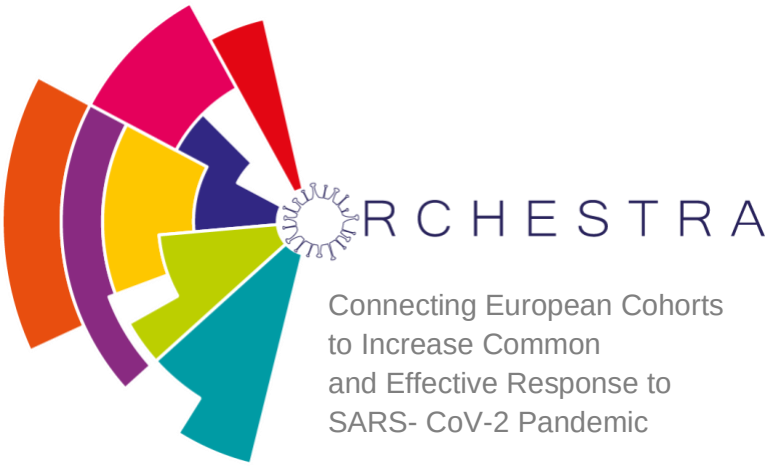

DELIVERABLE

WP5_D5.9

Final risk analysis of breakthrough infection

Ludwig-Maximilians-Universität Munich (LMU)

ORCHESTRA has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016167

Project Classification

Project Acronym:	ORCHESTRA
Project Title:	Connecting European Cohorts to Increase Common and Effective Response to SARS- CoV-2 Pandemic
Coordinator:	UNIVR
Grant Agreement Number:	101016167
Funding Scheme:	Horizon 2020
Start:	1st December 2020
Duration:	36 months
Website:	www.orchestra-cohort.eu
Email:	info@orchestra.eu

Document Classification

WP No:	WP5
Deliverable No:	D5.9
Title:	Final Risk Analysis of Breakthrough Infection
Lead Beneficiary:	LMU
Other Involved Beneficiaries:	All WP5 members
Nature:	Report
Dissemination Level:	Public
Due Delivery Date:	M23
Submission Date:	M25
Justification of delay:	Ongoing analyses of relevant data
Status:	Completed
Version:	1.0
Author(s):	Janke C, Porru S, Spiteri G, Reinkemeyer C, Castelletti N, Hoelscher M on behalf of the WP5 Study Group

History of Changes

Version	Date	Created/Modified by
0.1	30/12/2022	Janke C, Porru S, Spiteri G, Reinkemeyer C, Castelletti N, Hoelscher M
0.2	05/01/2023	Canziani LM
1.0	16/01/2023	Janke C, Porru S, Spiteri G, Reinkemeyer C, Castelletti N, Hoelscher M on behalf of the WP5 Study Group

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Executive summary

Breakthrough infections (BI) were analysed for all contributing Orchestra WP5 cohorts. We report on respective results for particular cohorts (here the Munich KoCoImpf cohort) as well as findings of the joint WP5 analysis done by the Section of Occupational Medicine in the Department of Diagnostics and Public Health at the University of Verona.

In either or both analyses, significant positive correlations with the occurrence of BI could be shown for younger participants and a household size of more than 4 persons. Furthermore, a higher baseline SARS-CoV-2 anti-Spike-RBD-antibody level was identified as a protective factor for BI.

The gender distribution among breakthrough and non-breakthrough infections differed among the participating study centres. As far as the different job titles are concerned, nurses had the highest incidence of BI. On the other hand, a previous infection with SARS-CoV-2 and a heterologous vaccination regime seem to be negatively correlated with the occurrence of breakthrough infections. However, non-occupational, social, and random exposure patterns (not covered by the questionnaires and the models) might have played a greater role for SARS-CoV-2 transmissions in the identified cases than the identified determinants.

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Core content

1. The Munich Cohort, Study Profile and Results

1.1 KoCoImpf Study: Prospective COVID-19 Post-Immunization Cohort in Munich

The aim of this study is to understand the serological short-, medium- and long-term immune response as well as the patterns of BI in vaccinated individuals with focus on healthcare workers in the greater Munich area. Detailed objectives are

- the determination of the baseline immune status and the prevalence of individuals (silently and symptomatically) infected before vaccination
- the follow-up of SARS-CoV-2-antibody dynamics over time and in relation to the vaccine they received, pre-existing immunity, and other covariates
- the determination of relative risks of post-immunization infections with SARS-CoV-2 in relation to antibody titres at the time of infection and other covariates.

