7–70.0)	20	4.7% (3.1–7.3)
lrHPV	143	34% (29.4–38.7)	15	3.6% (2.0–5.8)
HPV6/11	110	26.1% (22.1–30.6)	12	2.8% (1.5–4.9)
2vHPV[b]	105	24.9% (21.0–29.3)	5	1.2% (0.5–2.8)
4vHPV[c]	184	43.7% (38.9–48.6)	17	4.0% (2.4–6.4)
9vHPV[d]	244	58.0% (53.2–62.6)	23	5.5% (3.6–8.1)

[a]All specific HPV detected + HPVX.
[b]2vHPV: HPV16, 18.
[c]4vHPV: HPV6, 11, 16, 18.
[d]9vHPV: HPV6, 11, 16, 18, 31, 33, 45, 52, 58.

considered to have a good/average financial situation (89.1%). Most of them often or sometimes frequent gay meeting places (82.7%). Concomitant syphilis diagnosis, anal CT infection, and anal NG infection were observed in 10.5% (33/315), 5.4% (22/408), and 2.2% (13/407) of participants, respectively. Only two participants were tested HIV positive during the study period.

3.2 | HPV detection

HPV DNA was detected in 81.7% of anal samples (Table 2). Multiple types were detected in 71.2% of HPV-positive anal samples with a mean of 2.9 types; 2, 3, and 4 types were detected in 19.0%, 14.0%, and 11.0%, respectively, and 13.8% of HPV-positive samples had 5–10 types.

HPV DNA was detected in 10.7% of oropharyngeal samples (Table 2). In 91.1% of HPV-positive oropharyngeal samples, a single type was detected. Two, three, and five different HPV types were observed in two, one, and one samples, respectively. Up to five different HPV types have been retrieved from one sample.

Detection of any HPV (32 specific types plus HPVX), hrHPV, lrHPV, and vaccine-type HPV (i.e., HPV types covered by the bivalent [2vHPV], quadrivalent [4vHPV], and nonavalent [9vHPV] vaccines) is depicted in Table 2. Thus, hrHPV, 2vHPV, 4vHPV, and 9vHPV were detected in 65.6%, 24.9%, 43.7%, and 58.0% of anal samples, respectively, and in 4.7%, 1.2%, 4.0%, and 5.5% of oropharyngeal samples, respectively.

Figure 1 presents the proportion of HPV types in anal and oropharyngeal samples. HPV16 was the most prevalent type in anal samples (19.0%), followed by HPV6 (18.5%), HPV51 (16.4%), and HPV52 (12.8%) (Figure 1A). Oropharyngeal samples had a lower

