# Supplementary Online Content

Feng Q, Wei W-Q, Chaugai S, et al. A genetic approach to the association between *PCSK9* and sepsis. *JAMA Netw Open*. 2019;2(9):e1911130. doi:10.1001/jamanetworkopen.2019.11130



This supplementary material has been provided by the authors to give readers additional information about their work.

# eMethods. Cohort Identification and Power Calculations

**Cohort identification**

Sepsis was defined as concurrent infection and organ dysfunction occurring within one day of hospital admission (days -1, 0, and +1) using an algorithm to detect sepsis using EHR data[1] with minor modifications. The modifications were as follows: in the definition of sepsis we included (1) septic shock defined by presence of ICD9 codes 995.92 and 785.52, or ICD10 codes R65.20 and R65.21 because these codes are very specific; [1] (2) vasopressor initiation identified by use of levophed (noprepinephrine bitartrate), or use of dobutamine or dopamine and a billing code for administration of a vasopressor (ICD9-CM procedure code 00.17 or ICD10-PCS procedure codes 3E033XZ, 3E043XZ, 3E053XZ and 3E063XZ) because dobutamine or dopamine alone had low specificity for identifying patients in whom it was used as a pressor; (3) we did not use serum lactate levels as a criterion because they were seldom available; (4) we excluded individuals who had scheduled cardiothoracic surgery because the algorithms did not reliably identify the reason for artificial ventilation or ICU admission and some of these patients had an infection and received an antibiotic. The methods used have been described in detail previously.[2]

**Associations between LDL-C and (1)4 PCSK9 functional variants (2) PCSK9 GRS and (3) predicted PCSK9 expression**

For LDL-C analyses (other than the gene expression analysis), we did not restrict our analysis of the relationships between the genetic instruments and LDL-C to the sepsis cohort (n=10922) because some patients may only have had LDL-C measured after hospital admission (or during

sickness) which would confound the association test. Instead, we used all available data in BioVU for individuals with LDL-C and PCSK9 genotypes. We have used all BioVU individuals who had both PCSK9 genotypes and an LDL-C measurement. We used the median LDL-C for those with multiple measurements.

The number of individuals in each analysis varies due to data availability in BioVU. Specifically, the 4 functional PCSK9 variants were extracted from genome-wide platforms and the ExomeChip (N=22,995). The 6 SNPs for PCSK9 GRS were not available on the ExomeChip and could not be imputed from it. Therefore, the association between the PCSK9 GRS and measured LDL-C was evaluated within those on genome-wide platforms (n=15,387). For the association between estimated PCSK9 expression and LDL-C, we used the sepsis cohort because gene expression had been calculated in this cohorts. Within the sepsis cohort, 3630 individuals had both predicted PCSK9 expression and measured LDL-C.

**Power calculations**

There was adequate power to detect small differences between the study groups; the detectable odds ratio for sepsis in the loss-of-function (LOF) carriers relative to non-carriers was 1.15. We estimated this detectable difference as a post-hoc calculation using PC software[3] and the following assumptions: (1) 4,965 patients had >= one LOF PCSK9 variant; (2) 5,162 patients did not have any functional variants; and (3) 3,391 patients developed sepsis (31%). This estimation used an uncorrected chi-squared statistic to evaluate the null hypothesis and power of 0.84. The type I error probability associated with this test of the null hypothesis was 0.05. The figure below illustrates power for a range of true ORs for current cohort.

Patients who were gain-of-function carriers were excluded from this power calculation.

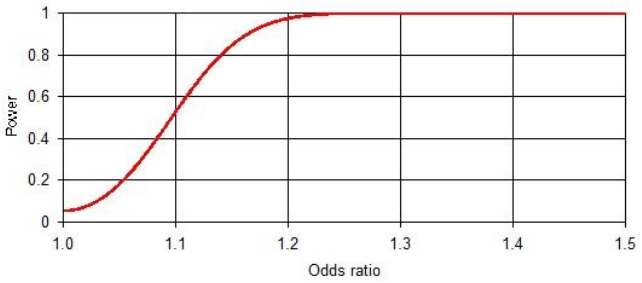



*Figure. statistical power in current study*

We adopted the PCSK9 GRS based on LDL-C levels from previous high impact publications.[4,5] Specifically, the four functional PCSK9 variants were used in Walley's paper in Science Translation Medicine.[4] Although there was no quantification of PCSK9's effect for removal of LPS in vivo, the same genetic instruments demonstrated the relationship between PCSK9 variants and mortality in a cohort of ~500 individuals with septic shock. Furthermore, the GRS used in our manuscript was also significantly associated with both myocardial infarction and type II diabetes mellitus. [5]

