Statins use and risk of mortality in patient with *Clostridium difficile* infection

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Abstract

Current evidence suggests that statins may improve outcome in infectious diseases. This study aims to assess whether statins use is associated with reduced risk of 30-day mortality in *Clostridium difficile* infection (CDI). Using the computerized database of Clalit, the largest healthcare provider in Israel, we identified a cohort of adult subjects (age ≥40 years) who tested positive on a *C. difficile* toxin assay performed between January 2011 and December 2012. Subjects were defined as current statins users if they filled at least one prescription during the 90 days before the laboratory assay date. Current users were classified into long-term users if at least one additional prescription was filled during the previous 91–180 days; otherwise, they were defined as short-term users. A total of 1888 patients with CDI were included. Of them, 340 (18.0%) died during the first 30 days after diagnosis. The 30-day mortality rate was lower among current statins users 89/669 (13.3%) compared with 251/1219 (20.6%) in non-users (p < 0.001). A significant reduced risk of 30-day mortality existed after adjustment for potential confounders; adjusted OR = 0.57 (95% CI 0.42–0.79) and was unique to long-term users; 0.53 (0.38–0.73) but not short-term users; 1.15 (0.56–2.34). The risk of 30-day mortality decreased with increasing number of filled statins prescriptions; adjusted OR = 0.77 (95% CI 0.67–0.89) for each additional prescription. Current aspirin use was also independently associated with reduced mortality; adjusted OR = 0.64 (95% CI 0.43–0.88). In conclusion, current statins use, particularly long-term use, has a dose–response protective effect on mortality in patients with CDI.

Keywords: Aspirin, *Clostridium difficile*, mortality, statins

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Introduction

*Clostridium difficile* is an anaerobic gram-positive, spore-forming, toxin-producing bacillus that causes intestinal inflammation and diarrhoea [1]. The clinical course of *C. difficile* infections (CDI) ranges from uncomplicated diarrhoea to pseudomembranous colitis, toxic megacolon and severe sepsis associated with organ failure and increased risk of death [2]. In recent years CDI was observed to be more frequent, severe, refractory to standard treatment, and more likely to relapse than previously described; this change in the pattern of the disease is probably caused by new more virulent strains of *C. difficile* [3,4]. All-cause 30-day mortality in CDI varies from 9% to 38% with most studies showing a mortality rate of 15% or greater [5]. When adjusted for age, sex and comorbidities CDI patients had a 2.5-fold increased 30-day mortality rate compared with controls without diarrhoea [6,7].

Increasing evidence suggests that besides their lipid-lowering effect, statins have potent anti-inflammatory effects that contribute to their beneficial effects in cardiovascular disease [8]. Statins use has been associated with reduced risk of infection including reduced risk of CDI [9–11]. Several observational studies have shown that statins use is associated
with decreased mortality in patients with sepsis [12–15]. However, it has been suggested that a healthy user effect may account for the observed benefit [16].

Death from CDI ultimately follows severe complications of sepsis. Because severe sepsis seems to be mediated by inflammatory cytokines, agents like statins that have anti-inflammatory and immunomodulatory properties might have a beneficial effect on mortality in patients with CDI. In this study we aimed to assess whether current statins use is associated with reduced risk of mortality in patients with CDI controlling for the possibility of healthy user effect.

Materials and Methods

The database

The Clalit Health Services (CHS) is a not-for-profit health-care provider covering more than half of the Israeli population (4 225 799 members) [17,18]. The centralized electronic database of the CHS includes data from multiple sources; primary-care physicians, specialty clinics in the community, hospitalizations, laboratories and pharmacies. A chronic disease registry is compiled from these data sources. Diagnoses are captured in the registry by diagnosis-specific algorithms, employing both code reading (e.g. ICD-9, ICPC) and text reading. A record is kept of the sources and dates are used to establish the diagnosis with the earliest recorded date being considered the starting date of the diagnosis.

Members of CHS may fill prescriptions at any pharmacy belonging to the CHS with low co-payment. All filled prescriptions are captured in the CHS computerized database. Because of the low co-payment it is unlikely that prescriptions are purchased in private, non-CHS, pharmacies. Hence, we can determine use of statins and other medications, as well as other medical background data with great precision. Moreover, the low co-payment makes statins use unlikely to be affected by affordability, which is a marker of mortality.

