Posaconazole for the Prevention of COVID-19 associated Pulmonary Aspergillosis in Critically-Ill Patients: A European Multicenter Case-Control Study (POSACOVID Trial)

Statistical Analysis Report

Version 1.0 (24th September 2023)

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POSACOVID study

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Statistical methods

The primary study analysis was aimed at assessing the impact of posaconazole prophylaxis on the risk of coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis (CAPA) in critically ill patients with COVID-19 in intensive care unit (ICU). To this aim, incidence rates (IR) of CAPA in the different centers were first calculated with standard methods, and compared between Graz (all patients undergoing posaconazole prophylaxis) and the other two centers (no patients undergoing posaconazole prophylaxis) for descriptive purposes and unadjusted for confounding factors, by means of the mid-P exact test [1]. Then, to minimize the impact of difference in the case-mix of patients between centers on the risk of CAPA, patients from Graz (i.e., undergoing posaconazole prophylaxis) were matched as a case with controls Genoa and controls from Rennes, according to demographic and relevant clinical variables. Before matching, demographic and clinical characteristics of patients in the three different centers were descriptively compared with the standardized difference (SD) of means and the Wilcoxon test for continuous variables, and with the SD of proportions and the chi-squared test for categorical variables. The SD of means/proportions was also reported for the comparison between cases (i.e., patients from Graz) and controls (i.e., patients from Rennes and Genoa).

Matching procedure

1:1 matching

Each patient from Graz (i.e., undergoing posaconazole prophylaxis) was matched as a case with one control from Genoa and one control from Rennes (i.e., not undergoing posaconazole prophylaxis), employing two separate 1:1 propensity score (PS) matching procedures (one for identifying the control from Genoa and one for identifying the control from Rennes) [2,3]. The following variables were considered for matching cases with controls: (i) age; (ii) sex; (iii) treatment with tocilizumab; (iv) time at risk. We did not match for systemic steroid treatment since all patients from Graz received systemic steroids, thus we consider for possible match only those controls receiving systemic steroids in Genoa and Rennes (i.e., the majority of patients in the two centers). Time at risk was defined as follows: (i) for cases: days on posaconazole prophylaxis plus, if present, days in intensive care unit before posaconazole prophylaxis initiation; (ii) for controls: days in intensive care unit. In order to guarantee equal time at risk in cases and respective controls, we started by matching the case with the longest time at risk (53 days) to possible controls with time at risk equal or longer than 53 days. Then, we selected the case with the second longest time at risk and possible remaining controls with equal or longer time at risk. The procedure was repeated until all cases were matched to one control from Genoa and one control from Rennes. Eventually, for the study analyses, we considered only the period of time at risk in controls (starting from intensive care unit admission) that was equal to the time at risk in the respective case (in order to have an exactly equal time at risk in cases and their respective controls).

1:1:1 matching (for sensitivity analysis)

The 1:1 PS matching described above is based on minimizing the distance between a case and a control. Therefore, for a given case from Graz, the two 1:1 PS matching procedures separately selected a control from Rennes and a control from Genoa. However,

this triplet of individuals may be not the one that minimizes the area or perimeter of the triangle formed by the three subjects [4]. Therefore, as sensitivity analysis, we conducted a logistic regression on all patients with observation time greater than or equal to the longest time at risk for the cases (thus, one case and all potential controls with equal or longer time at risk) to find for each subject the probability of belonging to the center of Graz (p1), the probability of belonging to the centre of Rennes (p2) and the probability of belonging to the centre of Genoa (p3). These probabilities sum up to 1, so we considered only the first two probabilities to figure each subject in a cartesian plane with x-axis p1 and y-axis p2. Then, we calculated the perimeter of the triangle resulting from each combination of the case with two controls (one from Genoa and one from Rennes) and chose the pair of controls for which that perimeter was the smallest. Subsequently, we repeated the procedure for the case with the second longest time at risk and all remaining potential controls with equal or longer time at risk. The procedure was then repeated until all cases were matched to one control from Genoa and one control from Rennes (1:1:1 matching)

Assessment of impact of posaconazole prophylaxis on the risk of CAPA after matching

After 1:1 matching, the risk of CAPA was compared between Graz and the other centers by means of multivariable logistic regression. Besides center, other variables included in the multivariable logistic regression model were: (i) European Organization for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) risk factor present at ICU admission; (*ii*) presence of extracorporeal membrane oxygenation (ECMO). A sensitivity analysis including the same variables was also conducted after 1:1:1 matching.

