Abstract

A clinical trial was conducted to assess the safety and efficacy of a new medication for the management of blood glucose in diabetes patients. Two treatments (drug and placebo) were administered to two different groups of diabetes patients for twelve weeks. Based on the design of the trial, we analyze whether i) treatment is associated with improved control of blood glucose levels over the twelve-week trial, and ii) whether drug treatment is associated with an improvement over and above that associated with placebo.

1 Introduction

A clinical trial was conducted to assess the safety and efficacy of a new medication for the management of diabetes using multi-center, randomized, placebo controlled, and parallel design. The primary objective was to discern the efficacy of drug treatment from placebo in improving long-term (several months) patient blood glucose control, as measured by glycated hemoglobin levels (HbA1c). Higher HbA1c is associated with increased risk for diabetes-related complications. Data on primary efficacy variable (HbA1c), secondary efficacy variable (Fasting Blood Glucose), and several safety variables were collected at week zero (pre-dose) and week twelve (post-dose).

Based on the design of the trial, we justify an analysis to determine whether i) treatment (drug or placebo) is associated with significantly improved blood glucose control as indicated by a change in HbA1c and/or Fasting Blood Glucose levels over the twelve-week trial, and ii) drug treatment is associated with significant improvement over and above that associated with placebo. The analysis comprises three steps:

1. Use baseline (pre-dose) measurements to validate randomization by comparing patients in the two treatment groups across all variables.

2. Detect for changes in HbA1c and Fasting Blood Glucose levels from pre-dose to post-dose within each treatment group.

3. Discern between the effects of drug treatment and placebo on changes in HbA1c and Fasting Blood Glucose levels over the course of the trial.
Employing the analysis described herein, we conclude that i) patients randomly administered either treatment experienced improved control of blood glucose levels as reflected by the twelve-week change in primary and secondary efficacy measures, and ii) patients administered drug treatment experienced an improvement over and above that associated with placebo. In the following sections we detail the methods employed to arrive at this conclusion, provide the theoretical justification and quantitative validation of model assumptions, and summarize the results of our analysis.

2 Methodology

2.1 Trial Design

Of primary interest is the effect of drug intervention on long-term blood glucose control in diabetes patients. Changes in blood glucose experienced by patients in the trial must be considered relative to the inherent variation in blood glucose levels observed in the population of all diabetes patients. Therefore, clinicians seek to quantify inherent variation for the purpose of qualifying treatment effects. Conducting the trial at three different centers is a means of blocking or separating inherent variation in blood glucose levels from systematic variation introduced by "nuisance factors," or sources of variability that are not of primary interest. Such "nuisance" factors may be as subtle as the time of day treatment is administered and the behaviours of employees at the center. In theory, blocking controls for such systematic sources of variation; thus, isolating the "true" variation.

This clinical trial can be classified as a two-factor randomized block experiment (Table 1), where the factors and respective levels are:

a. Treatment (factor-of-interest)
   i. Placebo
   ii. Drug
b. Center (blocking factor)
   i. Center 1
   ii. Center 2
   iii. Center 3

For each patient, measurements are repeated (one at pre-dose and post-dose, respectively) so observations are dependent in time. The measure $\Delta$HbA1c $\delta$ collapses data, taking advantage of the repeated measures structure to make data amenable to randomized block analysis (Table 1).

2.2 Analysis

With the specified trial design, we analyze the effect of drug intervention via a three-step approach.
Table 1: Two-factor randomized block design of clinical trial. Note the repeated measures (over time) structure.

1. Use baseline (pre-dose) measurements to validate randomization by comparing patients in the two treatment groups across all variables.

Validating the randomization assumption reasonably assures that patients in both treatment groups are selected from the same underlying population (diabetes patients); thus, facilitating comparison between groups. Therefore, we compare the two treatment groups using a t-test for two independent samples on all baseline measurements.

