Supplementary Online Content


**Appendix A.** Calculation of the Null Ratio
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This supplementary material has been provided by the authors to give readers additional information about their work.
APPENDIX A: **Calculation of the Null Ratio**

The null ratio is the expected crude sequence ratio in the event of no relationship between the two study drugs. It is used to correct the crude sequence ratio using the formula:

\[
Adjusted \ Sequence \ Ratio = \frac{Crude \ Sequence \ Ratio}{Null \ Ratio}
\]

The null ratio is created by examining the date of each quinine start among all eligible index-quinine recipients and calculating the probability, on that day, of an index prescription coming first. This probability is determined from the number of population index prescriptions observed in the year before, compared to the number in the year after, the date of that quinine start. Each participant has their index first probability calculated in this manner and the index first probabilities of all eligible participants are averaged to create the “null probability” – which is the mean probability that a prescription pair within the data set would be index first if no causal relationship exists.

The null ratio, the expected ratio of index first to index second pairs in the event of no relationship then becomes:

\[
null \ ratio = \frac{null \ probability}{1 - null \ probability}
\]
APPENDIX B: The Relationship between Relative Risk and Sequence Ratio

Let’s assume that you want to compare the effect of two drugs (Drug A and a control drug, Drug B) in causing a particular outcome. The outcome could be anything – an event or, in the case of our analysis, the subsequent prescribing of another drug such as quinine. You could potentially perform two separate sequence symmetry analyses, one for each of Drug A and Drug B, and compare results. Or you could perform a survival analysis using cohorts of Drug A and Drug B recipients and look at the relative risk of Drug A recipients having the outcome compared to those receiving Drug B. We can mathematically describe both the relative risk (RR), and the sequence ratio (SR), and easily combine these equations to show how the relative risk and sequence ratio relate to each other.

Assuming: 1) Negligible censoring (e.g. using a risk interval and selecting only subjects who were present in the database throughout the follow-up period).

2) Equivalent time intervals for analysis (i.e. the symmetry analysis looks both forward, and then backward, a period of time equivalent to the follow-up period in the survival analysis).

3) Use of the same subjects (and hence the same outcomes).

Let: \( T_A \) = the total time recipients of Drug A are in the survival analysis

\( T_B \) = the total time recipients of Drug B are in the survival analysis

\( O_A \) = # of outcomes that follow receipt of Drug A
\( O_B = \# \text{ of outcomes that follow receipt of Drug B} \)

\( P_A = \# \text{ of outcomes that precede receipt of Drug A} \)

\( P_B = \# \text{ of outcomes that precede receipt of Drug B} \)

\( SR_A = \text{the sequence ratio of the outcome surrounding initiation of Drug A} \)

\( SR_B = \text{the sequence ratio of the outcome surrounding initiation of Drug B} \)

\( RR_{A/B} = \text{relative risk of the outcome in recipients of Drug A compared to Drug B} \)

Given:

\[
RR_{A/B} = \frac{O_A/T_A}{O_B/T_B} \tag{1}
\]

And:

\[
SR_A = \frac{O_A}{P_A} \quad \text{&} \quad SR_B = \frac{O_B}{P_B} \tag{2}
\]

(which can also be written \( O_A = SR_A \times P_A \) and \( O_B = SR_B \times P_B \))

Then substituting (2) into (1) gives:

\[
RR_{A/B} = \frac{O_A/T_A}{O_B/T_B} = \frac{SR_A \times P_A/T_A}{SR_B \times P_B/T_B} \tag{3}
\]

Or:

\[
RR_{A/B} = \frac{SR_A}{SR_B} \times \frac{P_A/T_A}{P_B/T_B} \tag{4}
\]

But, since we assume little or no censoring, both \( T_A \) and \( T_B \) are roughly equal to the number of individuals recruited into each cohort multiplied by the duration of follow-up.

\[
i.e. \ T_A = N_A \times t \quad \text{&} \quad T_B = N_B \times t \tag{5}\]
Where \( t \) = the duration of the follow-up window (e.g. 1 year)

\( N_A, N_B \) = the number of subjects recruited for survival analysis in each arm

Substituting equations (5) into equation (4) and cancelling out \( t \) we find:

\[
RR_{A/B} = \frac{S_{RA}}{S_{RB}} \times \frac{P_{A/N_A}}{P_{B/N_B}}
\]  

(6)

But \( P_{A/N_A} \) and \( P_{B/N_B} \) are the proportion of subjects with outcomes in an interval of time \( t \) before the study drugs are given. If the populations are chosen to have equivalent risk, outside of that incurred by the administration of Drug A or B, then these proportions are identical and cancel out. Hence we are left with:

\[
RR_{A/B} = \frac{S_{RA}}{S_{RB}}
\]  

(7)

But if Drug B has no association to the outcome \( S_{RB} = 1 \)

Hence  

\[
Relative \ Risk_{A/B} = Sequence \ Ratio_A
\]  

(8)

When

1) Receipt of Drug B is not correlated with occurrence of the outcome.