Secondary analyses

To assess the prognostic impact of CAPA, 90-day survival in patients with and without CAPA after 1:1 matching was presented graphically using Kaplan-Meier curves, and compared with the log-rank test. To partly avoid immortal time bias, Kaplan-Meier curves were built as landmark analyses with different time of origin (15, 30, and 45 days after ICU admission) including patients still on follow-up at the selected time of origin, and excluding patients developing CAPA after the selected time of origin. It should be acknowledged that residual immortal time bias was present in patients from Genoa and Rennes in this secondary analysis, owing to their matching for time at risk (for the development of CAPA) in the primary study analysis (i.e., patients from Genoa and Rennes always remained alive for at least the time at risk of their respective case from Graz in the primary analysis). A sensitivity survival analysis was also conducted after 1:1:1 matching.

References

1. Martin DO, Austin H: **Exact estimates for a rate ratio**. *Epidemiology* 1996, **7**(1):29-33.
2. Rosenbaum PR, Rubin DB. **The central role of the propensity score in observational studies for causal effects.** Biometrika 1983; 70: 41–55. https://doi.org/10.1093/biomet/70.1.41
3. Austin PC. **An introduction to propensity score methods for reducing the effects of confounding in observational studies.** Multivariate Behav Res 2011; 46: 399–424. https://doi.org/10.1080/00273171.2011.568786
4. Rassen JA, Shelat AA, Franklin JM, Glynn RJ, Solomon DH, Schneeweiss S. **Matching by propensity score in cohort studies with three treatment groups.** Epidemiology. 2013 May;24(3):401-9. doi: 10.1097/EDE.0b013e318289dedf. PMID: 23532053.

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Table 1. Demographic and clinical characteristics of patients before matching

Variables*	**Graz (n = 83)**	**Rennes (n = 192)**	**Genoa (n = 239)**	***P***	***SD (Graz vs. Rennes)***	***SD (Graz vs. Genoa)***	***SD (cases vs. controls)***
Posaconazole prophylaxis	83 (100)	0 (0)	0 (0)	**<0.001**	Inf.	Inf.	Inf.
Age at ICU admission in years, median (IQR)	65 (58-71)	64 (55-71)	64 (56-71)	0.640	0.122	0.072	0.095
Male sex	56 (68)	125 (65)	178 (75)	0.095	0.064	-0.156	-0.043
Treatment with tocilizumab	2 (2)	6 (3)	56 (23)	**<0.001**	-0.064	**-0.670**	**-0.454**
Systemic steroid treatment	83 (100)	169 (88)	187 (78)	**<0.001**	**0.522**	**0.751**	**0.640**
Length of ICU stay in days, median (IQR)	18 (13-33)	14 (10-27)	21 (12-42)	**<0.001**	**0.256**	-0.164	-0.008

ICU, intensive care unit; IQR, interquartile range; SD, standardized difference in means or proportions divided by standard error; imbalance defined as absolute value greater than 0.20 (small effect size)
* Expressed as n (%), unless otherwise indicated

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Table 2. Incidence rate and incidence rate ratio of CAPA before matching

Center	IR	
Graz (posaconazole prophylaxis)	1.69 CAPA/1000 patient-days in ICU	
Rennes (no posaconazole prophylaxis)	4.50 CAPA/1000 patient-days in ICU	
Genoa (no posaconazole prophylaxis)	3.01 CAPA/1000 patient-days in ICU	
Center	**IRR (95% CI)***	***P****
No prophylaxis (Rennes and Genoa) vs. prophylaxis (Graz, reference)	2.38 (0.87-9.08)	0.0720