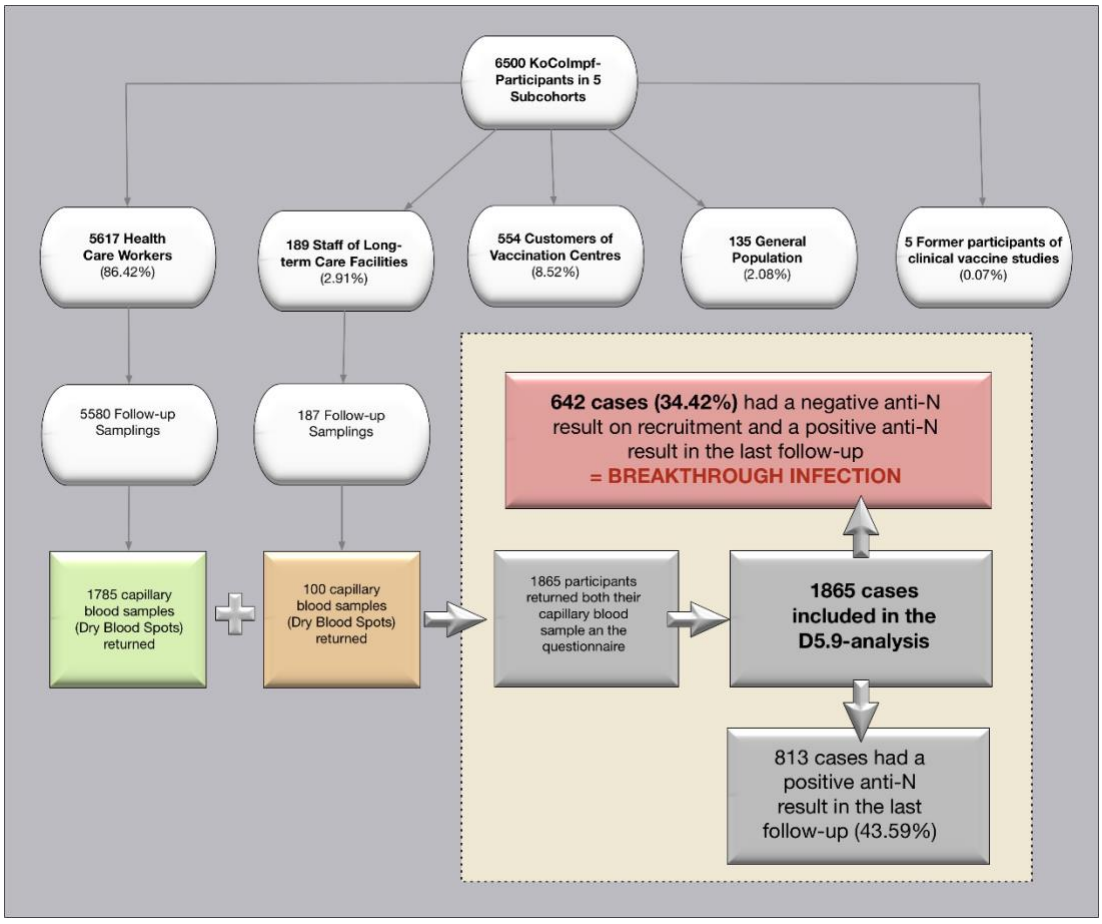

Figure 1. *The KoCoImpf-cohort and its contribution to D5.9*

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1.2 Methods Brief

Capillary blood samples were analysed for SARS-CoV-2 antibodies using the Elecsys® Anti-N SARS-CoV-2 (Roche) test. Antibody follow-ups were conducted from May to August 2022 based on participants self-sampled capillary blood (Dry Blood Spot). Anti-Spike-RBD-antibodies were additionally measured to quantify the overall serological response. Newly anti-Nucleocapsid positive cases with anti-Spike-RBD-antibodies in the initial round were considered breakthrough infections. Details concerning vaccinations were based on questionnaire data. The models were estimated using the R package mgcv (Gam) and considered several confounder variables. Since the recruitment took place over seven months in different hospitals during the course of the pandemic, it is crucial to take different waves of the pandemic into account, correcting for the infection risk. Therefore, the cumulative number of COVID-19 cases in Munich (Germany) was included from the beginning of the pandemic, as of 2020-01-27 to 2022-03-14 on the basis of a weekly rolling window. Since anti-N and anti-S antibodies need at least 2 weeks to develop, a time lag of 2 weeks was applied to the cumulative number of COVID-19 cases. Other independent variables comprised age, gender, contact with patients, smoking status, household size, intake of immunosuppressive drugs, intake of other drugs and vaccination status.