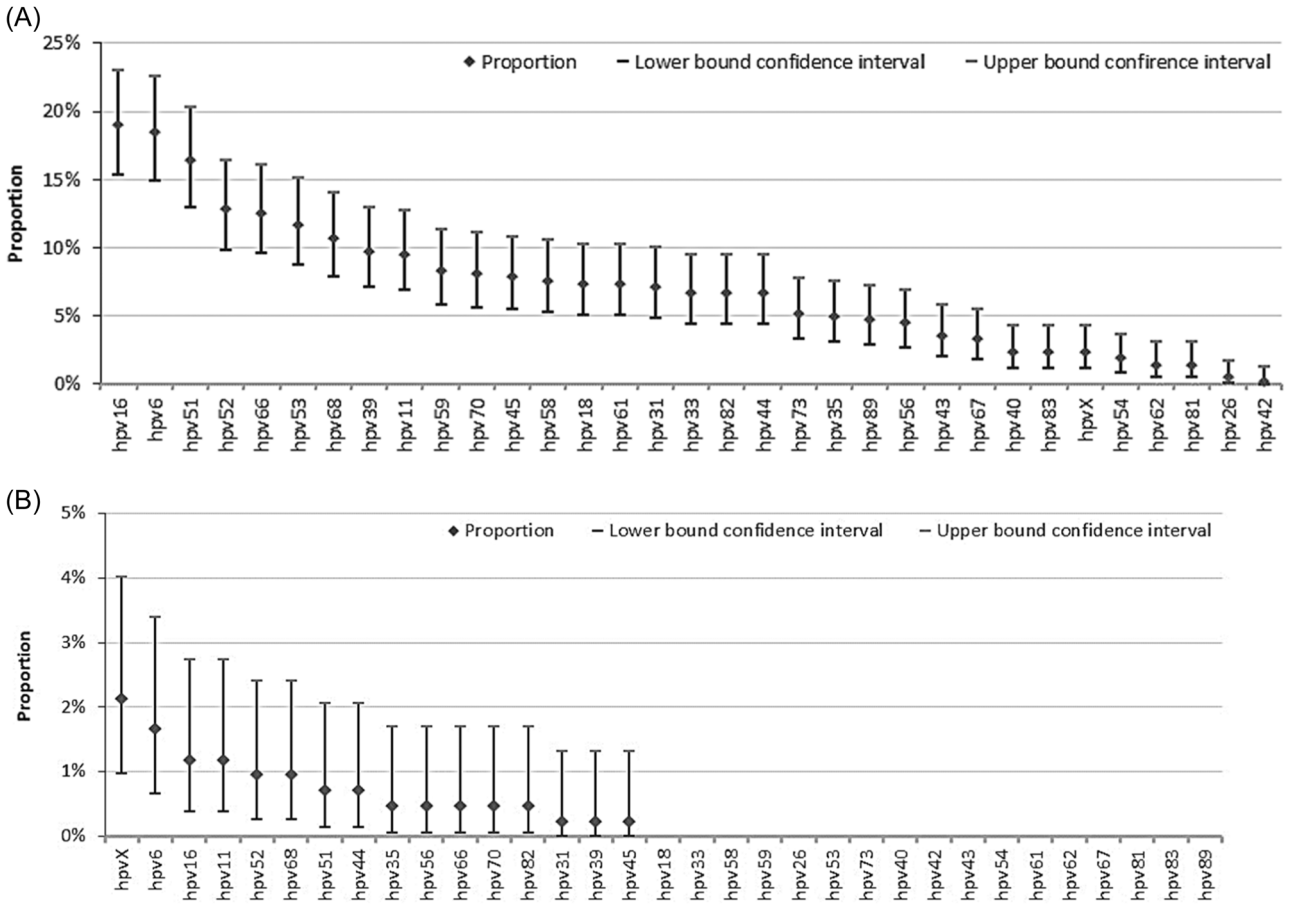

FIGURE 1 HPV proportion in anal (A) and oropharyngeal (B) samples. Error bars represent 95% confidence intervals.

prevalence and fewer types than anal samples (Figure 1B), and HPVX was the most prevalent (2.1%), followed by HPV6 (1.7%), HPV16 (1.2%), and HPV11 (1.2%).

A large proportion of participants (91%, 41/45) who were tested positive in oropharyngeal samples also had HPV detected in anal samples. Anal HPV was detected in 81% of participants who tested negative in oropharyngeal sample. Among 41 participants with HPV (any type) detected in oropharyngeal samples, 11 had at least one concordant HPV type detected in the anal sample.

3.3 | Risk factors associated with anal HPV detection in MSM

Risk factors for any HPV and hrHPV detection were assessed only for anal samples because of the low rate of positivity in oropharyngeal samples. As shown in Table 3, frequenting gay meeting places was significantly associated with HPV and hrHPV detection. The place of residence was associated with the detection of hrHPV.

Presenting with an intercurrent syphilis infection was not associated with the detection of an HPV infection, but anal CT or NG infection were both associated with hrHPV positivity (Table 3). All participants who had a CT or NG infection at anal level also tested positive for HPV (any type) in anal samples.