CAPA, coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis; CI, confidence interval; ICU, intensive care unit; IR, incidence rate, IRR, incidence rate ratio. Calculation of IR were as follows:
IR_{Graz} = (4 CAPA/2368 patient-days in ICU) * 1000 = 1.69 CAPA/1000 patient-days in ICU
IR_{Rennes} = (38 CAPA/8437 patient-days in ICU) * 1000 = 4.50 CAPA/1000 patient-days in ICU
IR_{Genoa} = (12 CAPA/3989 patient-days in ICU) * 1000 = 3.01 CAPA/1000 patient-days in ICU
* The exact Poisson method was employed for calculating 95% CI. The P-value is from exact mid-P test.

Overall, 4, 38, and 12 cases of CAPA were registered in Graz, Rennes, and Genoa, respectively. The distribution of proven, probable, and possible CAPA in the different centers was as follows: Graz (n = 0 proven, n = 3 probable, n = 1 possible); Rennes (n = 0 proven, n = 19 probable, n = 19 possible); Genoa (n = 0 proven, n = 12 probable, n = 0 possible).

Table 3. Demographic and clinical characteristics of patients after 1:1 matching

Variables*	**Graz (n = 83)**	**Rennes (n = 83)**	**Genoa (n = 83)**	***P***	***SD (Graz vs. Rennes)***	***SD (Graz vs. Genoa)***
Posaconazole prophylaxis	83 (100)	0 (0)	0 (0)	**<0.001**	Inf.	Inf.
Age at ICU admission in years, median (IQR)**	65 (58-71)	65 (57-72)	66 (61-71)	0.949	0.016	-0.122
Male sex**	56 (68)	55 (66)	54 (65)	0.946	0.043	0.064
EORTC/MSGERC risk factor present at ICU admission***	8 (10)	5 (6)	5 (6)	0.591	0.148	0.148
ECMO	13 (16)	3 (4)	2 (2)	**0.001**	**0.408**	**0.504**
Treatment with tocilizumab**	2 (2)	3 (4)	1 (1)	0.599	-0.117	0.082
Systemic steroid treatment	83 (100)	83 (100)	83 (100)	-	0	0
Time at risk in days, median (IQR)**	15 (10-23)	15 (10-23)	15 (10-23)	-	0	0

ECMO, extracorporeal membrane oxygenation; EORCT, European Organization for Research and Treatment of Cancer; ICU, intensive care unit; IQR, interquartile range; MSGERC, Mycoses Study Group Education and Research Consortium; SD, standardized difference in means or proportions divided by standard error; imbalance defined as absolute value greater than 0.20 (small effect size)

* Expressed as n (%), unless otherwise indicated

** Matching variables (for details and definition of time at risk, see methods)

*** Presence of missing values (0/83 for Graz, 1/83 for Rennes, 0/83 for Genoa)

Table 4 (sensitivity analysis). Demographic and clinical characteristics of patients after 1:1:1 matching

Variables*	**Graz (n = 83)**	**Rennes (n = 83)**	**Genoa (n = 83)**	***P***	***SD (cases vs. controls)***
Posaconazole prophylaxis	83 (100)	0 (0)	0 (0)	**<0.001**	Inf.
Age at ICU admission in years, median (IQR)**	65 (58-71)	65 (58-72)	65 (58-71)	0.988	-0.041
Male sex**	56 (68)	59 (71)	53 (64)	0.610	0
EORTC/MSGERC risk factor present at ICU admission***	8 (10)	4 (5)	7 (8)	0.490	0.108
ECMO	13 (16)	4 (5)	2 (2)	**0.003**	**0.408**
Treatment with tocilizumab**	2 (2)	2 (2)	1 (1)	0.815	0.042
Systemic steroid treatment	83 (100)	83 (100)	83 (100)	-	0
Time at risk in days, median (IQR)**	15 (10-23)	15 (10-23)	15 (10-23)	-	0