2) There is no loss to follow-up.
3) The survival analysis follow-up period matches the symmetry window (i.e. if the survival interval is t then the symmetry analysis looks forward t and backward t surrounding receipt of Drug A).

4) The baseline risk of the outcome is the same in both cohorts before drugs A and B are received.
APPENDIX C: Cox Proportional Hazards Analysis

We performed a Cox proportional hazards analysis comparing time to quinine start in new users of either inhaled LABA (exposed cohort) or inhaled anticholinergic (unexposed cohort) who first filled (and renewed within 1 year) their medication between Dec 1 2001 and Nov 30 2006. Subjects were excluded if they had prior renewed use of either cohort defining drug or a previous prescription for quinine. Censoring occurred upon addition of the other cohort defining medication (i.e a LABA cohort subject receiving inhaled anticholinergic, or vice-versa), after one year of follow-up and on Nov 30 2006. Subjects were excluded if they were less than 50 years old at the time they received their treatment and if they did not have evidence of medical services within the database for one year following, and two years preceding, receipt of the cohort defining medication.

We fit a model including all potential confounders of the exposure / outcome relationship. Increasing age, female gender and a number of diagnoses are known to be associated with cramps (including peripheral vascular disease, coronary artery disease, chronic kidney disease, dialysis, motor neuron disease, cirrhosis, radiculopathy / myelopathy and hypothyroidism). The number of patient visits to a family physician in the prior year and the number of distinct medication types received in the prior year were included as we thought this might indicate patients with more physician contact and a greater receptiveness to medication use. New starts of potassium-sparing and thiazide-like diuretics received within the prior year were also added as covariates based on our symmetry findings. Although not previously shown to be associated with cramps we included as covariates a diagnosis of either asthma or COPD in the prior 5
years, and use of salbutamol or inhaled steroids in the immediately prior year, in case these identified categories of LABA or anticholinergic users that were differentially susceptible to cramps. We also included a prior diagnosis of malaria since this is an alternate use for quinine.

Results of our Cox analysis are given in Table 3. The hazard ratio for LABA starters as compared to anticholinergic starters (HR 2.37 p <.0001 95%CI 1.73 to 3.23) is consistent with our symmetry results. Thiazide and potassium-sparing diuretic starts were not numerous enough for significance but had hazard ratios in a similar range to the ASR values calculated using symmetry. If only renewed diuretic starts are considered, the HR for potassium sparing diuretics becomes significant (HR 5.35 p=0.0009 95%CI 1.99 to 14.39), though this required searching for renewals during the risk interval. There was no significant interaction between LABA and potassium sparing diuretic starts. Cases of malaria and motor neuron disease were too few in number to include as covariates in the final model.

| Table 3. Cox Proportional Hazards Analysis of Time to Quinine Start in New Users of LABA Compared to New Users of Inhaled Anticholinergic |
|---|---|---|---|---|
| **Cohort** | **# of Subjects** | **# of Events** | **Covariates** |
| LABA | 15,423 | 196 |  |
| Anticholinergic | 8,307 | 58 |  |

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LABA</td>
<td>2.37</td>
<td>(1.73 to 3.23)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>1.03</td>
<td>(1.02 to 1.04)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Condition</td>
<td>Hazard Ratio</td>
<td>95% CI</td>
<td>P Value</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>Female Gender</td>
<td>1.40</td>
<td>(1.07 to 1.83)</td>
<td>.014</td>
</tr>
<tr>
<td># Distinct Drugs Used in Prior Yr</td>
<td>1.04^a</td>
<td>(1.01 to 1.06)</td>
<td>.002</td>
</tr>
<tr>
<td># Primary Care Visits in Prior Yr</td>
<td>1.00^b</td>
<td>(0.98 to 1.01)</td>
<td>.576</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>1.64</td>
<td>(1.11 to 2.43)</td>
<td>.013</td>
</tr>
<tr>
<td>Chronic Kidney Disease</td>
<td>2.22</td>
<td>(1.30 to 3.81)</td>
<td>.004</td>
</tr>
<tr>
<td>Dialysis</td>
<td>1.40</td>
<td>(0.18 to 10.71)</td>
<td>.746</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1.62</td>
<td>(0.60 to 4.37)</td>
<td>.341</td>
</tr>
<tr>
<td>Radiculopathy / myelopathy</td>
<td>2.19</td>
<td>(1.03 to 4.67)</td>
<td>.042</td>
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<tr>
<td>Hypothyroidism</td>
<td>1.67</td>
<td>(1.15 to 2.42)</td>
<td>.007</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>1.08</td>
<td>(0.81 to 1.42)</td>
<td>.614</td>
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<tr>
<td>Thiazide Diuretic Start In Prior Yr</td>
<td>1.50</td>
<td>(0.82 to 2.76)</td>
<td>.186</td>
</tr>
<tr>
<td>K-Sparing Diuretic Start in Prior Yr</td>
<td>2.47</td>
<td>(0.79 to 7.73)</td>
<td>.120</td>
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<tr>
<td>Asthma</td>
<td>1.05</td>
<td>(0.80 to 1.38)</td>
<td>.739</td>
</tr>
<tr>
<td>COPD</td>
<td>1.12</td>
<td>(0.71 to 1.76)</td>
<td>.626</td>
</tr>
<tr>
<td>Salbutamol Use In Prior Yr</td>
<td>0.76</td>
<td>(0.55 to 1.05)</td>
<td>.096</td>
</tr>
<tr>
<td>Inhaled Steroid Use In Prior Yr</td>
<td>1.28</td>
<td>(0.92 to 1.77)</td>
<td>.144</td>
</tr>
</tbody>
</table>

