

1 **ORIGINAL ARTICLES**

2 **Adherence to antiretroviral therapy through package-refill among HIV+ persons at “D.**  
3 **Cotugno” hospital, Naples, Italy.**

4

5 **Running title: ART adherence through package-refill**

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18 **Keywords:** antiretroviral therapy, adherence, package-refill, HIV/AIDS.

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## 20 **SUMMARY**

21 Background: A gold-standard for the measurement of adherence to antiretroviral therapy (ART) is  
22 lacking. Aim of this study is to verify the feasibility of a package-refill-based measurement of ART  
23 at “D. Cotugno” hospital, Naples, Italy, and the factors associated to adherence.

24 Methods: In the period January 2018-August 2020, we calculated the package-refill as the ratio  
25 between ART-packages actually withdrawn, and the ART packages needed to regularly take ART.  
26 Adherence was associated, through a univariate and multivariate logistic regression, to demographical,  
27 behavioural and clinical factors.

28 Results: 1140 HIV+ subjects were included. At univariate logistic regression inadequate package-  
29 refill-based adherence is associated with HIV-RNA higher than 50 copies/mmL (OR 3.77-IC95%  
30 2.76-5.13) and with HIV-RNA higher than 200 copies/mmL (OR 3.98-IC95% 2.69-5.90). Being  
31 not-Italian and Injective-drug-user are associated with low adherence, having HIV/AIDS for more  
32 than 8 years is associated with better adherence.

33 Conclusions: Package-refill is a suitable method for measuring adherence and is associated with the  
34 condition of viral failure.

35

## 36 **INTRODUCTION**

37 Adherence to antiretroviral therapy (ART) is one of the major determinants of sustained HIV  
38 virologic suppression, restoration of immune system, prevention of drug resistance and reduced risk  
39 of HIV transmission [1,2]. On the other side, studies suggest that long-term adherence is difficult to  
40 maintain and tools for the appropriate monitoring of adherence are needed, in order to promptly  
41 identify patients with poor adherence [3].

42 There is no gold standard for estimating ART adherence and several methods have been proposed.  
43 Most used methods to assess ART adherence in randomized controlled trials have included patient  
44 self-report, electronic monitoring and pill count [4]. In real-life, other methods have included  
45 pharmacy refill data, communication instruments such as SMS-recall and assessing pharmacologic  
46 drug levels in biological samples. Most feasible and acceptable measure may change place by place,  
47 based on the local organization and resources.

48 Pharmacy-based measures include pill-refill count and/or package-refill count. These measures are  
49 based on the ratio between the number of pills/packages actually withdrawn compared to those that  
50 should have been withdrawn to ensure optimal adherence. These measures have several advantages:  
51 the assessment of pill/package refills is not expensive, easy to conduct, since data are already  
52 included in pharmacy records and may provide an overall long-term estimation of adherence.[5]  
53 Among these measures, the package-refill is easier to be assessed.

54 The aim of this study is to verify the overall ART adherence measured through a package-refill-  
55 based adherence measure in a large sample of Persons Living With HIV (PLWH) stably followed-  
56 up at “D. Cotugno” hospital, Naples, South Italy. As secondary end points, we explored the  
57 demographical, behavioural and clinical factors associated to inadequate adherence to ART.

## 58 **PATIENTS AND METHODS**

### 59 *Study design*

60 This is a retrospective observational study exploring (i) the feasibility of a measure for ART  
61 adherence monitoring, the package-refill count, (II) the adherence to ART and (iii) the factors  
62 associated to inadequate adherence among a population of PLWH in care at “D. Cotugno” hospital,  
63 Naples, Southern Italy.

### 64 *Setting*

65 The study setting is the “D. Cotugno” hospital, a mono-specialistic infectious diseases referral  
66 centre sited in Naples, Campania, Southern Italy. Campania is the most populous region in Southern  
67 Italy, counting for 6 million of inhabitants. The “D. Cotugno” hospital has a long-term tradition in  
68 HIV/AIDS care and some Medical Units are mainly dedicated to in-patient and out-patient  
69 management of PLWH. Specific HIV diagnostics are performed within the hospital laboratory. A  
70 total of about 2500 PLWH are in care at “D. Cotugno” hospital, accounting for about 70% of  
71 PLWH in Campania Region.