## eTable 1. Event Counts in Each Analysis

| Predictor | Outcomes | Gender | |
|---|---|---|---|
| | | F | M |
| PCSK9 functional variants (4 SNPs) | sepsis | 1485 | 1906 |
| | cardiovascular failure | 308 | 527 |
| | In hospital death | 148 | 218 |
| PCSK9 GRS | sepsis | 1015 | 1456 |
| | cardiovascular failure | 222 | 424 |
| | In hospital death | 90 | 149 |
| PCSK9 expression | sepsis | 826 | 1152 |
| | cardiovascular failure | 189 | 337 |
| | In hospital death | 76 | 131 |

## eTable 2. Event Counts by Genotypes

| | | sepsis | | | | cardiovascular failure | | | | inhospital death | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N of minor allele | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No | Yes |
| | | Counts | | percent (%) | | Counts | | percent (%) | | Counts | | percent (%) | |
| rs11591147_T | 0 | 7309 | 3299 | 68.9 | 31.1 | 9803 | 805 | 92.4 | 7.6 | 10250 | 358 | 96.6 | 3.4 |
| | 1 | 214 | 86 | 71.3 | 28.7 | 272 | 28 | 90.7 | 9.3 | 292 | 8 | 97.3 | 2.7 |
| | | | | | | | | | | | | | |
| rs11583680_T | 0 | 5591 | 2514 | 69.0 | 31.0 | 7471 | 634 | 92.2 | 7.8 | 7832 | 273 | 96.6 | 3.4 |
| | 1 | 1777 | 793 | 69.1 | 30.9 | 2390 | 180 | 93.0 | 7.0 | 2489 | 81 | 96.8 | 3.2 |
| | 2 | 152 | 73 | 67.6 | 32.4 | 205 | 20 | 91.1 | 8.9 | 213 | 12 | 94.7 | 5.3 |
| | | | | | | | | | | | | | |
| rs562556_G | 0 | 5168 | 2314 | 69.1 | 30.9 | 6920 | 562 | 92.5 | 7.5 | 7229 | 253 | 96.6 | 3.4 |
| | 1 | 2112 | 971 | 68.5 | 31.5 | 2835 | 248 | 92.0 | 8.0 | 2986 | 97 | 96.9 | 3.1 |
| | 2 | 247 | 103 | 70.6 | 29.4 | 325 | 25 | 92.9 | 7.1 | 334 | 16 | 95.4 | 4.6 |
| | | | | | | | | | | | | | |
| rs505151_G | 0 | 6998 | 3165 | 68.9 | 31.1 | 9390 | 773 | 92.4 | 7.6 | 9817 | 346 | 96.6 | 3.4 |
| | 1 | 512 | 221 | 69.8 | 30.2 | 671 | 62 | 91.5 | 8.5 | 713 | 20 | 97.3 | 2.7 |
| | 2 | 18 | 4 | 81.8 | 18.2 | 22 | 0 | 100.0 | 0.0 | 22 | 0 | 100.0 | 0.0 |

**eTable 3. Associations Between Comorbidity Score and Sepsis and Related Outcomes**

| | Odds Ratio | 95% confidence interval | | P-value |
|---|---|---|---|---|
| **Sepsis** | 1.27 | 1.26 | 1.29 | <2e-16 |
| **Cardiovascular Failure** | 1.29 | 1.26 | 1.31 | <2e-16 |
| **Death** | 1.39 | 1.37 | 1.42 | <2e-16 |

## eTable 4. SNPs Included in *PCSK9* Genetic Risk Score

| SNPs | minor allele | effectsize mg/dL |
|---|---|---|
| rs2479394 | G | 1.2352 |
| rs11206510 | C | -2.6592 |
| rs2479409 | G | 2.0544 |
| rs10888897 | T | -1.6224 |
| rs7552841 | T | 1.1776 |
| rs562556 | G | -2.048 |

(Ference, B. A. *et al.* Variation in PCSK9 and HMGCR and Risk of Cardiovascular Disease and Diabetes. *N. Engl. J. Med.* **375**, 2144–2153 (2016).)

