

## Supplementary material

### *Environment And Diabetes: Is there an Association Between The COVID-19 Pandemic And Phenotypic Variation In Diabetes?*

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## *Statistical Analysis Plan*

### Overview

The COVID pandemic had wideranging impact on health care with both immediate and long-term consequences. We were interested in studying whether the pandemic led to differences in presentation of newly diagnosed diabetes in adults

Study Hypothesis: whether adult individuals diagnosed with diabetes in Lombardy during the COVID pandemic presented a different demographic and cardiovascular profile from individuals diagnosed in the pre-pandemic era.

Primary objective: assess differences incidence of newly diagnosed diabetes and prevalence of cardiovascular diseases at diagnosis between the prepandemic (2018-2019) and the pandemic cohort (2020-2021) of adult individuals

Secondary Objectives: assess differences in mortality rates and Disability-Adjusted Life Years (DALY) of newly diagnosed patients between the prepandemic (2018-2019) and the pandemic cohort (2020-2021); establish the proportion of patients with COVID diagnosed prior to exemption for diabetes in the 2020-2021 cohort.

Study setting: retrospective fixed-cohort study

Study population: all adult (40-65 years of age) individuals granted exemption for diabetes by the Lombard Regional Health Care system from January 1<sup>st</sup> 2018 to December 31<sup>st</sup>, 2021.

Exclusion criteria: women with gestational diabetes

Data Source: data from individuals with exemption for diabetes within the specified time frame was provided by the Regional Health Care Database upon approval of the study protocol (Agreement ID 244080756). Data were made available on the Database warehouse platform with password-restricted access and patient identifiers anonymized by the Database warehouse.

Time frame: Data provided by the Regional Health Care Datawarehouse for individuals exempted for diabetes encompassed records pertaining to access to the Health Care system for drug administrations, exams, other exemptions and death. Only events occurring within 730 days (2 years) after exemption for diabetes were considered in order to ensure comparable follow-up in both cohorts.

Grouping: Data from men and women were analysed separately. Further, individuals were grouped in quartiles according to age at diagnosis for age-related analyses: I quartile:

40-46 y.o. individuals, II quartile: 47-52 y.o. individuals, III quartile: 53-58 y.o. individuals, IV quartile: 59-65 y.o. individuals.

#### Methods for analyses

- Incidence of newly diagnosed diabetes was calculated over 2 years for both cohorts. Data was standardized according to age using the data from the Lombard Registry (<https://www.dati.lombardia.it/dataset>). Individuals enrolled in the Regional Health care system as of January 1<sup>st</sup> of 2018 and 2020 were considered at-risk population.
- Mortality was calculated as person-year with 730 days follow-up for both groups. 95% confidence interval and Chi-statistics for mortality rate ratios were calculated according to Sahai <sup>[1]</sup>. Survival was estimated with Kaplan-Meier function.
- Disability-Adjusted Life Years (DALY): calculation of Years Lost due to Disability (YLD) was performed as described by Devleeschauwer <sup>[2]</sup>. Life expectancy was extracted from the Italian Institute of Statistics ([www.istat.it](http://www.istat.it)) for 2018 and 2020: 80.8 and 85.2 years and 79.8 and 84.5 years for men and women, respectively. Disease weight was considered 0.2 according to the Dutch Burden of Disease Group <sup>[3]</sup>.
- Differences between cohorts and quartiles were assessed with Student's T test for unpaired data or Wilcoxon's Test for continuous variables, multiple way ANOVA, according to data distribution. Chi-square statistics was used for qualitative variables, e.g., proportion of individuals with hypertension, cardiovascular risk factors. Analyses were carried out using SAS Studio software, Release 3.8, SAS Institute, Cary, NC.

Outcome measures: Size effect statistics was estimated with Cohen's d, i.e., standardized mean difference, for continuous variables, and rate ratios with 95% confidence interval for measures of association. Significance was accepted for  $p < 0.05$ .

#### Power:

- for continuous variables, the minimum standardized mean difference (Cohen's d) required to achieve significance was calculated <sup>[4]</sup>. A standardized difference greater than 0.034 achieved two-tailed probability less than 0.05 with 90% potency in men; in women given the same error probabilities (alpha 0.05, beta 0.10), standardized mean difference greater than 0.045 was required for significance. Corresponding values for quartile analysis are given below (Statistical Analysis Plan Table 1).
- for qualitative data, the smallest significant rate ratio was calculated according to Schlesselman <sup>[5]</sup> for both men and women and age-quartile group. and reported below

(Statistical Analysis Plan Table 1). Prevalences in the control cohort (i.e., 2018-2019) were used for calculation [5].