1.3 Incidence of breakthrough infections

In the last follow-up (concluded August 2022) 813 of 1 865 participant (43.6%) were found to have anti-Nucleocapsid-antibodies representing a natural SARS-CoV-2 infection in the past. In the period between recruitment (cut-off date December 16th, 2021) and the last follow-up, 642 out of these 1865 study participants (who took part in both the baseline investigation and the last follow up, and for whom both questionnaire and serological data were available for the relevant time points) had an anti-Nucleocapsid seroconversion during this period. This number of breakthrough infections corresponds to an incidence of 34.4%. In other words, the assessment of breakthrough infections in KoCoImpf relies mainly on the detection of anti-Nucleocapsid-seroconversions during short-term follow-up periods (as opposed to self-reported COVID-19 or reported positive PCR results) thereby covering both symptomatic and asymptomatic BI. The substantial contribution of asymptomatic and oligosymptomatic infections in the transmission of SARS-CoV-2 infections has been shown in many studies.

1.4 Risk analysis of breakthrough infections

Based to the multivariate model described above, a significant correlation ($p < 0.01$) with the occurrence of breakthrough infections could be shown for younger participants and a household size of more than 4 persons. A negative correlation was seen for a higher baseline SARS-CoV-2 anti-Spike-RBD-antibody titre and (rather unexpectedly) for those participants who had no second vaccination on recruitment. Theoretically, this could be explained by infections that

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occurred before the second vaccination. However, in our cohort, this was only the case in few participants. Possibly, these participants spent less time under risk with waning antibodies during the observation period since their second vaccination was due shortly after recruitment. This assumption will inform the refinement of our model. Furthermore, the influence of household sizes likely represents the importance of social contact patterns on the transmission dynamics. The same might be true for the age distribution of SARS-CoV-2 infections in vaccinated health care workers with younger persons. However, based on the given model, no other significant factors of risk or prediction of breakthrough infections could be identified with the "household size of one" and "female sex" showing merely insignificant trends towards a "protective" association. In summary it may be said that non-occupational, social, and random exposure patterns (not covered by the questionnaires and the model) might have played a greater role for SARS-CoV-2-transmissions in the identified cases than pre-infection antibody levels or the non-serological determinants collected in the KoCoImpf questionnaires.

Table 1. *Variables that showed a significant correlation with the occurrence of breakthrough infections ($p < 0.01$) or a respective trend ($p < 0.1$)*

Variable	Odds Ratio	p-value
year of birth	1.032	<0.0001
household size > 4 persons	1.941	0.0052
household size 1 person	0.779	0.0605
anti-S1-RBD IgG antibody titre	0.999	0.0015
female sex	0.811	0.0873

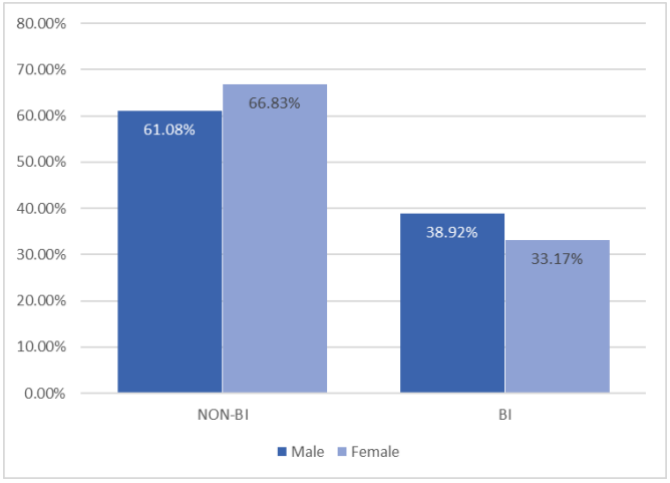

Figure 2.
The gender distribution of breakthrough infections (BI) in the Munich KoCoImpf cohort

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2. The Joint BI Analysis of all Work Package 5 Cohorts

This chapter briefly reflects on the joint analysis of all participating WP5-cohorts. The analysis was planned, coordinated, and executed by the Section of Occupational Medicine in the Department of Diagnostics and Public Health at the University of Verona. A respective publication is in preparation.