4 | DISCUSSION

The French study "MémoDepistages" allowed us to describe the HPV distribution at anal and oropharyngeal sites in a large cohort of 421 HIV-negative and multipartner MSM. Anal HPV detection was very frequent since more than 80% of the participants had detectable HPV DNA (corresponding to a hrHPV in two of the three cases) in this anatomical site. This prevalence is higher than the prevalence previously documented in HIV-negative MSM (47%–73%).[7,17–20] It is, however, in line with data obtained in an HIV-negative, high-risk population of MSM taking PrEP.[21] Because of the modality of recruitment and inclusion criteria, the studied population is probably not representative of the general population of HIV-negative MSM. Rather, it represents a peculiar population highly exposed to STI[15,16] and to HPV infections.

HPV16 was the most frequently detected type in the anal samples, as observed in previous studies.[5,7,17–19,22] It was followed by HPV51 and 52, also found to be the most prevalent types

TABLE 3 Factors associated with overall HPV and hrHPV positivity in anal samples.

	Any HPV+ *n* (%)	*p* Value	hrHPV+ *n* (%)	*p* Value
Characteristics				
Age		0.7		0.052
19–24 years (*n* = 69)	53 (76.8%)		40 (58.0%)	
25–29 years (*n* = 76)	59 (77.6%)		49 (64.5%)	
30–34 years (*n* = 74)	61 (82.4%)		51 (68.9%)	
35–39 years (*n* = 62)	54 (87.1%)		44 (71.0%)	
40–44 years (*n* = 48)	39 (81.3%)		24 (50.0%)	
45–49 years (*n* = 43)	36 (83.7%)		29 (67.4%)	
>50 years (*n* = 49)	42 (85.7%)		39 (79.6%)	
Place of birth		0.7		0.4
France (*n* = 389)	317 (81.5%)		253 (65.0%)	
Overseas (*n* = 32)	27 (84.4%)		23 (71.9%)	
Place of residence (inhabitants)		0.08		**0.021**
<2000 (*n* = 18)	11 (61.1%)		7 (38.9%)	
2000–20 000 (*n* = 65)	57 (87.7%)		50 (76.9%)	
20 000–100 000 (*n* = 125)	103 (82.4%)		83 (66.4%)	
≥100 000 (*n* = 213)	173 (81.2%)		136 (63.8%)	
Level of education		0.15		0.07
High school or less (*n* = 91)	79 (86.8%)		67 (73.6%)	
College or more (*n* = 330)	265 (80.3%)		209 (63.3%)	
Perceived financial situation		0.14		0.36
Good (*n* = 227)	178 (78.4%)		142 (62.6%)	
Average (*n* = 148)	128 (86.5%)		103 (69.6%)	
Bad (*n* = 46)	38 (82.6)		31 (67.4%)	
Self-identified sexual orientation		0.5		0.25
Homosexual (*n* = 359)	293 (81.6%)		238 (66.3%)	
Bisexual (*n* = 38)	33 (86.8%)		26 (68.4%)	
Other (*n* = 24)	18 (75.0%)		12 (50.0%)	
Frequenting gay meeting places		**0.02**		**0.01**
Often (*n* = 114)	101 (88.6%)		83 (72.8%)	
Sometimes (*n* = 234)	190 (81.2%)		155 (66.2%)	
Never (*n* = 73)	53 (72.6%)		38 (52.1%)	
Syphilis infection		0.35		0.19
Yes (*n* = 33)	29 (87.9%)		25 (75.8%)	
No (*n* = 282)	229 (81.2%)		181 (64.2%)	
Anal CT infection		**0.02**		**0.01**
Yes (*n* = 22)	22 (100.0%)		20 (90.9%)	
No (*n* = 386)	311 (80.6%)		254 (64.5%)	

(Continues)

TABLE 3 (Continued)

	Any HPV+ *n* (%)	*p* Value	hrHPV+ *n* (%)	*p* Value
Anal NG infection		0.14		**0.04**
Yes (*n* = 13)	13 (100.0%)		12 (92.3%)	
No (*n* = 394)	319 (81.0%)		254 (64.5%)	

Abbreviations: CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*.

detected in anal samples, after HPV16, in a recent meta-analysis.[23] Finally, detection of multiple types of HPV was also very frequent[17,21] and had to be related to the high sexual exposure with multiple partners.[24] Furthermore, the wide variety of HPV detected at the anal level likely reflects multiple exposures of the participants related to their sexual behavior. Indeed, in the MémoDepistage cohort, most participants had at least four different partners in the last 6 months at inclusion.[16] Because of the timing of the phases in this study, we could not evaluate the association between the number of sexual partners (collected in 2018) and the genital prevalence of HPV (samples collected in 2019). Unfortunately, no cytology data was available since self-sampling is not adequate for cytology evaluation.