### 72 *Participants and data source*

73 A sample of patients with HIV/AIDS in care at “D. Cotugno” hospital, those referring to 2  
74 HIV/AIDS Units and accounting for 50% of the entire PLWH hospital population, were included in  
75 the study. Among the patients selected, only subjects stably in care in the period January 2018-  
76 August 2020 were included, defined as patients with at least one access per year for laboratory  
77 assessment and at least one access per year for ART package withdraw. Data sources were  
78 represented by clinical electronic database for patients’ records, and by pharmacy electronic  
79 database for package-refill data.

### 80 *Variables*

81 The main outcome of the study is represented by ART adherence measured by package-refill.  
82 Package refill is calculated as the ratio between ART packages actually withdrawn, compared to the  
83 number of ART packages needed to regularly take the therapy. We considered adequate a package-  
84 refill  $\geq 95\%$  of packages withdrawn in the study period, partial a package-refill of 70%-94%;  
85 inadequate a package-refill less than 70%.

86 In order to verify the accountability of this measure, we included HIV-RNA in study period as main  
87 dependent variable. We classified HIV-RNA as: always less than 50 copies/mmL; at least one  
88 record between 51-200 copies/mmL; and at least one record higher than 200 copies/mmL.

89 Other variables of interest included in the study were: demographical data (age, gender, origin); risk  
90 factor for HIV acquisition; clinical data (CD4 count, CDC classification, AIDS event in medical  
91 history, years of HIV/AIDS, comorbidities); ART-related data (type of ART, pill burden).

#### 92 *Statistical methods*

93 Package-refill have been calculated as the ratio between the number of packages of ART actually  
94 withdrawn in hospital pharmacy compared to the expected number of packages to be withdrawn (32  
95 packages for the period January 2018-August 2020). For patients with first diagnosis (or first  
96 referring to Cotugno hospital) during the study period, the number of packages to be withdrawn for  
97 the first year were considered as 6 in 2018 and 2019, and 4 packages in 2020.

98 Statistical differences among frequencies and percentages were calculated with the Student's Chi-  
99 squared test or t student test, as appropriate, using a significance level of 0.05. Factors associated to  
100 inadequate adherence were calculated by a univariate and multivariate logistic regression. Data  
101 were analysed using the free PSPP software.

102

## 103 **RESULTS**

104 A total of 1210 PLWH were randomly selected and included in the database. Among these patients,  
105 those stably in care were 1140, who represent the population included in the study. Demographical,  
106 behavioural and clinical characteristics of these PLWH are summarized in Table 1. The population  
107 is mainly constituted by males, median age is 46-years-old for both males and females. Patients of  
108 foreign origin were 20% of the whole population, foreign origin is more present among female  
109 patients (44% of females are not Italian, compared with 10% of males). The population has a long  
110 HIV/AIDS history, with median of 8 years of infections. At study time, level of CD4 shows a mean  
111 of 702 cell/mmL, but 48% of study population experienced at least one AIDS event in their life.  
112 Infective comorbidities are present in a limited extension.

113 Main study outcome is presented in Table 2. Subjects with inadequate adherence have an increased  
114 risk for viral failure, with OR 3.77 (IC95% 2.76-5.13) for having HIV-RNA 50-200 copies/mmL at  
115 least one time in study period, and OR 3.98 (IC95% 2.69-5.90) for having HIV-RNA > 200  
116 copies/mmL at least one time in study period.

117 At univariate logistic regression (Table 2), being not-Italian and injective drug users are risk factors  
118 for inadequate adherence (OR 4.1, IC95% 3.02-5.55 and OR 2.6, IC95% 1.72-3.93, respectively).  
119 Instead, HIV infection for more than 8 years (OR 0.62, IC95% 0.44-0.87), having more than 200

120 CD4 cells/mmL at last observation of study period (OR 0.31, IC95% 0.18-0.53), and taking ART  
121 based on Abacavir + Integrase Inhibitors (ABC+INI, OR 0.43, IC95% 0.20-0.95) are protective  
122 factors for inadequate adherence.

123 A multivariate analysis has been performed including gender, age, and all factors emerged as  
124 significant at univariate analysis. Among risk factors, the origin of the patient is confirmed  
125 (adjusted OR for having inadequate adherence in not-Italian 3.45, IC95% 2.23-5.35), while being  
126 HIV+ for more than 8 years is protective (adjusted OR 0.60, IC95% 0.41-0.88). Moreover, a  
127 protective role of female gender (adjusted OR 0.64, IC95% 0.43-0.96) emerges.

128

## 129 **DISCUSSION**

130 We explored the applicability and suitability of a package-refill-based measure for ART adherence  
131 among a population of PLWH. According to data emerged, this measure is feasible to be applied in  
132 clinical settings: it is easy and inexpensive to apply, because records are already available in  
133 patients' clinical records and in hospital pharmacy database. The package-refill measure is easy to  
134 calculate and may be routinely applied in clinical practice.