ECMO, extracorporeal membrane oxygenation; EORCT, European Organization for Research and Treatment of Cancer; ICU, intensive care unit; IQR, interquartile range; MSGERC, Mycoses Study Group Education and Research Consortium; SD, standardized difference in means or proportions divided by standard error; imbalance defined as absolute value greater than 0.20 (small effect size)
* Expressed as n (%), unless otherwise indicated
** Matching variables (for details and definition of time at risk, see methods)
*** Presence of missing values (0/83 for Graz, 1/83 for Rennes, 0/83 for Genoa)

Table 5. Multivariable logistic regression of factors associated with development of CAPA after 1:1 matching

Variables	OR (95% CI)	*P*
EORTC/MSGERC risk factor present at ICU admission	4.35 (1.15-16.49)	**0.031**
ECMO	1.85 (0.34-9.99)	0.475
Center		**0.007**
Rennes (vs. Graz as reference)	6.07 (1.76-20.91)	**0.004**
Genoa (vs. Graz as reference)	0.59 (0.10-3.53)	0.566

CI, confidence interval; CAPA, coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis; ECMO, extracorporeal membrane oxygenation; EORCT, European Organization for Research and Treatment of Cancer; ICU, intensive care unit; IQR, interquartile range; MSGERC, Mycoses Study Group Education and Research Consortium; OR, odds ratio.
Overall, 4, 17, and 2 cases of CAPA were registered in Graz, Rennes, and Genoa, respectively, after 1:1 matching. The distribution of proven, probable, and possible CAPA in the different centers was as follows: Graz (n = 0 proven, n = 3 probable, n = 1 possible); Rennes (n = 0 proven, n = 10 probable, n = 7 possible); Genoa (n = 0 proven, n = 2 probable, n = 0 possible).

Table 6 (sensitivity analysis). Multivariable logistic regression of factors associated with development of CAPA after 1:1:1 matching

Variables	OR (95% CI)	*P*
EORTC/MSGERC risk factor present at ICU admission	2.84 (0.70-11.63)	0.146
ECMO	1.48 (0.29-7.50)	0.639
Center		**0.002**
Rennes (vs. Graz as reference)	5.10 (1.54-16.90)	**0.008**
Genoa (vs. Graz as reference)	0.80 (0.17-3.80)	0.776

CI, confidence interval; CAPA, coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis; ECMO, extracorporeal membrane oxygenation; EORCT, European Organization for Research and Treatment of Cancer; ICU, intensive care unit; IQR, interquartile range; MSGERC, Mycoses Study Group Education and Research Consortium; OR, odds ratio.
Overall, 4, 16, and 3 cases of CAPA were registered in Graz, Rennes, and Genoa, respectively, after 1:1:1 matching. The distribution of proven, probable, and possible CAPA in the different centers was as follows: Graz (n = 0 proven, n = 3 probable, n = 1 possible); Rennes (n = 0 proven, n = 9 probable, n = 7 possible); Genoa (n = 0 proven, n = 3 probable, n = 0 possible).