Study population

The CHS laboratory database was searched for all C. difficile toxin assays performed between 1 January 2011 and 31 December 2012. The first positive assay during the study period was selected (index date). We identified 1888 subjects, age ≥40 years at index date, who tested positive for CDI during this period.

Follow-up

The cohort of subjects with positive C. difficile assay was followed-up for all-cause mortality occurring during the 30 days starting on the index date. The date of death was extracted from the vital status records of CHS, which are updated monthly against the National Death Index. The cause of death was unavailable.

Statins exposure definition

Exposure to statins treatment was analysed in three ways: (i) Current statins users, (dichotomous variable, yes versus no) if at least one prescription was filled during the 90 days before the index date; (ii) and (iii) Current statins users were further classified into two categories, short-term users and long-term users; (ii) Short-term statins use was defined as at least one prescription filled during the 90 days before the index date and no prescription filled between 91 and 180 days before the index date, (iii) Long-term users were defined as those who had at least one prescription filled during each of the periods. To further assess the effect of adherence to statins use (a proxy for dose–response effect) on mortality, we calculated the number of statins prescriptions filled during the 90 days before the index date (ordinal variable) among current users. Generally the number of prescriptions filled during this period ranged from none (non-users) to four prescriptions, considering that a maximum of three statins prescriptions could be used during 90 days, this variable was grouped into four categories (0, 1, 2 and ≥3 prescriptions).

Covariates

The following covariates were considered as potential confounders: comorbid conditions present before the index date including ischaemic heart disease, congestive heart failure, stroke, diabetes, renal failure, hypertension, chronic obstructive pulmonary disease, obesity, liver cirrhosis, inflammatory bowel disease and cancer in the previous year, were identified from the CHS chronic disease registry. Hospitalizations (for any cause) within 90 days before the index date were identified from the CHS hospitalization database.

Treatment with antibiotics, chemotherapy, proton pump inhibitors and aspirin were ascertained if any such prescription was filled during the 90 days before the index date.

Clostridium difficile assay

The diagnosis of C. difficile at CHS laboratories is done using a two-step algorithm: the first step is an enzyme immunoassay for C. difficile toxins A and B and an enzyme immunoassay for C. difficile glutamate dehydrogenase (GDH). If the toxin assay is positive then the test is considered positive. If the toxin assay is negative and the GDH assay is positive, a second test (second step) is performed consisting of PCR to detect toxin B genes.
Statistical methods
Comparisons of means between two groups were tested with the Student’s t-test. The chi-squared test was used to compare proportions between categorical variables.

We used logistic regression to assess the univariate and multivariate association between statins use and 30-day all-cause mortality. All covariates, included in the multivariate model, were checked against one another for collinearity. We assessed the association of 30-day all-cause mortality both with statins use as one group (any statin) and with the specific statin type (simvastatin, atorvastatin, pravastatin, rosuvastatin). A p-value <0.05 for the two-tailed test was considered statistically significant. All statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

Correcting for a possible healthy user effect
As patients who use statins for primary or secondary prevention are more likely to also seek other preventive services and to engage in healthy behaviours leading to a healthy user effect [19,20], measures were taken to correct for such a possible effect.

One approach involves the evaluation of negative control exposure [19,21]. In such a design the exposure would not be expected to have a biological effect on study outcome, but may be influenced by health status or health-seeking behaviour in the same way as the study exposure (statins in our study). In an unbiased analysis, there would be no association between the negative control exposure and the study outcome. For this purpose we used calcium-channel blockers as a possible negative control exposure given the lack of supporting biological mechanism or clinical evidence that could explain a protective effect (if present) on CDI mortality. The presence of a healthy user effect would be suggested if a protective effect were also found for calcium-channel blocker users. On other hand, if no association is detected for calcium-channel blockers, then if a protective effect of statins is found then such an effect may be unlikely to be attributable to healthy-user effect.

Results

Our study cohort includes 1888 subjects who were eligible to be included in the study. The mean age of the patients was 75.7 ± 12.6 years, and 1164 (61.7%) were females. The baseline characteristics of the study population are presented in Table 1.

Statins use and all-cause 30-day mortality
Overall 340 (18.0%) subjects with CDI died within 30 days of CDI diagnosis. All subjects had complete 6-month follow up when the data were retrieved from the database, during this period 668 (35.4%) subjects died. It follows that about half of deaths occurring during 6 months occurred during the first 30 days.