135 The reliability of this measure is confirmed by the outcomes of other variables associated to  
136 adherence: package-refill is able to predict the risk of viral failure. Indeed, prevalence of PLWH  
137 with HIV-RNA higher than 50 copies or higher than 200 copies is significantly associated to  
138 inadequate adherence measured by package-refill.

139 This measure has some limits, too. Indeed, package-refill does not reflect daily assumption, and it is  
140 not a direct measure of drug ingestion. The estimation over a long period (at least one year) is  
141 necessary to obtain accurate adherence measure. In our case, a 32-months-period has been applied  
142 in most patients.

143 The correlation between adherence and viral suppression is demonstrated in several studies, among  
144 which some conducted in Italy or involving Italy in a multi-country study [6-9].

145 On the other hand, adherence measurement is not the only determinant of viral failure. In our study  
146 population, 11% of patients has at least one HIV-RNA higher than 200 copies, despite an optimal  
147 adherence measured by package-refill. Other reasons, such as drug-to-drug interaction, and  
148 resistance to ongoing ART may explain this data.

149 Some factors are associated to inadequate adherence in our population. Foreign origin and being an  
150 IDU as risk factor for HIV are all associated to inadequate adherence. Foreign persons are often  
151 associated with poor retention in care and adherence, because of their mobility, and because of  
152 frequent health and social marginality [10-12]. Similarly, people who inject drugs have been  
153 already described among those group at higher risk for inadequate adherence and poor retention in

154 care [13,14]. The association of low adherence, measured by package-refill, with factors and  
155 populations well known to be associated with a poor adherence, confirm the reliability of this  
156 measure, again.

157 In our population, longer HIV/AIDS history is associated with better adherence. This finding, is in  
158 contrast with other recent finding in other study, where long-term adherence is generally poor [3].

159 A number of CD4 less or equal to 200 cells/mmL is significantly more present among PLWH with  
160 inadequate adherence. This last evidence is probably a consequence of adherence, and not a  
161 determining associated factor.

162 Of note, pill burden is not associated to adherence in our population. PLWH taking a single-tablet-  
163 regimen has the same adherence of those taking more than one pill. This finding is common to  
164 another Italian study where adherence and related-health status was not different among patients  
165 taking qd and bid therapies [8].

166 Some limitations are present in this study. It is a monocentric study, addressing the half only of  
167 entire HIV/AIDS population in care. Moreover, the suitability of the package-refill has been  
168 measures in comparison to an outcome, the HIV-RNA level, and not to other adherence measures.

169 The absence of a gold standard for adherence measure is the main reason for this choice.

170

## 171 **CONCLUSIONS**

172 Package-refill is a simple and suitable method for measuring adherence, especially is a long-time  
173 observation is possible. Factors associated with inadequate adherence in our population is similar to  
174 those already described in other adherence studies conducted in Italy.

175

## 176 **AUTHORS' CONTRIBUTIONS**

177 FMF conceived the study, analyzed data, and drafted the paper; NS collected the data, contributed  
178 to draft the paper and to data analysis; NP and NC contributed to data collection and analysis; OT,  
179 VI, VB, MAC, VR, MDL, MS, RV contributed to data collection; VS and VE coordinated the  
180 study. All authors gave their contribute for important intellectual contents and all authors saw and  
181 approved the final version of the manuscript.

182

## 183 **DATA AVAILABILITY STATEMENT**

184 The database of the study is available for review, if required

185

186 **FUNDING STATEMENT**

187 This study was supported by an unrestricted GILEAD Grant 20200320, N° 10055. Title of the  
188 grant: Evaluation of adherence to antiretroviral therapy by measuring the pill-refill and impact on  
189 the viro-immunological profile: retrospective data at the D. Cotugno Hospital, period 2017-2019.

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191 **CONFLICT OF INTEREST DISCLOSURE**

192 All authors declare to have not conflicts of interest about the contents of the study

193

194 **ETHICS APPROVAL STATEMENT**

195 Due to the observational nature of the study and the presentation of anonymized aggregated data  
196 only, no specific ethic approval has been obtained.

197

198 **PATIENT CONSENT STATEMENT**

199 For all patients, a generic authorization is obtained to use, exclusively anonymously and  
200 collectively, data deriving from clinical practice. This article presents overall data, completely  
201 anonymous, from which it is not possible in any way to trace the identity of the subjects involved.  
202 For this reason, we have not found it necessary to ask for specific consent.