2. Detect for changes in HbA1c and Fasting Blood Glucose levels from pre-dose to post-dose within each treatment group.

For each patient, we take the twelve-week change in efficacy variables as the primary and secondary response measures (Table 2). Using the twelve-week change takes advantage of the “automatic blocking” introduced when repeated measurements are taken on the same experimental unit (patient) over time. For each treatment group, we separately utilize a t-test for pairwise dependent observations to determine if treatment is associated with a significant improvement (over time) in blood glucose management.

3. Discern between the effects of drug treatment and placebo on changes in HbA1c and Fasting Blood Glucose levels.

Only if significant changes are detected from pre-dose to post-dose, need we proceed to the question of whether drug is more effective than placebo in improving blood glucose management. Furthermore, if the randomization assumption is valid, we may address this question using simple techniques. Based on the results of steps 1 and 2, we use the two-sample
pooled t-test to determine if the change in blood glucose levels observed in patients administered the drug is significantly different from the corresponding change observed in patients administered the placebo.

<table>
<thead>
<tr>
<th>Patient</th>
<th>HbA1c</th>
<th>Pre-dose</th>
<th>Post-dose</th>
<th>ΔHbA1c</th>
<th>Δ</th>
<th>$\bar{D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>1</td>
<td>HbA1c₁</td>
<td>HbA1c₁</td>
<td>$\Delta_1$</td>
<td>$\Delta$</td>
<td>$\frac{1}{29} \sum_{i} \Delta_i$</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>HbA1c₂₉</td>
<td>HbA1c₂₉</td>
<td>$\Delta_{29}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1</td>
<td>HbA1c₁</td>
<td>HbA1c₁</td>
<td>$\Delta_1$</td>
<td>$\Delta$</td>
<td>$\frac{1}{30} \sum_{i} \Delta_i$</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>HbA1c₃₀</td>
<td>HbA1c₃₀</td>
<td>$\Delta_{30}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\delta = \bar{D} - \bar{P}$

Table 2: Primary response, $\Delta$HbA1c, and parameter of interest, $\delta$, for detecting and discerning between treatment effects on glycated hemoglobin.

### 3 Data Description

- Data were provided in two flat files, one for each treatment group. A flat file contains a single record for each patient in a given treatment group, with Patient Number as the primary key.

- Patients were assigned to one of three different centers so patient numbers are unique at each center, as well as across all centers.

- For each patient, eighteen efficacy and safety variables were observed at pre-dose and post-dose, respectively. Therefore, each record has values in thirty-six measurement fields, eighteen fields corresponding to each time point (Table 2).

- There are several missing values in both files, so data are incomplete. However, complete data are available for several fields, including the primary efficacy variable.

- Treatment groups are analyzed in order to test the single population hypothesis by i) merging records for both treatment groups ii) sorting by time (week zero and week twelve), then iii) creating two separate flat files, one for week zero and
week twelve, respectively. Each resulting flat file contains a single record for each patient in the trial, with pre-dose measurements in one file and corresponding post-dose measurements in the other.

4 Analysis

In order to validate the randomization assumption, we plot pre-dose efficacy measures by treatment group and center (Figure 1). Figure 1 does not suggest gross violations of the randomization assumption; however, the location and spread of observations for Center 2 differ from those of Centers 1 and 3. We formally test the randomization assumption using the t-test for independent samples across all variables using Proc ttest, with partial output shown in Table 3. Specifying $\alpha = 0.05$ for the remainder of the analysis, we find no significant difference between patients when separated by treatment group or center, based on individual t-tests. Based on this result, we address the question of whether either drug or placebo is associated with a significant change in efficacy measures over the twelve-week trial.

![Pre-Dose Efficacy Measures](image)

Figure 1: Pre-dose efficacy measures by Treatment Group and Center.