# eTable 5. SNPs Included in Estimated *PCSK9* Expression

| POS | ID | WEIGHT | ref_allele | eff_allele |
|---|---|---|---|---|
| 1:54677786-54677786 | rs17392549 | 0.04947 | G | A |
| 1:56132837-56132837 | rs116532018 | -0.07378 | A | C |
| 1:56073566-56073566 | rs112931677 | -0.02218 | T | C |
| 1:55524601-55524601 | rs565436 | 0.069746 | G | A |
| 1:54532687-54532687 | rs61777599 | -0.11534 | A | G |
| 1:54637417-54637417 | rs682705 | -2.05E-05 | G | A |
| 1:55789748-55789748 | rs71637889 | 0.006645 | G | A |
| 1:54654129-54654129 | rs4244643 | -0.05393 | G | T |
| 1:54994972-54994972 | rs6675210 | 0.140809 | T | C |
| 1:54803850-54803850 | rs66916204 | -0.10365 | A | C |
| 1:54535919-54535919 | rs72664136 | 0.155025 | G | A |
| 1:56413577-56413577 | rs56375406 | -0.20066 | G | A |
| 1:55522674-55522674 | rs11587071 | -0.01967 | C | T |
| 1:55442357-55442357 | rs12062838 | -0.01175 | A | G |
| 1:54632658-54632658 | rs113535797 | 0.064701 | G | A |
| 1:55519015-55519015 | rs639750 | 0.021681 | G | T |
| 1:54572175-54572175 | rs12046178 | 0.01663 | T | C |
| 1:55757418-55757418 | rs35818377 | 0.009532 | A | G |
| 1:55720674-55720674 | rs12039195 | -0.08535 | C | T |
| 1:55518166-55518166 | rs625619 | 0.045018 | G | A |
| 1:54566310-54566310 | rs6694397 | 0.017712 | G | A |
| 1:54762343-54762343 | rs12118138 | -0.14263 | C | T |
| 1:55647272-55647272 | rs11206521 | -0.09513 | T | C |
| 1:55757519-55757519 | rs34262463 | 0.0363 | T | C |
| 1:54660252-54660252 | rs148520561 | 0.01326 | C | T |
| 1:55785774-55785774 | rs17111909 | 0.038863 | A | G |
| 1:55431601-55431601 | rs1076528 | 0.02762 | A | G |
| 1:55246035-55246035 | rs12144319 | -0.02505 | T | C |
| 1:56170178-56170178 | rs72671422 | -0.14367 | G | A |
| 1:55115501-55115501 | rs1655523 | -0.0922 | T | G |
| 1:54557290-54557290 | rs12046885 | 0.017157 | C | T |
| 1:55518752-55518752 | rs7552841 | 0.194478 | C | T |
| 1:55789351-55789351 | rs17111925 | 0.010158 | G | A |
| 1:56187341-56187341 | rs2986585 | 0.065122 | A | G |
| 1:55366346-55366346 | rs4424550 | -0.026 | T | C |

## eTable 6. Associations Between Median Measured LDL-C Levels and 4 Functional *PCSK9* Variants

| CHR | SNP | BP | A1 | BETA | STAT | P |
|---|---|---|---|---|---|---|
| 1 | rs11591147 | 55505647 | T | -13.03 | -1.02E+01 | 2.87E-24 |
| 1 | rs11583680 | 55505668 | T | -0.1629 | -0.3759 | 0.707 |
| 1 | rs562556 | 55524237 | G | -0.6824 | -1.75E+00 | 0.08027 |
| 1 | rs505151 | 55529187 | G | 1.633 | 2.005 | 0.04499 |

## eTable 7. Associations Between Median Measured LDL-C Levels and *PCSK9* GRS

| predictor | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| PCSK9 GRS (n=15387) | 0.7983 | 0.2593 | 3.079 | 0.00208 |

## eTable 8. Associations Between Median Measured LDL-C Levels and *PCSK9* Expression

| predictor | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| predicted PCSK9 expression (n=3630) | -0.07969 | 0.53808 | -0.148 | 0.882 |