Of the total 669 current statins users, 89 (13.3%) died within 30 days, compared with 251/1219 (20.6%) among non-users (p <0.001; Table 2). The decreased risk of 30-day mortality persisted on multivariate analysis; adjusted OR = 0.57 (95% CI 0.42–0.79; Table 2 and see Supporting information, Table S1). The multivariate logistic regression model showed that current aspirin use was also associated with decreased risk of 30-day mortality; adjusted OR = 0.64 (95% CI 0.43–0.88). Similar risk estimates of aspirin effect were reached on stratified analysis according to current statins use; OR = 0.62 (95% CI 0.40–0.96) among non-statins users, and 0.63 (95% CI 0.38–1.04) among statins users (see Supporting information, Table S2). In addition, the effect of statins use persisted on stratified analysis according to aspirin treatment; OR = 0.55 (95% CI 0.32–0.95) among aspirin users, and 0.57 (95% CI 0.38–0.86) among non-aspirin users.

Compared with non-statins users, the crude odds for 30-day mortality was 0.54 (95% CI 0.41–0.72) for long-term statins users, and 1.22 (95% CI 0.65–2.32) for short-term statins users.
(Table 2). Similar risk estimates were reached on multivariate analysis. Compared with non-statin users, the odds for 30-day mortality was 0.53 (95% CI 0.38–0.73) for long-term statins users, and 1.15 (95% CI 0.56–2.34) for short-term statins users (Table 2 and Table S1).

All-cause 30-day mortality rate decreased with increasing number of prescriptions filled during the previous 90 days among current statins users (p < 0.001; Table 2). The significant association with 30-day mortality and the number of prescriptions persisted on multivariate analysis; adjusted OR = 0.77 (95% CI 0.67–0.89) for each additional prescription (Table 2 and Table S1). We performed sensitivity analysis to assess the relationship between the number of prescriptions filled during the previous 180 days and 30-day mortality, and similarly the adjusted OR was 0.88 (95% CI 0.82–0.94) for each additional prescription.

Statin type and 30-day mortality
Simvastatin was the most frequently used statin (437; 65.3%) followed by atorvastatin (117; 17.5%), pravastatin (72; 10.8%) and rosuvastatin (43; 6.4%). Except for the less frequently used rosuvastatin, all other statins were independently associated with reduced risk of 30-day mortality, the adjusted OR ranged from 0.24 (pravastatin) to 0.64 (simvastatin; Table 3).

The negative control exposure and 30-day mortality
We employed the same exposure criteria to analyse exposure to calcium-channel blockers, which were used as negative control exposure. Calcium-channel blockers were not associated with 30-day mortality in any of the three different exposure definition categories (Table 4 and see Supporting information, Table S3).

### Discussion

Our study shows that current statins use is associated with reduced risk of 30-day mortality in patients with CDI. This finding is consistent with other studies that showed protective effect of statins against mortality in patients with sepsis [12,13,15]. Although only a few patients were short-term users, our findings suggest that long-term statins use but not short-term use is associated with reduced risk of 30-day mortality in patients with CDI. Long-term statins use was found to better reduce 30-day mortality than short-term use in patients with bacteremia [13]. In addition, a dose–response relationship of decreased 30-day mortality risk with increasing number of prescriptions filled by statins users was demonstrated in our study. Further, the lack of association between 30-day mortality and the negative exposure control (calcium-channel blockers) makes the possibility of a healthy user effect less likely. This fact is also supported by the finding
that aspirin use which shares common anti-inflammatory properties with statins was also associated with reduced risk of 30-day mortality in patients with CDI.

Different aspects of the relationship between statins and CDI were previously studied. Previous statins use was found to be associated with reduced risk of CDI [10,11]. Previous statins use in patients with CDI was also found to be associated with successful response to treatment and reduced risk of CDI recurrence [22]. However, the same study did not find a statistically significant association between statins use and 30-day mortality among patients with CDI, but authors suggested that their study was underpowered to detect survival benefit [22].

Plausible explanations for our findings are the anti-inflammatory and immunomodulatory properties of statins [8], through which they may reduce the severity of CDI and consequently reduce mortality from CDI. This theory is further supported by our finding of reduced risk of mortality associated with aspirin, which shares anti-inflammatory properties with statins. In addition, both C. difficile toxin B and statins potentiate cytokine-induced nitric oxide synthase II expression through inhibition of small G proteins of the Rho family. A sudden high nitric oxide synthase II induction can lead to septic shock and death [23]. Hence, people who have been taking statins over a long period (but not short-term users) may be protected because their bodies have a constant basal state of inhibition of these small G proteins, so that C. difficile toxin B exposure is less harmful. However, the persistence of the protective effect of aspirin among non-statins users makes this explanation less likely.