Risk analysis on BI after booster dose involved 14 European centres. Data were collected from the following healthcare settings: Germany (Munich), Italy (Bari, Bologna, Brescia, Modena, Padova, Perugia, Torino, Trieste, and Verona), Romania, Slovakia, and Spain (Barcelona and Oviedo). The analysis was conducted on Health Workers (HW) that had received the 3rd dose (booster dose) from September 2021 to May 2022. Data on 80 463 HW were available. Of these, 63 565 boosted HW were included in the analysis. The proportion of HW who received the booster dose was higher in women, in HWs younger than 50 years, in nurses, and in subjects with comorbidities. Data on previous SARS-CoV-2 infections and socio-demographic characteristics are reported in Table 2.

Table 2. *Data socio-demographic characteristics of HW*

Center	HW Full sample	Sex (%)		Job Title (%)					Age Classes				
		Male	Female	Physician	Nurse	Other HW	Technician	Admin-istrative	<30	30–39	40–49	50-59	≥60
Turin	10 748	2799 (28.6)	6975 (71.4)	1862 (21.6)	3142 (36.5)	1158 (13.4)	1501 (17.4)	957 (11.1)	1651 (16.8)	1529 (15.6)	1941 (19.8)	3226 (32.9)	1469 (15.0)
Brescia	8 903	2255 (27.5)	5957 (72.5)	2432 (29.6)	2669 (32.5)	1537 (18.7)	664 (8.1)	910 (11.1)	871 (10.6)	1964 (23.9)	1703 (20.7)	2631 (32.0)	1043 (12.7)
Verona	6 377	1641 (29.6)	3894 (70.4)	1764 (31.9)	1994 (36.0)	840 (15.2)	514 (9.3)	423 (7.6)	950 (17.2)	1237 (22.3)	1183 (21.4)	1703 (30.8)	462 (8.3)
Padua	8 511	1976 (30.2)	4576 (69.8)	2065 (31.5)	2453 (37.5)	950 (14.5)	591 (9.0)	491 (7.5)	891 (13.6)	1514 (23.1)	1203 (18.4)	2187 (33.4)	757 (11.5)
Trieste	7 959	1939 (31.7)	4181 (68.3)	1328 (21.7)	2202 (35.9)	1253 (20.5)	886 (14.5)	451 (7.4)	587 (9.6)	1175 (19.2)	1355 (22.1)	2025 (33.1)	978 (16.0)
Modena	5 267	1466 (29.3)	3542 (70.7)	1418 (31.1)	1767 (38.7)	952 (20.9)	160 (3.5)	267 (5.8)	705 (14.1)	1387 (27.7)	1079 (21.5)	1268 (25.3)	569 (11.4)
Bologna	7 597	2246 (31.8)	4819 (68.2)	2342 (33.2)	2428 (34.4)	1295 (18.4)	641 (9.1)	345 (4.9)	890 (12.6)	2069 (29.3)	1479 (20.9)	1792 (25.4)	835 (11.8)
Perugia	3 805	760 (34.7)	1432 (65.3)	612 (28.4)	834 (38.6)	305 (14.1)	275 (12.7)	133 (6.2)	37 (1.7)	364 (16.6)	436 (19.9)	725 (33.1)	630 (28.7)
Bari	6 196	2218 (39.3)	3423 (60.7)	2728 (48.4)	1582 (28.0)	794 (14.1)	198 (3.5)	339 (6.0)	720 (12.8)	1464 (25.9)	953 (16.9)	1478 (26.2)	1026 (18.2)
Oviedo	8 226	549 (21.4)	2013 (78.6)	NA	NA	NA	NA	NA	82 (3.2)	430 (16.8)	820 (32.0)	741 (28.9)	490 (19.1)
Barcelona	848	125 (24.4)	388 (75.6)	166 (34.9)	162 (34.0)	78 (16.4)	0 (0.00)	70 (14.7)	54 (10.5)	74 (14.4)	168 (32.8)	122 (23.8)	95 (18.5)
Munich	3 282	856 (26.3)	2398 (73.7)	NA	NA	NA	NA	NA	669 (20.5)	876 (26.9)	640 (19.6)	700 (21.5)	374 (11.5)
Slovakia	1 072	414 (82.8)	86 (17.2)	75 (15.0)	164 (32.9)	171 (34.3)	37 (7.4)	52 (10.4)	56 (11.2)	60 (12.0)	139 (27.8)	144 (28.8)	101 (20.2)
Romania	1 672	114 (19.4)	475 (80.6)	421 (71.5)	70 (11.9)	11 (1.9)	67 (11.4)	20 (3.4)	28 (4.7)	56 (9.5)	156 (26.5)	216 (36.7)	133 (22.6)
Total	**80 463**	**19 358 (30.5)**	**44 159 (69.5)**	**17 213 (30.7)**	**19 467 (34.7)**	**9 344 (16.7)**	**5 534 (9.9)**	**4 465 (8.0)**	**8 191 (12.9)**	**14 199 (22.3)**	**13 255 (20.9)**	**18 958 (29.8)**	**8 962 (14.1)**