# eTable 9. Associations Between *PCSK9* Candidate SNPs and Sepsis and Related Adverse Outcomes

| CHR | SNP | Location | Minor Allele Frequency | Minor Allele | Amino acid change | Sepsis | | Cardiovascular Failure | | Death | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Odds Ratio | P | Odds Ratio | P | Odds Ratio | P |
| **unadj.** | | | | | | | | | | | |
| 1 | rs11591147 | 55505647 | 0.013 | T | p.Arg46Leu | 0.8903 (0.6909-1.147) | 0.3694 | 1.254 (0.844-1.862) | 0.2628 | 0.7844 (0.3856-1.596) | 0.5028 |
| 1 | rs11583680 | 55505668 | 0.14 | T | p.Ala53Val | 1.004 (0.9249-1.091) | 0.9154 | 0.9411 (0.8129-1.09) | 0.417 | 1.042 (0.8457-1.285) | 0.6974 |
| 1 | rs562556 | 55524237 | 0.17 | G | p.Ile474Val | 1.004 (0.9314-1.082) | 0.9156 | 1.039 (0.9125-1.182) | 0.5657 | 1.021 (0.8426-1.237) | 0.8321 |
| 1 | rs505151 | 55529187 | 0.036 | G | p.Gly670Glu | 0.927 (0.7936-1.083) | 0.3386 | 1.048 (0.8067-1.361) | 0.7262 | 0.759 (0.4857-1.186) | 0.2262 |
| | Any LOF | - | - | - | - | 0.9667 (0.8886-1.0516) | 0.43 | 1.0489 (0.9055-1.2150) | 0.524 | 0.8908 (0.7180-1.1041) | 0.292 |
| **adj. gender and sex** | | | | | | | | | | | |
| 1 | rs11591147 | 55505647 | 0.013 | T | p.Arg46Leu | 0.8922 (0.692-1.15) | 0.379 | 1.261 (0.8482-1.875) | 0.2516 | 0.7835 (0.3846-1.596) | 0.5017 |
| 1 | rs11583680 | 55505668 | 0.14 | T | p.Ala53Val | 1.006 (0.9263-1.093) | 0.8837 | 0.9443 (0.8154-1.094) | 0.4447 | 1.046 (0.8489-1.29) | 0.6715 |
| 1 | rs562556 | 55524237 | 0.17 | G | p.Ile474Val | 1.008 (0.9345-1.086) | 0.8441 | 1.046 (0.9185-1.191) | 0.498 | 1.026 (0.8468-1.244) | 0.7919 |
| 1 | rs505151 | 55529187 | 0.036 | G | p.Gly670Glu | 0.9269 (0.7933-1.083) | 0.339 | 1.052 (0.8094-1.368) | 0.7038 | 0.771 (0.4921-1.208) | 0.2562 |
| | Any LOF | - | - | - | - | 0.9707 (0.8921 - 1.0561) | 0.4894 | 1.0611 (0.9157 - 1.2296) | 0.43 | 0.9077 (0.7311 - 1.1257) | 0.3785 |
| **adj. age, sex and comorbidity groups** | | | | | | | | | | | |
| 1 | rs11591147 | 55505647 | 0.013 | T | p.Arg46Leu | 0.8374 (0.6441-1.089) | 0.1851 | 1.235 (0.8237-1.851) | 0.3072 | 0.734 (0.3571-1.509) | 0.4003 |
| 1 | rs11583680 | 55505668 | 0.14 | T | p.Ala53Val | 1.007 (0.9252-1.097) | 0.8648 | 0.9351 (0.8053-1.086) | 0.3786 | 1.063 (0.8605-1.314) | 0.57 |
| 1 | rs562556 | 55524237 | 0.17 | G | p.Ile474Val | 0.9912 (0.9171-1.071) | 0.8245 | 1.024 (0.8971-1.169) | 0.7264 | 0.9907 (0.8147-1.205) | 0.9257 |
| 1 | rs505151 | 55529187 | 0.036 | G | p.Gly670Glu | 0.917 (0.7811-1.077) | 0.2896 | 1.048 (0.8012-1.371) | 0.7326 | 0.7973 (0.5061-1.256) | 0.3284 |
| | Any LOF | - | - | - | - | 0.9555 (0.8759-1.0423) | 0.3047 | 1.0461 (0.9002-1.2157) | 0.5566 | 0.8919 (0.7158-1.1102) | 0.3066 |
| **adj. age, sex, comorbidity groups and 6PCs** | | | | | | | | | | | |
| 1 | rs11591147 | 55505647 | 0.013 | T | p.Arg46Leu | 0.8654 (0.6594-1.136) | 0.2976 | 1.334 (0.887-2.005) | 0.1664 | 0.817 (0.3965-1.683) | 0.5836 |
| 1 | rs11583680 | 55505668 | 0.14 | T | p.Ala53Val | 1.01 (0.9239-1.05) | 0.8241 | 0.9356 (0.8012-1.092) | 0.3997 | 1.063 (0.8513-1.326) | 0.5914 |
| 1 | rs562556 | 55524237 | 0.17 | G | p.Ile474Val | 0.9979 (0.9195-1.083) | 0.9601 | 1.037 (0.9038-1.189) | 0.6054 | 1.016 (0.8282-1.246) | 0.8802 |
| 1 | rs505151 | 55529187 | 0.036 | G | p.Gly670Glu | 0.9184 (0.7765-1.086) | 0.3201 | 1.018 (0.7696-1.346) | 0.9014 | 0.6528 (0.3921-1.087) | 0.1011 |
| | Any LOF | - | - | - | - | 0.9642 (0.8801-1.0564) | 0.4341 | 1.0666 (0.9127-1.2465) | 0.4175 | 0.9128 (0.7258-1.1470) | 0.4343 |