Figure 1. Flow-chart of the patient selection process

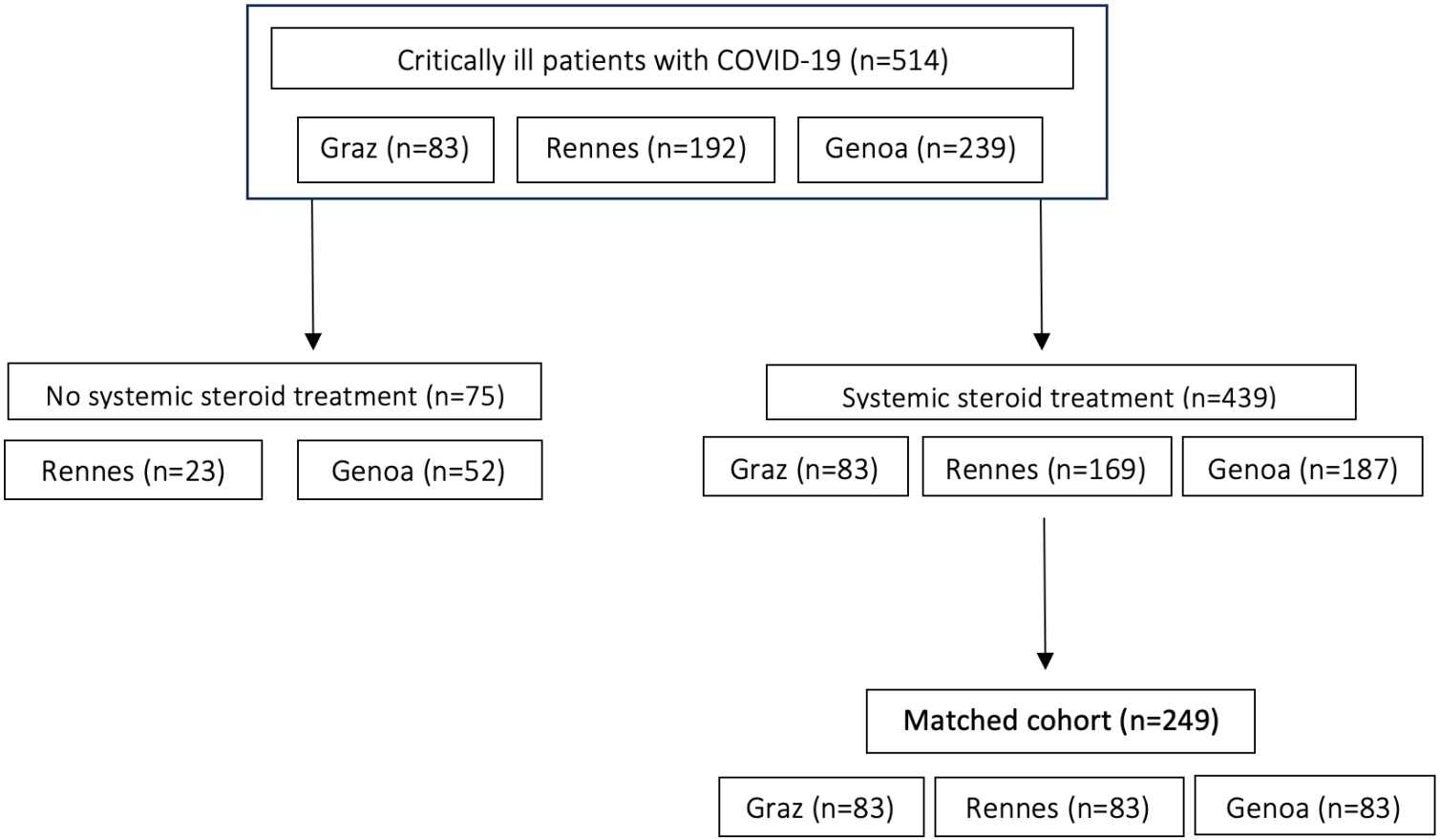

Figure 1 legend. Matched cohorts after 1:1 and 1:1:1 matching were both composed of 249 patients, although some different controls could have been selected (for details, see matching methods). COVID-19, coronavirus disease 2019.

Figure 2. Landmark analysis of 90-day survival in patients with and without CAPA after 1:1 matching