203

204 **REFERENCES**

- 205 1. Bangsberg DR, Perry S, Charlebois ED, et al. Non-adherence to highly active antiretroviral  
206 therapy predicts progression to AIDS. *Aids* 2001;15(9):1181-1183.
- 207 2. Lima VD, Harrigan R, Bangsberg DR, et al. The combined effect of modern highly active  
208 antiretroviral therapy regimens and adherence on mortality over time. *J Acquir Immune*  
209 *Defic Syndr.* 2009;50(5):529-536.
- 210 3. Saberi P, Ming K, Legnitto D, Neilands TB, Gandhi M, Johnson MO. Feasibility and  
211 acceptability of novel methods to estimate antiretroviral adherence: A longitudinal study.  
212 *PLoS One.* 2019;14(1):e0210791.

- 213 4. Marcellin F, Spire B, Carrieri MP, Roux P. Assessing adherence to antiretroviral therapy in  
214 randomized HIV clinical trials: a review of currently used methods. *Expert Rev Anti-Infect.*  
215 2013;11(3):239-250.
- 216 5. Saberi P, Chakravarty D, Ming K, et al. Moving antiretroviral adherence assessments to the  
217 modern era: correlations among three novel measures of adherence. *AIDS Behav.* 2020  
218 Jan;24(1):284-290.
- 219 6. de Los Rios P, Okoli C, Punekar Y, et al. Prevalence, determinants, and impact of  
220 suboptimal adherence to HIV medication in 25 countries. *Prev Med.* 2020;139:106182.
- 221 7. Maggiolo F, Di Filippo E, Comi L, et al. Reduced adherence to antiretroviral therapy is  
222 associated with residual low-level viremia. *Pragmat Obs Res.* 2017 May 26;8:91-97.
- 223 8. Gianotti N, Galli L, Bocchiola B, et al. Number of daily pills, dosing schedule, self-reported  
224 adherence and health status in 2010: a large cross-sectional study of HIV-infected patients  
225 on antiretroviral therapy. *HIV Med* 2013;14(3):153-160.
- 226 9. Aloisi MS, Arici C, Balzano R, et al. Behavioral correlates of adherence to antiretroviral  
227 therapy. *J Acquir Immune Defic Syndr.* 2002;31 Suppl 3:S145-148.
- 228 10. Taylor BS, Reyes E, Levine EA, et al. Patterns of geographic mobility predict barriers to  
229 engagement in HIV care and antiretroviral treatment adherence. *AIDS Patient Care STDS.*  
230 2014;28(6):284-95.
- 231 11. Been SK, van de Vijver DA, Nieuwkerk PT, et al. Risk Factors for Non-Adherence to  
232 cART in Immigrants with HIV Living in the Netherlands: Results from the ROTterdam  
233 ADherence (ROAD) Project. *PLoS One.* 2016;11(10):e0162800.
- 234 12. Woolley I, Bialy C. Visiting friends and relatives may be a risk for non-adherence for HIV-  
235 positive travellers. *Int J STD AIDS.* 2012;23(11):833-834.
- 236 13. Davis A, McCrimmon T, Dasgupta A, et al. Individual, social, and structural factors  
237 affecting antiretroviral therapy adherence among HIV-positive people who inject drugs in  
238 Kazakhstan. *Int J Drug Policy.* 2018;62:43-50.
- 239 14. Chan PY, Joseph MA, Des Jarlais DC, Uusküla A. Perceived effectiveness of antiretroviral  
240 therapy, self-rated health and treatment adherence among HIV-positive people who inject  
241 drugs in Estonia. *Int J STD AIDS* 2018;29(1):13-22.
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243 **Table 1 - Demographical, behavioral and clinical characteristics of 1140 Persons Living With**  
 244 **HIV followed up at “D. Cotugno” hospital, Naples, Italy in 2018-August 2020**

