**Statistical Analysis Plan (Translation)**

STUDY: Androcan

Author: JF Dreyfus, MD, PhD

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Scientific Coordinator: Henry Botto, MD

Sponsor: Hôpital Foch, 92150−Suresnes, France

# Protocol

Version 10, dated Octobler 20th, 2015.

The present Statistical Analysis Plan only details operations that are to be conducted once the collection of data from the preoperative and peroperative period is considered complete.

An independent plan will be prepared to detail the statistical processes to be used once the 1−year postoperative data are available.

Data from the twin study conducted in the French Antilles (Martinique) are available but will not be used in a separate analysis.

# Data management

Hôpital Foch is responsible for the data quality control, data qualitiy assurance and regulatory adherence. The Statistician guarantees adherence to the MR−001 methodology as set un by the Frenc CNIL[[1]](#footnote-1).

Data are collected:

− on an electronic file for data gathered preoperatively including questionnaires (as individual item scores). Data entry is done by local staff;

− from the hormonology assay cenre of CHU Mondor (Pr Fiet) as Excel® workbooks directly output by assay automata;

− on an electronic file after central reviewing of slides and, if needed, consultation with the on−site pathologist.

In all the files, patients will be indentified by a unique number with 2 digits for the centre and 3 digits for each new patient, in ascending oeqseitinl order.

Files will be sent to the Statistics Unit of the Delegation for Clinical Research and Innovation, Hôpital  Foch as data collection advances.

The files received will be merged under the responsibility of JF Dreyfus, MD, PhD, head of the Statistics Department.

Once recruitment is terminated, after monitoring visits have ensured that the file content is acceptable, the complete file will be sent with summary data (including stem−and−leaf diagrams and listings) to the Steering Committee of the study; the Committee will review the data and make decisions on which data are to be included in the analysis.

It is anticipated that the following data will be excluded from the analysis:

− data from centres, if any, that are excluded from the trial for recruitment or quality issues;

− data from patients that did not sign an niformed consent form in keeping with the French regulations;

− data from patients for whom a gross violation of eligibility criteria is detected which was obvious at patients' inclusion. In case a patient is excluded based of this provision, eligibility criteria will be reviewed for all study cases so as to ensure a fair and uniformized approach for all cases.

Unaccepted patients' data will be quarantined so as not to be usable for analysis but to remain accessible in the database to allow for audits or inspections.

The database will then be locked and among other precautions to secure the data in this version of the database, listings will be printed to be kept by the sponsor and by the Steering Committee.

An electronic copy of this file will be used as the starting point for amalyses and to report modifications, if any, in the analysis logbook that is part of the final statistical report.

The following parameters will be considered of interest :

| **Variable name** | **Origin[[2]](#footnote-2)** | **Type[[3]](#footnote-3)** |
| --- | --- | --- |
| Patient Identification | c | ALP |
| Centre | c | CAT |
| Ethnic group | c | CAT |
| Height | c | NUM |
| Weight | c | NUM |
| BMI | d | NUM |
| Obesity | d | CAT |
| Overweight | d | CAT |
| Fatty mass | c | NUM |
| Age | d | NUM |
| Waistline | c | NUM |
| History of cancer | c | CAT |
| Biopsy stage | c | CAT |
| Biopsy grade | c | CAT |
| Biopsy Gleason score | c | ORD |
| PSA | c | NUM |
| Diabetes | c | CAT |
| Cardiovascular disease | c | CAT |
| Hypertension | c | CAT |
| Total cholesterol | b | NUM |
| HDL cholesterol | b | NUM |
| Triglycerides | b | NUM |
| Blood sugar | b | NUM |
| Medication (× 6) | d | CAT |
| At least one medication | d | CAT |
| LH | h | NUM |
| FSH | h | NUM |
| SHBG | h | NUM |
| Bioavailable testosterone | h | NUM |
| Low bioavailable testosteron | d | CAT |
| Testosterone | h | NUM |
| Low testosterone | d | NUM |
| Free TT | d | NUM |
| DHT | h | NUM |
| DHEA | h | NUM |
| Δ5 | h | NUM |
| D4 | h | NUM |
| E1 | h | NUM |
| E2 | h | NUM |
| DHEA sulfate | h | NUM |
| E1 sulfate | h | NUM |
| Weight of prostate specimen | p | NUM |
| Dominating grade of specimen | d | CAT |
| Grade category of specimen | p | CAT |
| Gleason score of specimen | p | ORD |
| Margins | p | CAT |
| Ganglia | p | CAT |
| Hypogonadism | d | CAT |
| IIEF score | q | NUM |
| AMS somatic subscore | d | NUM |
| AMS psychological subscore | d | NUM |
| AMS sexual score | d | NUM |
| AMS global score | q | NUM |