Statistical Analysis Plan Table 1. Smallest detectable significant rate ratio and Cohen's d across the entire cohort and quartiles for men and women. **Smallest detectable significant rate ratio** was calculated according to Schlesselman [5] for estimated prevalences of 40%, 15% and 6% ( $p_0$ ) at given samples size per group (n) for the entire and quartile series with two-sided alpha equal to 0.05 and power 0.9. **Cohen's d**, i.e., standardized mean difference required to reach statistcance with two-sided alpha equal to 0.05 and power 0.9 across quartiles was calculated according to Faul [4]

<b>All men (n ~18400)</b>	
estimated prevalence	smallest detectable rate ratio
40%	<0.933 or >1.071
15%	<0.908 or >1.097
6%	<0.863 or >1.148
<u>40-46 y.o men (n ~2300)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.821 or >1.213
15%	<0.754 or >1.291
6%	<0.640 or >1.450
<u>47-52 y.o men (n ~3600)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.854 or >1.167
15%	<0.800 or >1.229
6%	<0.706 or >1.351
<u>53-58 y.o men (n ~5300)</u>	
estimated prevalence	smallest detectable rate ratio

prevalence	
40%	<0.877 or >1.136
15%	<0.855 or >1.186
6%	<0.754 or >1.285
<u>59-65 y.o men (n ~7000)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.893 or >1.117
15%	<0.854 or >1.161
6%	<0.786 or >1.246
<b>Mortality (all men)</b>	
estimated prevalence	smallest detectable rate ratio
0.00167	<0.328 or >2.013

Quartile	Cohen's d
40-46 y.o men (n ~2300)	0.096
47-52 y.o men (n ~3600)	0.076
53-58 y.o men (n ~5300)	0.063
59-65 y.o men (n ~7000)	0.054

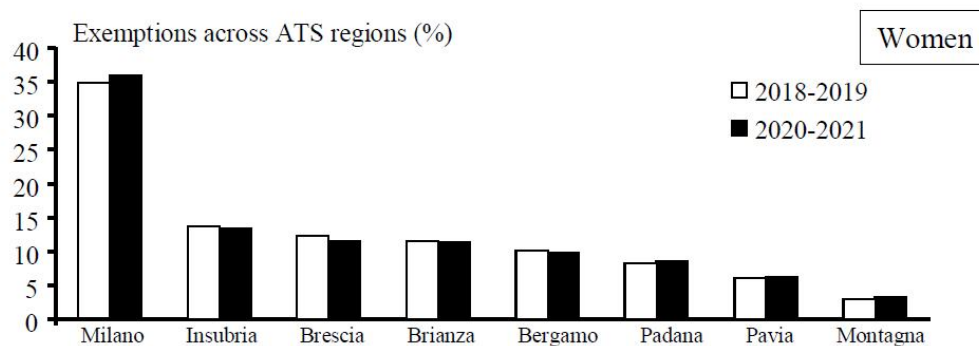
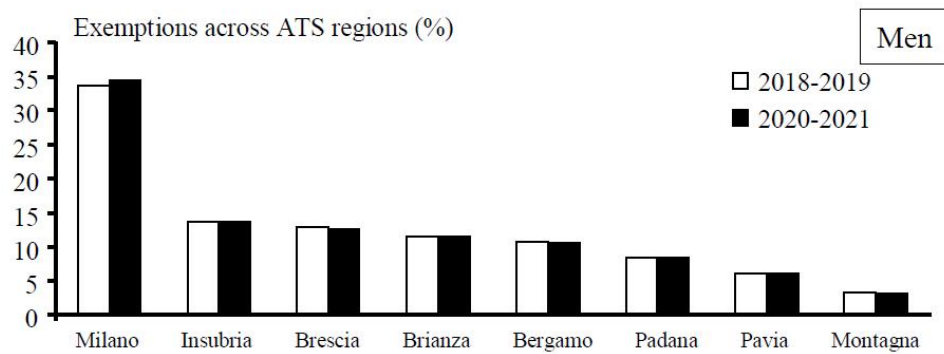
<b>All women (n ~10500)</b>	
estimated prevalence	smallest detectable rate ratio
40%	<0.912 r >1.095
15%	<0.879 or >1.130
6%	<0.821 or >1.198
<u>40-46 y.o women (n ~1030)</u>	
estimated prevalence	smallest detectable rate ratio