245

Male gender, n(%)	802 (70,3)
Age (median (SD))	46 (11,2)
Origin, male, Italian, n(%)	722 (90)
Origin, female, Italian, n(%)	190 (56)
Risk Factor per HIV:	
Heterosexual contacts, n(%)	423 (37)
MSM, n(%)	347 (30)
IDU, n(%)	191 (17)
Other/Not known, n(%)	179 (16)
Years of HIV (on 854 subjects, median)	8
CDC classification (on 1074 subjects):	
A, n(%)	554 (52)
B, n(%)	191 (18)
C, n(%)	331 (31)
CD4 nadir (on 882 subjects, mean, SD):	364 ( $\pm$ 278)
CD4 last observation in study period (on 1093 subjects, mean, SD):	702 ( $\pm$ 345)
Presence of AIDS event in clinical history (on 1071 subjects, n, %)	519 (48)
Co-morbidities in clinical history:	
HBsAg+ (on 1097 subjects, n, %)	17 (2)
HCV-Ab+ (on 1102 subjects, n, %)	98 (9)
Syphilis (on 1047 subjects, n, %)	137 (13)
ART at beginning of Study Period (on 1081 subjects):	
TDF+INI, n(%)	248 (23)
TDF+NNRTI, n(%)	204 (19)
TDF+PI, n(%)	212 (20)
ABC+INI, n(%)	201 (19)
ABC+NNRTI, n(%)	8 (1)
ABC+PI, n(%)	41 (4)
2DR, n(%)	99 (9)
Other, n(%)	68 (6)

ART at end of Study Period (on 1079 subjects):	
TDF+INI, n(%)	343 (32)
TDF+NNRTI, n(%)	173 (16)
TDF+PI, n(%)	176 (16)
ABC+INI, n(%)	208 (19)
ABC+NNRTI, n(%)	3 (0)
ABC+PI, n(%)	15 (1)
2DR, n(%)	112 (10)
Other, n(%)	51 (5)
MSM: Men who have Sex with Men; IDU: Injective Drug Users; TDF: Tenofovir; ABC: Abacavir; INI: INtegrase Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; 2DR: 2 Drug Regimen	

246

247 **Table 2 - Factors associated to inadequate package-refill in January 2018-Agust 2020 among**  
248 **1140 PLWH at “D. Cotugno” hospital, Naples, Italy**

249

	Optimal or partial package refill; N (%)	Inadequate package refill; N (%)	OR (CI 95%)
N° of patients	818 (72)	322 (28)	
Gender:			
Male	630 (78)	172 (53)	1
Female	188 (22)	150 (47)	0.75 (0.56-1.0)
Age:			
Less than 40-year-old	213 (26)	126 (39)	1
More or equal to 40-year-old	605 (74)	196 (61)	1.01 (0.77-1.34)
Origin:			
Italian	712 (87)	200 (62)	1
Not-Italian	106 (13)	122 (38)	<b>4.1 (3.02-5.55)</b>
Risk Factor per HIV:			
Heterosexual contacts	275 (34)	148 (46)	1
MSM	287 (35)	60 (17)	1.01 (0.70-1.46)

IDU	144 (18)	51 (16)	<b>2.6 (1.72-3.93)</b>
Other/Not known	112 (14)	63 (20)	1.49 (0.96-2.34)
Years of HIV (on 854 subjects):			
8 years or less	336 (49)	110 (62)	1
More than 8 years	344 (51)	66 (38)	<b>0.62 (0.44-0.87)</b>
CD4 last observation in study period (on 1093 subjects):			
Less or equal to 200 cells/mmL	26 (3)	30 (9)	1
More than 200 cells/mmL	792 (97)	292 (91)	<b>0.31 (0.18-0.53)</b>
Viral load:			
Always $\leq$ 50 copies/mmL	499 (61)	140 (43)	1
At least one 51-200 copies/mmL	196 (24)	52 (16)	<b>3.77 (2.76-5.13)</b>
At least one >200 copies/mmL	123 (15)	130 (40)	<b>3.98 (2.69-5.90)</b>
Presence of AIDS event in clinical history (on 1071 subjects):			
No	390 (50)	162 (45)	1
Yes	387 (50)	132 (55)	0.82 (0.63-1.07)
ART at end of Study Period (on 1079 subjects):			
TDF+INI	271 (33)	72 (27)	1
TDF+NNRTI	128 (16)	45 (17)	0.81 (0.38-1.73)
TDF+PI	117 (14)	59 (22)	0.61 (0.27-1.35)
ABC+INI	158 (19)	50 (19)	<b>0.43 (0.2-0.95)</b>
ABC+NNRTI	1 (0)	2 (1)	0.66 (0.30-1.46)
ABC+PI	13 (2)	2 (1)	0.11 (0.01-1.31)
2DR	88 (11)	24 (9)	1.79 (0.27-7.28)
Other	42 (5)	9 (3)	0.79 (0.34-1.84)
Pill burden (on 1079 subjects):			
Single-Tablet Regimen	550 (67)	190 (72)	1
More than 1 pill	268 (33)	73 (28)	0.79 (0.58-1.07)