The plot of $\Delta$HbA1c and $\Delta$Fasting Blood Glucose by treatment group and center provides visual evidence of treatment effects (Figure 2). There is strong indication
Table 3: Pre-dose t-tests for $H_{0A}: \overline{HbA1c}_D = \overline{HbA1c}_P$ and $H_{0B}: \overline{Fbg}_D = \overline{Fbg}_P$ at $\alpha = 5\%$

| Measure | Treatment | Mean  | S.E.  | $Pr(T > |t_0|)$ | Pooled (d.f.) | Satterthwaite (d.f.) |
|---------|-----------|-------|-------|----------------|---------------|---------------------|
| HbA1c   | Drug      | 0.0926| 0.00398| 0.1112          | 0.1130        | (57)                | (53.325)            |
|         | Placebo   | 0.0845| 0.0031 | (               )|               |         |
| Fbg     | Drug      | 182.2 | 10.9741| 0.5006          | 0.5006        | (49)                | (48.923)            |
|         | Placebo   | 171.8 | 10.7655| (               )|               |         |

that patients administered the drug experienced greater decreases in both HbA1c and Fasting Blood Glucose, in comparison to those administered the placebo. In order to assess whether either drug or placebo is associated with decreases in primary and sec-
Secondary efficacy measures, we utilize the t-test for pairwise dependent data. Formally, we test $H_{0A}: \Delta D = 0$ and $H_{0B}: \Delta P = 0$. This test, for both $\Delta HbA1c$ and $\Delta Fasting Blood Glucose$, corroborates visual evidence; hence, we conclude that drug intervention is associated with significant change in both efficacy measures (Table 4), while placebo is associated with a significant change in $HbA1c$. Since significant changes are detected from pre-dose to post-dose, we proceed to the question of whether drug is more effective than placebo in improving blood glucose management.

| Measure  | Treatment | Mean  | S.E. | d.f. | Pr($T > |t_0|$)  |
|----------|-----------|-------|------|------|-----------------|
| $\Delta HbA1c$ | Drug      | -0.0174 | 0.00312 | 28   | < 0.0001        |
|           | Placebo   | -0.00613 | 0.00233 | 29   | 0.0133          |
| $\Delta Fbg$ | Drug      | -43.4  | 14.7773 | 24   | 0.0073          |
|           | Placebo   | -1.8    | 13.6867 | 24   | 0.8965          |

Table 4: Post-dose t-tests for $H_{0A}: \Delta D = 0$ and $H_{0B}: \Delta P = 0$ at $\alpha = 5%$

From Figure 3, it appears that patients with higher pre-dose primary and secondary efficacy measures experience a greater response to drug treatment than placebo. This is expected and justifies using the change in efficacy measures as the appropriate value upon which to base conclusions. Performing a two-sample pooled t-test for both efficacy measures, we conclude that change in blood glucose levels observed in patients administered the drug is significantly different from the corresponding change observed in patients administered the placebo. Based on the sign of the this difference, we may further state that drug intervention is associated with an improvement over and above that associated with placebo.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean</th>
<th>S.E.</th>
<th>Pooled $(d.f.)$</th>
<th>Satterthwaite $(d.f.)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta_{HbA1c}$</td>
<td>-0.0113</td>
<td>0.00387</td>
<td>0.0050 (57)</td>
<td>0.0053 (52.243)</td>
</tr>
<tr>
<td>$\delta_{Fbg}$</td>
<td>-45.2</td>
<td>20.1419</td>
<td>0.0295 (48)</td>
<td>0.0295 (47.721)</td>
</tr>
</tbody>
</table>

Table 5: Post-dose t-test for $H_{0A}: \delta_{HbA1c} = 0$ and $H_{0b}: \delta_{Fbg} = 0$ at $\alpha = 5%$
5 Summary of Findings

The result of t-test of two independent samples yields $p-value = 0.5006$ using pooled and separate variances, indicating that patients entered the trial with the similar blood glucose profiles. This validates the assumption that patients were randomly assigned to each treatment group and center. Consequently, when we detect for changes in efficacy measures within each treatment group, we observe that both drug and placebo are associated with a significant decrease HbA1c. Additionally, drug is associated with significant decrease with Fasting Blood Glucose. As a result, we seek to discern between the effects of drug treatment and placebo on changes in HbA1c over the course of the trial.