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Table 3. Data on previous SARS-CoV-2 infections of HW

Center	HW Full sample	HW vaccinated with 3 doses (%)	BI after 3rd dose (%)
Turin	10 748	9 816 (91.3)	1 712 (17.4)
Brescia	8 903	8 212 (92.2)	1 691 (20.6)
Verona	6 377	5 535 (86.8)	1 721 (31.1)
Padua	8 511	6 552 (77.0)	1 943 (29.4)
Trieste	7 959	6 120 (76.9)	1 761 (28.8)
Modena	5 267	5 008 (95.1)	695 (13.9)
Bologna	7 597	7 065 (93.0)	1 358 (19.2)
Perugia	3 805	2 192 (57.6)	317 (14.45)
Bari	6 196	5 641 (91.0)	862 (15.3)
Oviedo	8 226	2 563 (31.2)	599 (23.3)
Barcelona	848	513 (60.5)	71 (13.8)
Munich	3 282	3 259 (99.3)	214 (6.6)
Slovakia	1 072	500 (46.6)	145 (29.0)
Romania	1 672	589 (35.2)	4 (0.7)
Total	**80 463**	**63 565 (79.0)**	**13,093 (20.6)**

As regards type of vaccine, most HW (n=50 217, 86.55%) had received BNT162b2 and 11.78% (n=6 834) different types of vaccine (heterologous vaccine). A negligible proportion received other types of vaccine. Over 13 000 HW had BI, yielding a cumulative incidence of 20.6% (95% Confidence Interval 20.3-20.9%). Cumulative incidence was the highest in Slovakia 29.9% (95% CI 25.0-33.2%) and Northern Italy (31.1%; 95% CI 29.9-32.3% in Verona, 29.7%; 28.6-30.8% in Padua, 28.8%; 27.6-29.9% in Trieste). The association between main demographic and clinical characteristics and BI is presented in Table 3.

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Table 3. *Analysis of sex, age class, job title, previous infections, type of vaccine and comorbidities effect on the risk of BI*

	Overall (N=63 565)	Cumulative incidence of BI (N= 13 093)	P Value
Gender			**p<0.001**
Men	10 358	19.45% (3765/19 358)	
Women	44 159	**21.12%** (9328/44 159)	
Age Classes			**p<0.001**
18-29	8,191	**25.52%** (2091/8191)	
30-39	14 199	**22.42%** (3183/14 199)	
40-49	13 255	**24.01%** (3201/13 255)	
50-59	18 958	18.25% (3460/18 958)	
≥60	8 962	12.92% (1158/8962)	
Job Title			**p<0.001**
Physicians	17 213	21.07% (3627/17 213)	
Nurse	19 467	**24.67**% (4803/19 467)	
Other Health Workers	9 344	20.95% (1958/9344)	
Technicians	5 534	19.53% (1081/5534)	
Administrative	4 465	16.14% (721/4465)	
Previous Infection			**p<0.001**
none	57 416	**21.15%** (12 143/57 416)	
<1st dose	4 612	16.61% (766/4612)	
≥1st dose<3rd dose	1 537	11.97% (184/1537)	
Type of Vaccine			**p<0.001**
Pfizer	50 217	**23.36%** (11 733/50 217)	
Heterologous	6 834	13.03% (822/6834)	
Other	967	20.37% (197/967)	
Comorbidity			p=0.859
No	14 197	24.57% (3488/14 197)	
Yes	2 831	24.73% (700/2831)	