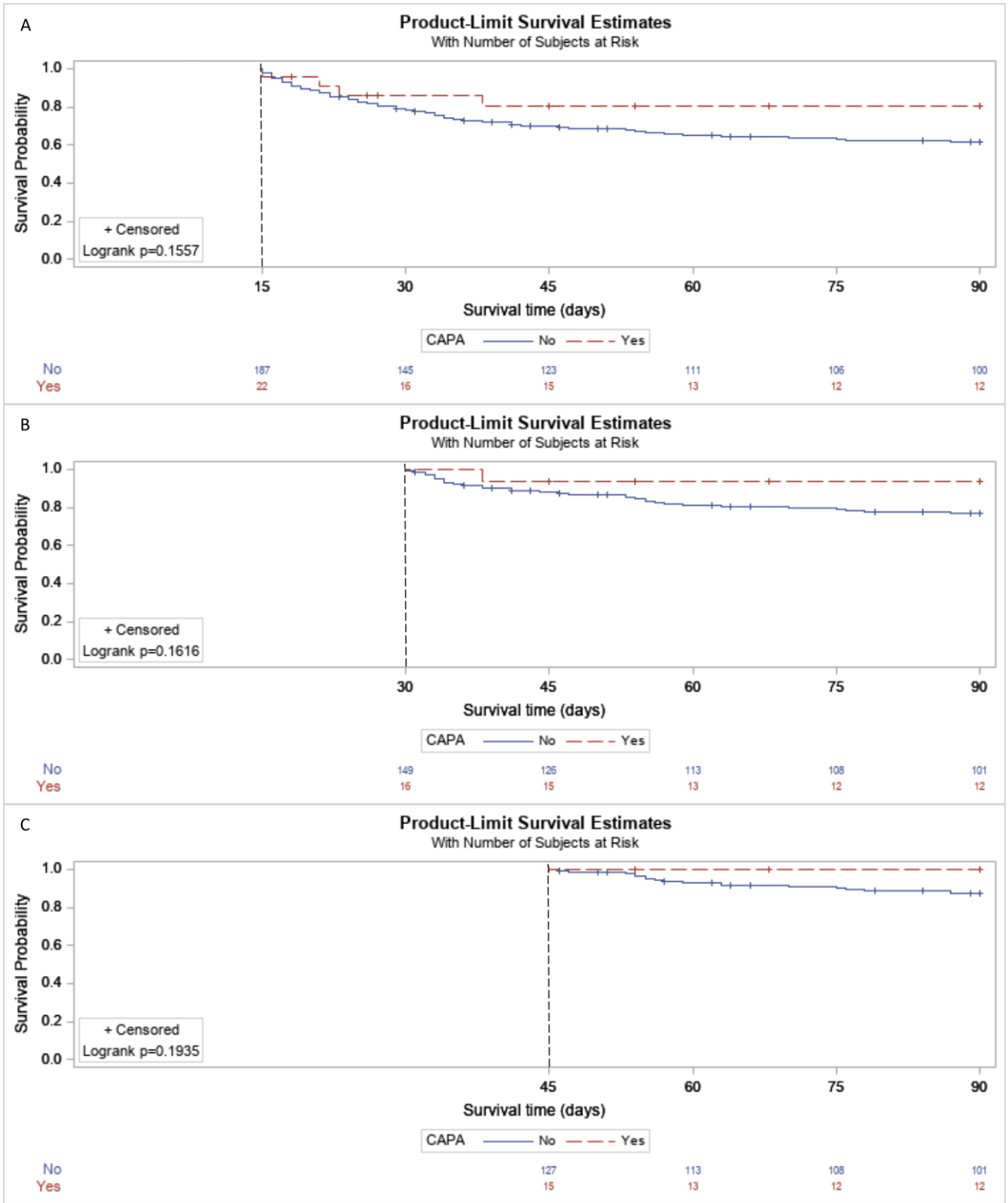

Figure 2 legend. Landmark survival analysis after 1:1 matching, with different time of origin (15, 30, and 45 days in ICU for panel A, B, and C, respectively). CAPA, coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis; ICU, intensive care unit.