After twelve weeks, when testing for a significant difference in means between the two treatment groups, we find such a difference exists when measuring Fasting Blood Glucose but not when measuring Hb1Ac. The difference in Fasting Blood Glucose between drug and placebo is $31.8523$ with $s.e. = 15.6226$, and the $p-value = 0.0469$ using pooled variance and $0.0465$ using separate variances. The difference in means of Hb1Ac is $0.00319$ with $s.e. = 0.005$, and the $p-value = 0.5258$ using pooled variance and $0.5252$ using separate variances. The contrast between the two primary efficacy
measures may be explained by the examining baseline patient profiles. The baseline difference between treatment groups in Hb1Ac is greater than the difference in Fasting Blood Glucose by comparing the \textit{p-values} of the test (0.11 for Hb1Ac is and 0.5006 for Fasting Blood Glucose). The baseline difference between the two treatment groups with respect to Hb1Ac makes it difficult to compare the difference after twelve weeks. In order to overcome this, we utilize the change in efficacy measures to test whether differences exist between the two treatments. Using the pairwise t-test we observe that the mean of Hb1Ac is $-0.0113$ with $s.e. = 0.00387$, and \textit{p-value} = 0.0053, while the mean of Fasting Blood Glucose is $-45.2000$ with $s.e. = 20.1419$ and associated \textit{p-value} = 0.0295. Thus, we see there is a significant difference between treatments in measuring both Hb1Ac and Fasting Blood Glucose. Hence, graphical evidence and formal tests support the conclusion that patients administered drug treatment experienced an improvement in blood glucose control over and above that associated with placebo.
SAS Code

*Import the data set;

PROC IMPORT OUT= WORK.week0
   DATAFILE= "F:\week0.csv"
   DBMS=CSV REPLACE;
   GETNAMES=YES;
   DATAROW=2;
RUN;
PROC IMPORT OUT= WORK.week12
   DATAFILE= "F:\week12.csv"
   DBMS=CSV REPLACE;
   GETNAMES=YES;
   DATAROW=2;
RUN;

Data diabetes;
Merge week0 week12; by time treat center;
run;

PROC IMPORT OUT= WORK.drug
   DATAFILE= "F:\drug.csv"
   DBMS=CSV REPLACE;
   GETNAMES=YES;
   DATAROW=2;
RUN;

PROC IMPORT OUT= WORK.placebo
   DATAFILE= "F:\placebo.csv"
   DBMS=CSV REPLACE;
   GETNAMES=YES;
   DATAROW=2;
RUN;

data drug;
set drug;
dHbA1cwk=HbA1cwk12-Hba1cwk0;
dFBGWk=FBGwk12-FBGWk0;
dWTwk=WTwk12-WTwk0;
dBMIwk=BMIIwk12-BMIwk0;
dTCHOLwk=TCHOLwk12-TCHOLwk0;
dHDLwk=HDLwk12-HDLwk0;
dLDLwk=LDLwk12-LDLwk0;
dTrigwk=Trigwk12-Trigwk0;
dSBPwk=SBPwk12-SBPwk0;
dDBPwk=DBPwk12-DBPwk0;
dUreawk=Ureawk12-Ureawk0;
dUAcidwk=UAcidwk12-UAcidwk0;
dMicroalbuminwk=Microalbuminwk12-Microalbuminwk0;
run;
data placebo;
set placebo;
dHbA1cwk=HbA1cwk12-HbA1cwk0;
dFBGWk=FBGWk12-FBGwk0;
dWTwk=WTwk12-WTwk0;
dBMIwk=BMIwk12-BMIwk0;
dTCHOLwk=TCHOLwk12-TCHOLwk0;
dHDLwk=HDLwk12-HDLwk0;
dLDLwk=LDLwk12-LDLwk0;
dTrigwk=Trigwk12-Trigwk0;
dSBPwk=SBPwk12-SBPwk0;
dDBPwk=DBPwk12-DBPwk0;
dUreawk=Ureawk12-Ureawk0;
dUAcidwk=UAcidwk12-UAcidwk0;
dMicroalbuminwk=Microalbuminwk12-Microalbuminwk0;
run;
proc ttest data=week0;
var HbA1c Fasting_Blood_Glucose;
class treat;
run;
proc ttest data=week12;
var HbA1c Fasting_Blood_Glucose;
class treat;
run;
proc univariate data=drug;
var dHbA1cwk dFBGWk;
run;
proc univariate data=placebo;
var dHbA1cwk dFBGWk;
run;
data treatment;
Merge drug placebo; by Treat center;
run;
proc ttest data=treatment;
var dHbAlcwk dFBGWk;
class treat;
run;

proc contents data = drug out =druginf;
run;