* rs505151 is a gain-of-function variant; other SNPs are loss-of-function variants

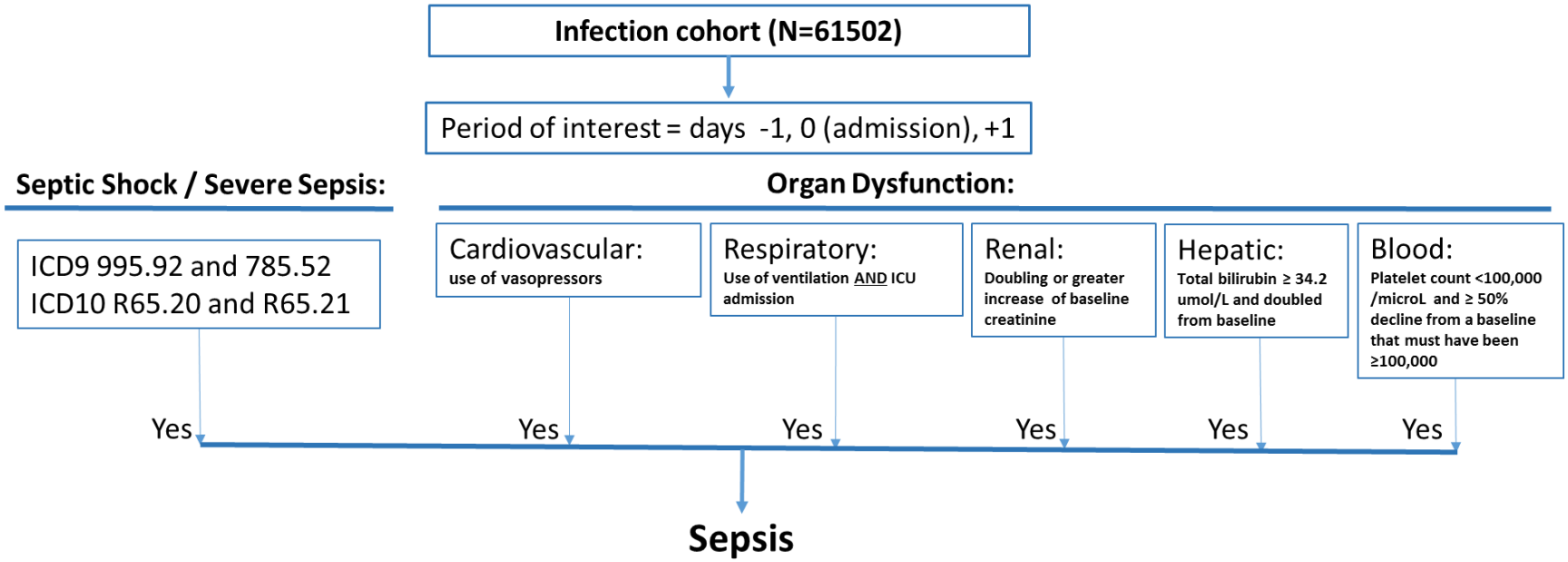
## eTable 10. Associations Between *PCSK9* GRS and Sepsis and Related Adverse Outcomes