At the oropharyngeal level, HPV DNA was detected in approximately 10% of participants, and mostly single HPV types were detected. This frequency of HPV DNA detection was of the same order of magnitude (7%–8%) as previously reported in populations of HIV-negative MSM.[25,26] hrHPV were found in 5% of the cohort, a prevalence slightly higher than that documented in the general population[27,28] reflecting again the high sexual exposure of the study population. The most frequently detected type remains HPV16, whatever the study is, with an approximate prevalence of 1% of the population.[25,28]

It has to be pointed out that the observed prevalence of HPV oropharyngeal detection in our cohort could be underestimated due to the use of oropharyngeal self-sampling instead of gargles, which have shown a higher sensitivity for HPV detection.[25,29] Swabbing the oropharynx is also more difficult to perform than swabbing the oral cavity, particularly in self-sampling. Thus, the collection method may be an important source of variation in prevalence studies.[29–31]

As for risk factors, frequenting gay meeting places and living in a large city (for hrHPV) were associated with HPV detection. HPV detection was also significantly associated with intercurrent anal CT/NG infection. This is consistent with the fact that current or past history of other STIs have been found to be risk factors for HPV detection at the cervix or penis.[32–34] Moreover, a recent meta-analysis revealed that CT and HPV may act as reciprocal risk factors.[35] In this study, the prevalence of STI other than HPV was of the same magnitude, even if slightly lower (10%–15% vs. 25%–30%), than that observed in a similar cohort.[21]

The main strengths of our study were the number of participants, the focus on a population of HIV-negative MSM (who are often more difficult to investigate than HIV-positive people), and the exhaustive collection of epidemiological, behavioral, and STI-related data. A large number of HPV types were investigated using a highly sensitive technique and the exploration of two major anatomical sites of HPV infection. Among the weaknesses, we could note the loss of participants between the first and second phase of the study (1948 vs. 444 persons) and the type of oropharyngeal sampling (oropharyngeal swabs could be less sensitive than gargles). Slightly discrepant results could also have been observed with different commercial HPV tests due to the difference in sensitivity between the kits.

In conclusion, we confirmed that multipartner HIV-negative MSM is at high risk of HPV/hrHPV detection at the anal level and, to a lesser extent, at the oropharyngeal level. The prevalence of HPV types targeted by the nonavalent vaccine is 58% for anal samples and 5.5% for oropharyngeal samples. Promoting the vaccination as recommended in France to MSM since 2016 is a priority for this population. Since 2021, universal HPV vaccination before sexual debut has been recommended, targeting both adolescent boys and girls aged 11–14 years old in France. This strategy is a major progress, and it will protect MSM before they engage in sexual activities. Secondary prevention through anal lesion screening should also be encouraged, as recently proposed for populations at very high risk of anal cancer by the French National Society of Coloproctology.[36]

AUTHOR CONTRIBUTIONS

Jean-Luc Prétet: Conceptualization; investigation; resources; supervision; writing—original draft. **Alice Baraquin**: Investigation; data curation; writing—review and editing. **Anne-Sophie Barret**: Conceptualization; formal analysis; writing—review and editing. **Béatrice Bercotd**: Investigation; writing—review and editing. **Delphine Rahib**: Initial conceptualization of the MémoDépistages study; writing—review and editing. **Nathalie Lydié**: Initial conceptualization of the MémoDépistages study. **Line Pépin-Puget**: Investigation; data curation; writing—review and editing. **Quentin Lepiller**: Data curation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The protocol was approved by local authorities under number ID RCB 2017-A00838-45 and by the ethics committee CPP-Ouest II ANGERS. Informed consent was obtained for all participants.

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