Figure 3 (sensitivity analysis). Landmark analysis of 90-day survival in patients with and without CAPA after 1:1:1 matching

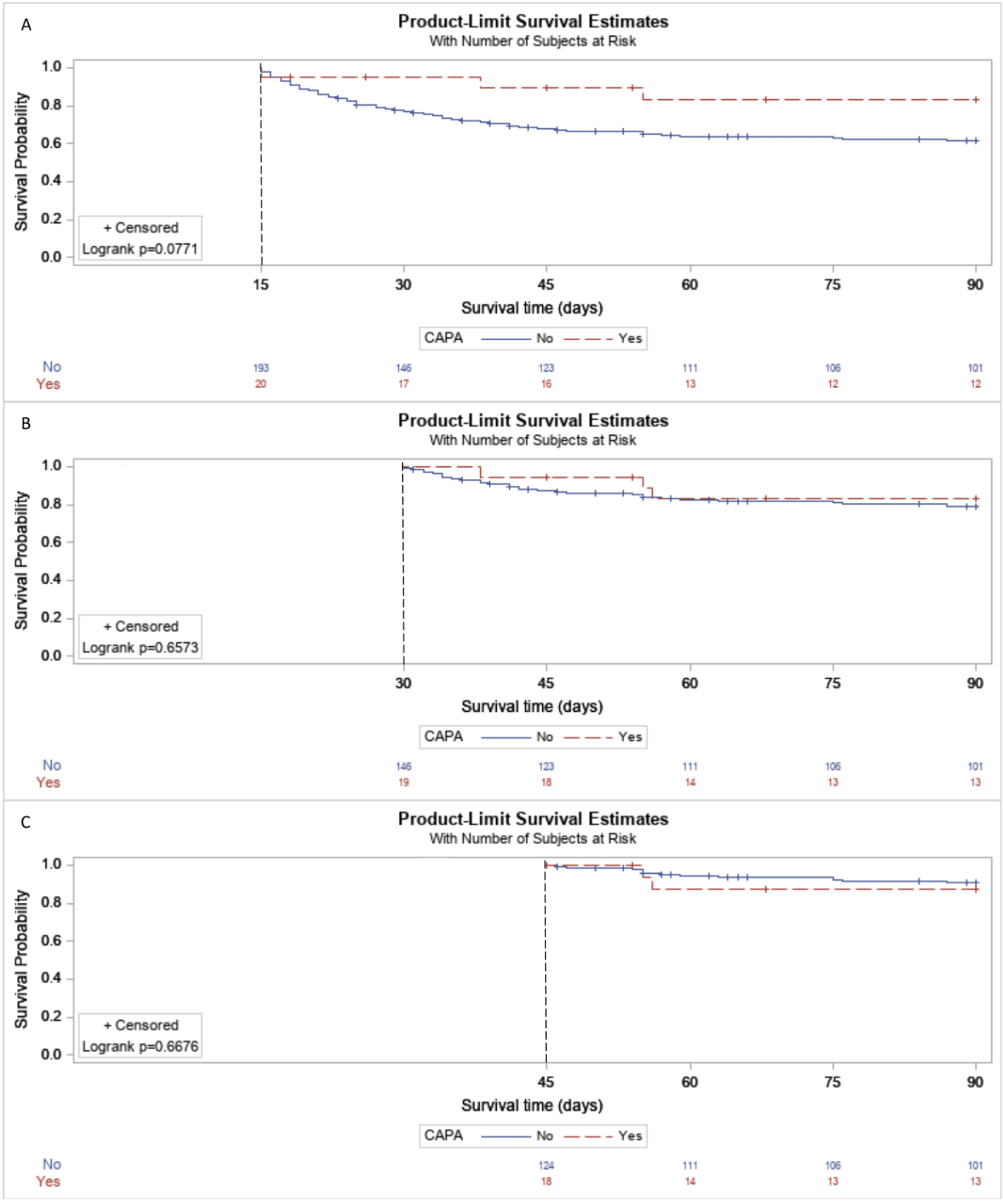

Figure 3 legend. Landmark survival analysis after 1:1:1 matching, with different time of origin (15, 30, and 45 days in ICU for panel A, B, and C, respectively). CAPA, coronavirus disease 2019 (COVID-19)-associated pulmonary aspergillosis; ICU, intensive care unit.