| PCSK9 Tertiles | Sepsis | | Cardiovasular Failure | | Death | |
|---|---|---|---|---|---|---|
| | Odds Ratio | P | Odds Ratio | P | Odds Ratio | P |
| **unadj.** | | | | | | |
| Low | 0.9944 (0.8839-1.1188) | 0.926 | 0.9868 (0.8089-1.2038) | 0.896 | 0.8840 (0.6446-1.2103) | 0.442 |
| Middle | 1.0424 (0.9269-1.1722) | 0.489 | 1.0331 (0.8485-1.2581) | 0.746 | 0.9084 (0.6638-1.2413) | 0.546 |
| High | 1 | - | 1 | - | 1 | - |
| **adj. age, sex and comorbidity groups** | | | | | | |
| Low | 0.9892 (0.8759-1.1172) | 0.86165 | 0.9790 (0.7989-1.1997) | 0.838029 | 0.9012 (0.6534-1.2410) | 0.52436 |
| Middle | 1.0285 (0.9110-1.1611) | 0.64959 | 1.0191 (0.8332-1.2468) | 0.853655 | 0.9285 (0.6744-1.2766) | 0.6478 |
| High | 1 | - | 1 | - | 1 | - |
| **adj. age, sex, comorbidity groups, and 6PCs** | | | | | | |
| Low | 0.9884 (0.8750 - 1.1165) | 0.85112 | 0.9739 (0.7944-1.1939) | 0.799039 | 0.8879 (0.6428-1.2243) | 0.46866 |
| Middle | 1.0275 (0.9100 - 1.1602) | 0.66128 | 1.0179 (0.8320-1.2456) | 0.862745 | 0.9202 (0.6678-1.2665) | 0.60988 |
| High | 1 | - | 1 | - | 1 | - |

## eTable 11. Associations Between Genetically Estimated *PCSK9* Expression Tertiles and Sepsis and Related Adverse Outcomes

| PCSK9 Tertiles | Sepsis | | Cardiovascular Failure | | Death | |
|---|---|---|---|---|---|---|
| | Odds Ratio | P | Odds Ratio | P | Odds Ratio | P |
| **unadj.** | | | | | | |
| Low | 0.9977 (0.8742-1.1387) | 0.973 | 1.0618 (0.8468-1.3318) | 0.6036 | 0.9318 (0.6693-1.2957) | 0.674 |
| Middle | 1.0674 (0.9359-1.2173) | 0.331 | 1.2792 (1.0289-1.5927) | 0.0271 | 0.7830 (0.5533-1.1032) | 0.164 |
| High | 1 | - | 1 | - | 1 | - |
| **adj. age, sex and comorbidity groups** | | | | | | |
| Low | 1.0078 (0.8789-1.1556) | 0.911203 | 1.0816 (0.8583-1.3635) | 0.506235 | 0.9492 (0.6772-1.3290) | 0.76145 |
| Middle | 1.0608 (0.9259-1.2155) | 0.394932 | 1.2637 (1.0112-1.5814) | 0.040103 | 0.7558 (0.5305-1.0722) | 0.11809 |
| High | 1 | - | 1 | - | 1 | - |
| **adj. age, sex, comorbidity groups and 6PCs** | | | | | | |
| Low | 1.0071 (0.8782-1.1550) | 0.919352 | 1.0793 (0.8561-1.3613) | 0.518633 | 0.9328 (0.6643-1.3080) | 0.68657 |
| Middle | 1.0621 (0.9269-1.2171) | 0.38602 | 1.2676 (1.0140-1.5868) | 0.037783 | 0.7496 (0.5256-1.0646) | 0.10882 |
| High | 1 | - | 1 | - | 1 | - |

## eFigure. Algorithm to Identify Sepsis Within Infection Cohort



eReferences.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