The analysis on determinants of asymptomatic/symptomatic BI was carried out on 14 195 HW (information available from 5 centers: Verona, Padua, Perugia, Barcelona, and Slovakia). Previous infection protected against asymptomatic infection (RRR of old infection vs no infection = 0.39, 95%CI 0.23-0.66 and RRR of recent infection = 0.25, 95%CI 0.11-0.60) and even more against symptomatic infections (RRR =0.36, 95%CI 0.27-0.48 and 0.05, 95%CI 0.02-0.12, respectively). With respect to Pfizer vaccine, heterologous vaccination protected against symptomatic infections (RRR =0.51, 95%CI 0.28-0.91), but not against asymptomatic ones (Figure 3).

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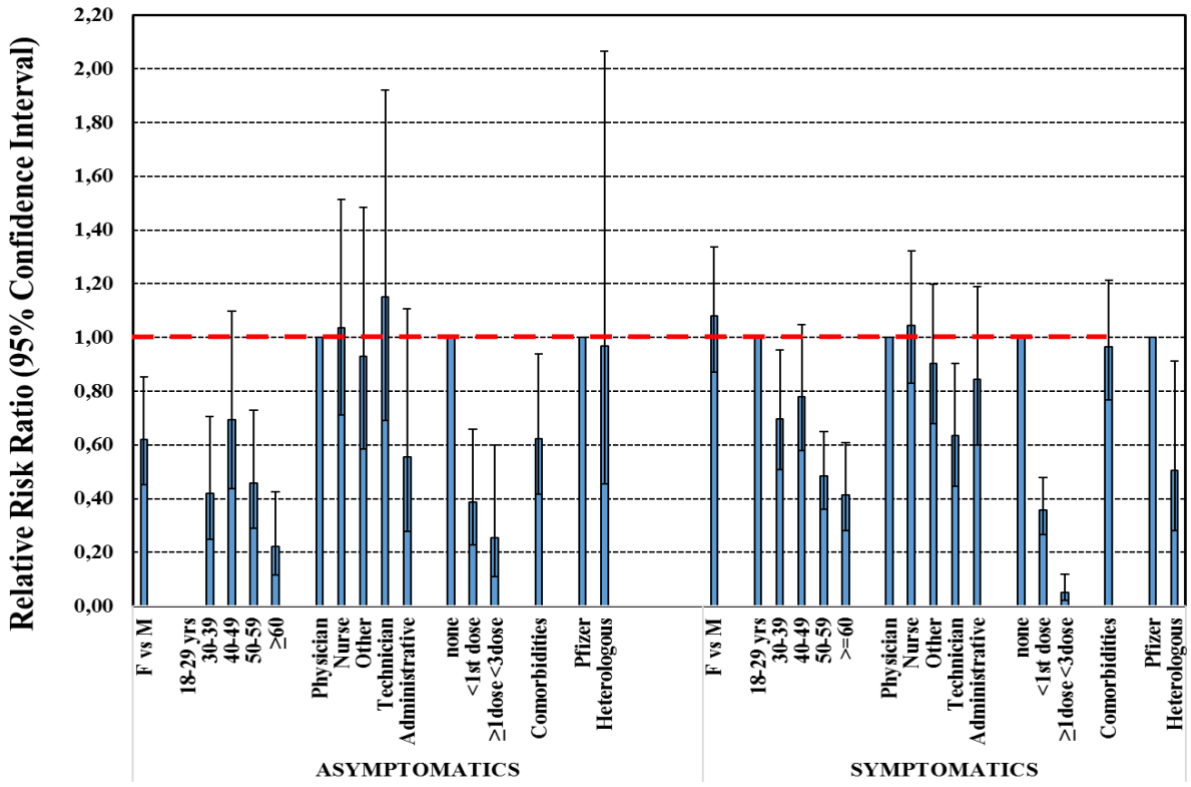
***Figure 3**. Determinants of BI investigated by a two-level multinomial logistic regression model (outcome where 0 = no infection, 1 = asymptomatic infection, 2 = symptomatic infection), where level-1 units (HW) were nested into level-2 units (participating centers).*



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