The protective effect on acquiring CDI was found to be similar between the various statin types with an overall estimate of OR = 0.78 (95% CI 0.75–0.81) [11]. In line with these findings the beneficial effect on mortality in our study seems to be common to all statins (except rosuvastatin) with some variability in the individual statins-specific estimates (ranging from 0.24 to 0.64). Larger studies are needed to best assess the association between the individuals statins and 30-day mortality in patients with CDI.

Our study has several limitations; the main possible limitation of our study design is the case definition of CDI in that it was based only on the presence of positive C. difficile toxin assay as we had no data to ascertain whether the clinical presentation was also consistent with CDI. However, the instructions for all CHS microbiological laboratories are to perform C. difficile toxin assay only on fluid stool samples. Although this may result in misclassification it is likely to be non-differential and is expected to bias the estimates toward the null. In this study we used 30-day all-cause mortality because we did not have data on specific causes of death, and therefore we could not state whether the reduced risk of mortality associated with statins use is a direct result of reduced CDI mortality. However, 30-day all-cause mortality is acceptable in studies that assessed mortality in patients with CDI [5,24]. Further, a previous study has found that CDI-related deaths occurred mainly within 30 days after diagnosis [6]. In addition, this study shows that more than half of deaths detected in the first 6 months have occurred during the first 30 days which strongly suggests that most deaths occurring during the first 30 days may be attributable to CDI. Although we have adjusted for a large number of risk factors of mortality, our study may still suffer from residual confounding, therefore a cause and effect relationship cannot be proven by our study.

In conclusion, current and long-term statins use provides protection with a dose–response relationship against 30-day

### TABLE 4. Crude and adjusted ORs for the association of negative control exposure (calcium-channel blockers) with 30-day mortality in patients with Clostridium difficile infection

<table>
<thead>
<tr>
<th>Current calcium-channel blockers use category</th>
<th>Number of deaths (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted^a OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current users (two categories)^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 1493)</td>
<td>271 (18.2)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Yes (n = 395)</td>
<td>69 (17.5)</td>
<td>0.95 (0.71–1.28)</td>
<td>0.94 (0.60–1.18)</td>
</tr>
<tr>
<td>Current users (three categories)^c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n = 1493)</td>
<td>271 (18.2)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Short-term users (n = 52)</td>
<td>11 (21.2)</td>
<td>1.21 (0.61–2.38)</td>
<td>1.30 (0.62–2.75)</td>
</tr>
<tr>
<td>Long-term users (n = 343)</td>
<td>58 (16.9)</td>
<td>0.92 (0.67–1.25)</td>
<td>0.79 (0.55–1.13)</td>
</tr>
<tr>
<td>Prescription number^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR for increase in 1 prescription</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (n = 1493)</td>
<td>271 (18.2)</td>
<td>0.93 (0.81–1.07)</td>
<td>0.90 (0.77–1.05)</td>
</tr>
<tr>
<td>1 prescription (n = 129)</td>
<td>27 (20.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 prescriptions (n = 123)</td>
<td>24 (19.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3 prescriptions (n = 143)</td>
<td>18 (12.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\^aAdjusted for demographic variables, comorbidities and medications use included in Table 1.
\^bCurrent calcium-channel blocker use (yes) was defined if at least one prescription was filled during the 90 days before the index date.
\^cShort-term calcium-channel blocker use was defined if at least one prescription was filled during the 90 days before the index date.
\^dThe number of calcium-channel blocker prescriptions filled during the 90 days before the index date.

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mortality in patients with CDI. The beneficial effect of statins appears to be common to most statins with an additional suggestive beneficial effect of aspirin.

**Transparency Declaration**

The authors declare no conflict of interest.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Multivariate analysis of the association between statins use and 30-day mortality in patients with *Clostridium difficile* infection.

**Table S2.** Multivariate analysis of the association between aspirin use and 30-day mortality, in patients with *Clostridium difficile* infection.

**Table S3.** Multivariate analysis of the association between negative control exposure (calcium channel blockers) and 30-day mortality in patients with *Clostridium difficile* infection.

**References**