40%	<0.743 or >1.333
15%	<0.645 or >1.456
6%	<0.491 or >1.711
<u>47-52 y.o women (n ~1870)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.803 or >1.234
15%	<0.730 or >1.327
6%	<0.606 or >1.505
<u>53-58 y.o women (n ~3100)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.844 or >1.181
15%	<0.786 or >1.248
6%	<0.685 or >1.382
<u>59-65 y.o women (n ~4450)</u>	
estimated prevalence	smallest detectable rate ratio
40%	<0.868 or >1.149
15%	<0.819 or >1.205
6%	<0.733 or >1.313
<u>Mortality (all women)</u>	
estimated prevalence	smallest detectable rate ratio
0.00156	<0.146 or >2.493

Quartile	Cohen's d
40-46 y.o women (n ~1030)	0.142
47-52 y.o women (n ~1870)	0.106
53-58 y.o women (n ~3100)	0.082
59-65 y.o women (n ~4450)	0.068

## References

1. Sahai H, Khurshid A. Formulas and tables for the determination of sample sizes and power in clinical trials for testing differences in proportions for the matched pair design: a review. *Fundam Clin Pharmacol* 1996; **10**:554–563. doi: 10.1111/j.1472-8206.1996.tb00614.x
2. Devleeschauwer B, Havelaar AH, Maertens de Noordhout C, Haagsma JA, Praet N, Dorny P, Duchateau L, Torgerson PR, Van Oyen H, Speybroeck N. DALY calculation in practice: a stepwise approach. *Int J Public Health* 2014; **59**:571–4. doi: 10.1007/s00038-014-0553-y.
3. Melse JM, Essink-Bot ML, Kramers PG, Hoeymans N. A national burden of disease calculation: Dutch disability-adjusted life-years. Dutch Burden of Disease Group. *Am J Public Health* 2000 **90**:1241–7. doi: 10.2105/ajph.90.8.1241.
4. Faul F, Erdfelder E, Lang AG, Buchner A. G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**:175–91. doi: 10.3758/bf03193146
5. Schlesselman JJ, Stolley PD. Case-control studies: design, conduct, analysis. Oxford University Press, New York, 1982



**Supplementary Figure 1 Proportion of new exemptions in the two cohorts across Health Care Agencies (ATS) in Lombardy.**

**Supplementary Table 1 Number of individuals with diabetes in age quartiles**

	2018-2019	2020-2021
<b>Men</b>		
40-46 years	2307	2326
47-52 years	3767	3624
53-58 years	5291	5437
59-65 years	7141	6970
<b>Women</b>		
40-46 years	1078	1018
47-52 years	1883	1854
53-58 years	3097	3153
59-65 years	4497	4407

**Supplementary Table 2 Incidence of exemption for diabetes across Health Care Agencies (ATS) in Lombardy**

	2018-2019	2020-2021	size effect statistic	<i>P</i> value
Men				
Bergamo	435.8 (415.8; 455.8)	419.7 (400.4; 439.1)	RR 0.963 (0.902; 1.057)	0.66
Brescia	479.3 (459.5; 499.1)	449.2 (430.4; 468.1)	RR 0.937 (0.883; 1.050)	0.14
Brianza	441.5 (433.3; 449.8)	429.8 (421.2; 438.5)	RR 0.973 (0.915; 1.053)	0.94
Insubria	435.1 (417.7; 452.6)	420.9 (403.9; 437.8)	RR 0.967 (0.914; 1.048)	0.76
Milano	463.4 (451.6; 475.3)	459.9 (447.2; 470.5)	RR 0.989 (0.955; 1.026)	0.53
Montagna	832.6 (763.9; 901.5)	799.3 (732.5; 866.1)	RR 0.959 (0.853; 1.114)	0.80
Padana	510.9 (484.9; 537.1)	491.7 (466.4; 517.1)	RR 0.962 (0.894; 1.065)	0.59
Pavia	498.7 (468.3; 529.1)	491.1 (461.3; 521.0)	RR 0.984 (0.903; 1.079)	0.88
Women				
Bergamo	244.3 (229.1; 259.6)	232.4 (217.7; 247.1)	RR 0.951 (0.870; 1.085)	0.52
Brescia	269.9 (254.8; 284.9)	238.1 (224.3; 252.1)	RR 0.883 (0.814; 0.957)	0.014
Brianza	247.5 (232.9; 262.0)	236.1 (222.1; 250.1)	RR 0.954 (0.877; 1.079)	0.61
Insubria	243.1 (230.1; 256.1)	225.1 (212.8; 237.5)	RR 0.926 (0.857; 1.072)	0.20
Milano	265.6 (256.8; 274.5)	263.8 (255.1; 272.6)	RR 0.993 (0.947; 1.041)	0.47
Montagna	439.6 (389.7; 489.5)	480.9 (429.2; 532.7)	RR 1.094 (0.935; 1.162)	0.14
Padana	289.8 (270.1; 309.7)	293.2 (273.4; 312.9)	RR 1.011 (0.918; 1.094)	0.57
Pavia	291.1 (267.8; 314.6)	290.2 (267.1; 313.4)	RR 0.996 (0.889; 1.112)	0.74

Age standardized incidence is provided per 100000 inhabitants per 2 years. Rate ratio is given with 95%CI in parentheses.

