Abstract

Cholesterol levels are among the most important cardiovascular risk factors in humans. Patients with dyslipidemia suffer from low cholesterol levels and are at high risk of cardiovascular disease. It is hypothesized that Cholesteryl Ester Transfer Protein (CETP) increases breakdown of high density lipoprotein (HDL) cholesterol and that CETP can be inhibited through the use of choletrapib. To test this claim, we conducted a double-blinded randomized control trial on a group of 485 dyslipidemia patients. The treatment group received choletrapib, while the control received placebo. The measures of HDL cholesterol were taken at three time points: at the baseline, 1 year into the treatment and 2 years into the treatment. An ANCOVA with baseline HDL cholesterol levels as a covariate was fitted to the data. The results indicate that HDL levels of the treatment group 24 months into treatment were 30.5 mg/dl higher than the control group.

Methods

Study Design

A double-blinded randomized control trial was conducted to study the effects of choletrapib, an inhibitor of Cholesteryl Ester Transfer Protein (CETP), on HDL levels in patients with dyslipidemia. The primary endpoint was HDL cholesterol. The measured variables are: age at baseline, height at baseline, weight at baseline, gender, smoking status, diabetes mellitus (yes/no), baseline waist circumference, hypertension (during follow-up), SBP in mmHg (at baseline and month 24), DBP in mmHg (at baseline and month 24), HDL cholesterol in mg/dl (at baseline, month 12, and month 24), LDL cholesterol in mg/dl (at baseline, month 12, and month 24). Descriptive statistics (mean and standard deviation or frequencies) for all baseline variables were calculated per treatment arm.

Sample Size Calculation

We determined the sample size needed for the study using R version 4.1.3 and the *pwr.anova.test* function from the *pwr* library (version 1.3-0). This function can be used for statistical power analysis and sample size calculation for AN(C)OVA. Sample size calculation was based on a power of 80% and an one-sided significance level of 5%, assuming the expected difference in HDL cholesterol between the treatment and placebo group after 24 months is 4mg/dl, standard deviation $\sigma = 15$. Using this information, the expected effect size is $f = 0.13$. This yielded a minimal sample size of 444 participants. To account for the drop-out rate, we add 5% to each arm resulting in a minimum of 466 participants in total (so 233 per arm).

Statistical analysis

The aim of this study is to inspect if treatment with choletrapib increases breakdown of HDL in the liver. This is done by comparing HDL cholesterol levels between the treatment and placebo arms. Analysis was done on the intention-to-treat population. Cases with missing values were removed. We fit an ANCOVA model with baseline HDL cholesterol as a covariate in order to test for a significant difference in HDL cholesterol levels between the two condition at 24 months. HDL cholesterol levels at baseline are added as a covariate to control for possible baseline differences that might influence HDL cholesterol levels at 24 months. The mean difference in HDL cholesterol between the groups was calculated, a significance level of .05 is used for the ANCOVA analysis. Analyses were performed in R (version 4.1.3).

Results

Participants

The sample size calculation indicated that we need a total of 466 participants. We collected data on 485 participants that were randomized in one of the two treatment arms. The treatment arms consist of 230 patients taking choletrapib and 255 patients taking placebo. The mean age was 57.4 (*SD* = 7.5) and 58.1 (*SD* = 7.9) in the placebo and choletrapib groups, respectively. The mean HDL level measured at baseline was 47.6 (*SD* = 9.2) for the placebo group and 47.1 (*SD* = 11.4) for the choletrapib group. Participants in the two treatment arms show similar levels on the baseline characteristics (see Table 1).

Outcome

From the 485 participants, 12 were excluded from the analysis because of missing values on either the covariate (baseline HDL levels) or the outcome variable (HDL levels after 24 months of treatment). We also identified 19 outliers (on baseline HDL levels and/or HDL levels after 24 months of treatment) in our original 485 participants, 6 of whom also had missing values. Outliers were identified using the IQR method. Analyses were performed using both the full data as well as the data with these participants removed. Substantive results did not differ, however the data without outliers more closely followed the assumptions needed for using ANCOVA. Therefore, only the results on these data are reported.

To compare the means on the outcome HDL levels at 24 months into treatment while controlling for the baseline HDL levels, a one-way ANCOVA model was fit. From this model, it follows that there was a significant difference ($F(1, 457) = 385.7$, $p < 0.001$) in mean HDL level at 24 months between the placebo ($M = 46.4$ mg/dl, $SD = 9.9$) and the choletrapib ($M = 74.9$ mg/dl, $SD = 18.3$) groups. Controlling for baseline HDL levels, the mean difference in HDL levels at 24 months into treatment between the placebo and choletrapib groups is estimated to be 30.5 mg/dl (95% CI: 28.5 - 32.4).

Table 1: **Patient characteristics at baseline**

		Placebo group	Choletrapib group
n		255	230
Age (mean (SD))		57.39 (7.45)	58.13 (7.94)
Height (mean (SD))		171.94 (9.48)	171.32 (9.16)
Weight in kg (mean (SD))		88.19 (15.46)	87.83 (16.33)
Sex (n (%))	Male	171 (67.1)	152 (66.1)
	Female	84 (32.9)	78 (33.9)
Smoking (n (%))	No	223 (87.5)	193 (83.9)
	Yes	32 (12.5)	37 (16.1)
Diabetes mellitus (n (%))	No	195 (76.5)	192 (83.5)
	Yes	60 (23.5)	38 (16.5)
SBP in mmHg (mean (SD))		119.87 (9.96)	120.51 (10.79)
DBP in mmHg (mean (SD))		74.79 (6.41)	74.08 (6.90)
HDL cholesterol in mg/dl (mean (SD))		47.56 (9.21)	47.14 (11.44)
LDL cholesterol in mg/dl (mean (SD))		101.71 (19.14)	99.31 (21.83)
Waist circumference value (cm) (mean (SD))		100.82 (12.26)	99.10 (13.80)

References

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd Ed). Hillsdale, NJ: Lawrence Erlbaum Associates.

Appendix

During the assumption checks we ran into some deviations from normality. Since ANCOVA is rather robust against these deviations, we continued with the analysis.