^a Hazard ratio per distinct drug used in prior year.
^b Hazard ratio per patient visit to a general practitioner in prior year.
APPENDIX D: **Attributable Proportions:**

a) **Determining the proportion of quinine starters receiving an index start in the prior year that can be attributed to that index drug:**

Using \[ \text{Attributable Proportion} = \frac{\text{ASR} \times \text{baseline rate} - \text{baseline rate}}{\text{ASR} \times \text{baseline rate}} \]

Or \[ \text{Attributable Proportion} = \frac{\text{ASR} - 1}{\text{ASR}} = 1 - \frac{1}{\text{ASR}} \]

Then the proportion of quinine starts in the year following the start of an index drug that are attributable to that index drug are:

For thiazides = \(1 - \frac{1}{1.48} = 0.324 = 32\%\)

For k-sparing diuretics = \(1 - \frac{1}{2.12} = 0.528 = 53\%\)

For LABA = \(1 - \frac{1}{2.42} = 0.587 = 59\%\)

b) **Estimating the proportion of all quinine starts attributable to these three index drugs:** To make this estimation we examined all BC residents over the age of 50 on Dec 1 2001 who received a new Rx for quinine between Dec 1 2001 and Nov 30 2006 (i.e. our study period). We then looked at the year before each quinine start to see if our index drugs were prescribed. Since an examination of figure 2 suggested that the increase in quinine prescribing following thiazides and potassium sparing diuretics was sustained, we considered that whether prescriptions were starts or not, diuretic use had a similar influence on cramp promotion. This did not seem a reasonable assumption with LABA as the risk appeared to be lower (though still present) 6 to 12 months after
the LABA start. In the case of LABA we considered starts and renewals separately. If a
LABA was prescribed in the year before quinine but was not a new start we used an
ASR calculated using only the latter 6 months of prescribing seen in Figure 2 (producing
ASR = 1.48). Determining how many quinine starters had received each of these index
drugs in the year prior, and using the ASR for those drugs to estimate the number of
attributable quinine prescriptions, we were able to estimate the proportion of all quinine
starts collectively attributable to the three index drugs. To be conservative we assumed
that users of more than one of these drugs had a cramp promoting effect only from the
drug with the greater ASR (e.g. if both a potassium-sparing and thiazide diuretic were
received in the year prior to starting quinine we assumed only the potassium-sparing
diuretic contributed to cramp promotion – hence in the calculation below that individual
was counted as having a potassium-sparing diuretic in the year before but the thiazide
was not counted).

The proportion of all quinine starts attributable to the three index drugs was estimated
by:

\[
\text{Attributable Proportion}_{\text{all quinine starts}} = \left(1 - \frac{1}{\text{ASR}_L}\right) \times S_L + \left(1 - \frac{1}{\text{ASR}_{L*}}\right) \times (C_L - S_L) + \left(1 - \frac{1}{\text{ASR}_K}\right) \times C_K + \left(1 - \frac{1}{\text{ASR}_T}\right) \times C_T
\]

Where

- \(\text{ASR}_L\) = Adjusted Sequence Ratio for LABA = 2.42
- \(\text{ASR}_{L*}\) = ASR for LABA use that is not a new start = 1.48
- \(\text{ASR}_K\) = Adjusted Sequence Ratio for K-Sparing Diuretics = 2.12
- \(\text{ASR}_T\) = Adjusted Sequence Ratio for Thiazides = 1.48
\[ S_L = \text{Number of LABA starts prior to starting quinine} = 596 \]

\[ C_L = \text{Total number receiving LABA prior to starting quinine} = 1,731 \]

\[ C_T = \text{Total number receiving Thiazide prior to starting quinine} = 4,459 \]

\[ C_K = \text{Total number receiving K-Sparing prior to starting quinine} = 2,352 \]

\[ \# \text{ quinine starts} = 25,129 \]

Using these numbers in the above formula gives an \textbf{Attributable Proportion} = \textbf{13.6%}