**Supplementary Table 3 Data in men and women deceased after exemption for diabetes**

	2018-2019 (n=607)	2020-2021 (n=572)	size effect statistic	<i>P</i> value
<b>Men</b>				
follow-up (person-days)	13249516	13145253	--	--
mortality rate (1000 person-yr)	16.7 (15.4;18.1)	15.8 (14.5;17.2)	RR 0.949 (0.846;1.066)	0.38
age at diagnosis (yr)	58.3 ± 0.22 (57.8;58.7)	58.3 ± 0.24 (57.8; 58.7)	0.01 (-0.63;0.65)	0.98
age at death (yr)	59.1 ± 0.22 (58.6;59.5)	59.0 ± 0.24 (58.5; 59.5)	0.07 (-0.58;0.721)	0.82
time to death (d)	301.9 ± 8.58 (285.0;318.7)	283.6 ± 8.79 (266.3; 300.8)	18.3 (-5.79;42.4)	0.13
cardiovascular risk factors (%)	39.0 (32.8; 45.3)	41.6 (35.3;47.9)	RR 1.065 (0.886; 1.244)	0.37
<b>Women</b>				
	2018-2019 (n=322)	2020-2021 (n= 329)	size effect statistic	
follow-up (person-days)	7570527	7461248	--	--
mortality rate (1000 person-yr)	15.6 (13.9; 17.3)	16.1 (14.4; 17.9)	RR 1.033 (0.88;1.128)	0.64
age at diagnosis (yr)	58.32 ± 0.30 (57.7; 58.9)	57.9 ± 0.33 (57.3; 58.6)	0.36 (-0.52; 1.23)	0.43
age at death (yr)	59.0 ± 0.31 (58.4; 59.6)	58.6 ± 0.33 (58.0; 59.3)	0.32 (-0.56; 1.21)	0.47
time to death (d)	266.6 ± 12.0 (242.9; 290.2)	261.6 ± 11.3 (239.8; 283.9)	5.0 (-27.5; 37.5)	0.76
cardiovascular risk factors (%)	29.5 (20.3; 38.7)	25.5 (16.2; 34.9)	RR 0.865 (0.571; 1.159)	0.25

Continuous variables are provided as means ± S.E.M. 95% confidence interval for continuous and qualitative variables are shown in parentheses; yr: years; d: days.

**Supplementary Table 4 Data according to SARS-CoV-19 testing prior to exemption for diabetes in the 2020-2021 cohort**

	SARS-CoV-19 + (n=2163)	SARS-CoV-19 - (n=16194)	size effect statistic	P value
Men				
age at diagnosis (yr)	55.3 ± 0.14 (55.1; 55.6)	55.3 ± 0.05 (55.3; 55.5)	0.02 (-0.28; 0.32)	0.88
hypertension (%)	29.1 (25.5; 32.6)	30.8 (29.5; 32.1)	RR 0.94 (0.873; 1.012)	0.09
cardiovascular risk factors (%)	39.5 (36.2; 42.8)	40.8 (39.6; 42.0)	RR 0.97 (0.913; 1.023)	0.25
mortality rate (1000 person-yr)	19.6 (15.6;24.2)	16.0 (14.6;17.5)	RR 1.22 (0.956;1.543)	0.09
Women				
	SARS-CoV-19 + (n=1232)	SARS-CoV-19- (n=9200)	size effect statistic	
age at diagnosis (yr)	55.7 ± 0.18 (55.4; 56.2)	56.2 ± 0.07 (56.1; 56.3)	0.40 (0.02; 0.78)	0.04
hypertension (%)	34.6 (30.1; 39.2)	33.1 (31.5; 34.8)	RR 1.05 (0.964; 1.128)	0.28
cardiovascular risk factors (%)	38.7 (34.3; 44.1)	38.0 (36.4; 39.6)	RR 1.02 (0.943 1.093)	0.64
mortality rate (1000 person-yr)	17.4 (12.5; 23.5)	15.9 (14.1;17.9)	RR 1.09 (0.771;1.514)	0.58

Continuous variables are provided as means ± S.E.M. 95% confidence interval for continuous and qualitative variables are shown in parentheses; yr: years; d: days.