/**************************************************/
/*/First table, to find mean and count
of efficacy variables in week 0 */
data week00;
set week0 (keep = HbA1c Fasting_Blood_Glucose treat);
if nmiss(of _numeric_) then delete;
*get rid of the missing values
run;
data week00;
if treat = 0 then treatment = "drug";
else treatment = "placebo";
run;
proc print data = week00;
run;
proc tabulate data=week00;
class treat;
var HbA1c ;
table treat, HbA1c*(mean n)*f=comma8.;;
*why the mean is 0, check;
run;
proc tabulate data=week00;
class treat;
var Fasting_Blood_Glucose;
table treat, Fasting_Blood_Glucose*(mean n)*f=comma8.;
run;

/*/second table, to find the mean and n of
these two variable in week12*/
data week12;
set WORK.week12 (keep = HbA1c Fasting_Blood_Glucose treat);
if nmiss(of _numeric_) then delete;
*get rid of the missing values;
run;
proc print data = week12;
run;

proc tabulate data=week12;
class treat;
var HbA1c;
table treat, HbA1c*(mean n)*f=comma8.;
*why the mean is 0;
run;

proc tabulate data=week12;
class treat;
var Fasting_Blood_Glucose;
table treat, Fasting_Blood_Glucose*(mean n)*f=comma8.;
*why the mean is 0;
run;

/*third table, to find the difference of drug between week0 and week12*/
data drug1;
set drug (keep = dHbA1cwk dFBGWk);
if nmiss(of _numeric_) then delete;
*get rid of the missing values.*
run;

proc print data = drug1;
run;

proc tabulate data = drug1;
var dHbA1cwk;
table dHbA1cwk*(mean n)*f=comma8.;
*why the mean is 0;
run;

proc tabulate data = drug1;
var dFBGWk;
table dFBGWk*(mean std)*f=comma8.;
run;

/*fourth table, to find the difference of placebo between week0 and week12*/
data placebo1;
set placebo (keep = dHbA1cwk dFBGWk);
if nmiss(of _numeric_) then delete;
*get rid of the missing values;
run;

proc tabulate data = placebo1;
var dHbA1cwk;
table dHbA1cwk*(mean Std)*f=comma8.;
*why the mean is 0;
run;

proc tabulate data = placebo1;
var dFBGWk;
table dFBGWk*(mean Std)*f=comma8.;
run;

/*fifth table, combining the
data sets of two weeks*/
data treatment1;
set treatment (keep = dHbA1cwk dFBGWk Treat);
if nmiss(of _numeric_) then delete;
*get rid of the missing values;
run;

proc print data = treatment1;
run;

proc tabulate data=treatment1;
*find the mean and n of these two variables;
class Treat;
var dHbA1cwk ;
table treat, dHbA1cwk*(mean n)*f=comma8.;
*why the mean is 0, check;
run;

proc tabulate data=treatment1;
class Treat;
var dFBGWk;
table treat, dFBGWk*(mean n)*f=comma8.;
run;

/*sixth table, use t-tst to test if both dHbA1cwk and dFBGWk equal to 0.*/
proc tabulate data = treatment1;
var dHbA1cwk;
table dHbA1cwk*(mean Std)*f=comma8.;
*we need p-value in stead of mean here;
run;

proc tabulate data = treatment1;
var dFBGWk;
table dFBGWk*(mean Std)*f=comma8.;
*we need p-value in stead of mean here;
run;

/**************************
*we also can optput the contents of
the data, here we go....
*/

proc contents data = drug out =druginf;
run;

proc contents data = placebo out = placeboinf;
run;

proc contents data = treatment out = treatmentinf;
run;