# Statistical Analysis

The present study may be considered as a cohort study that should help determine actual proportions, locations and variabilities of the relevant parameters. When comparisons are performed between groups, the superiority paradigm should be used by default.

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**Schedule**

The final analysis should olny take place when the Steering Committee has locked the daatabase after declaring that the expected sample size has been reached.

**Significance level**

Since the study is not a Randomized Control Trials, the probability value ascribed to a difference or a correlation is to be taken with caution.

Nevertheless, a bilateral p < 0.05 will be considered significant while 0.05 < p <0.10 is to be considered as indicative of a trend (abridged to 'trend'.

Bootstrapping with k=3000 iterations will be used when a 95% CI is to be obtained.

If multiple comparisons are performed, the Bonferronni−Holms correction is to be used so as to preserve the anticipated experimentwise significance level.

No pairwise analysis is to be performed if the global difference for the relevant factor is not significant or does not show a trend.

Except for questionnaires there will be no imputation of data.

As to the questionnaires, the following rules will be used:

− IIEF5: 4 items must be scored for the questionnaire to be considered valid; when there is a signle missing item score, it will be imputed using the patient's mean for scored items rounded to the nearest integer;

−AMS: one missing item per subdomain is acceptable. The missing item is replaced by the mean value of the patient's remaining subdomain items (rounded to the nearest integer). The global score will be accepted if at most 3 items have not been scored whether these 3 items are from the same or from different subdomains. Therefore, a global score may be present even if a subdomain score is missing. Missing items will be imputed using the mean value (rounded to the nearest integer) of the remaining items for the patient considered.

**CONSORT flowchart**

A flowchart will summarize how many patients have been approached, how many were included and how many provided data for critical parameters.

The same chart will tally the number of cases that did not meet eligibility criteria or declined to participate, withdrew their consent after it was signed, could not be operated (no specimen), ol did not provide hormonal levels and why.

**Analysis population**

Since the completion rate of the questionnaires is likely to be around 85%, the Steering Committee has required that two independent analyses be performed:

− on patients for whom clinical, biology, hormonology and pathology data are available except if they were quarantined by the Steering Committee;

− on patients for whom, in addition, complete questionnaires will be available.

**Outcome criteria**

There are 3 types of outcole criteria that should be analysed:

− hormone levels: LH, FSH, SHBG, testosterone, bioavailable testosterone, free testosterone, DHT, DHEA, Δ5, Δ4, E1, E2, DHEA sulfate, E1 sulfate. All these variables are continuous but Hypo TT and Hypo BT that are categorical;

− pathology outcones: Gleason score, which is ordinal and may be treated as a continuous parameter, Grade, Stage, Margins, Predominant grade that are categorical ;

− questionnaire global scores (IIEF5 and AMS) and AMS subscores (somatic, psychological and sexual) that are continuous.

**Statistical procedures**

*Quality control of database*

Each qualitative parameter will be checked to ensure that its values are within the expected range; values that are outside the range will be imputed if there is an obvious explanation for the inappropriate value (for instance a « I » intead of a « 1 » or a missing letter in a label); for continuous parameters, Grubb's outlier test will be used. A list of outlying and/or inappropriate values will be submitted to the Steering Committee for it to decide whether they should be quarantined or imputed and in the latter case, how?

All changes made to the database are to be reported in the analysis logbook and the file with its modification should be stored as it replaces the active database for the forthcoming statistical processes.

*Assessment of normality of distribution for hormonal parameter*s

It is assumed that hormonology parameters have a nonnormal distribution; normality will be tested using the Shapiro−Wilks test.

Whatever the results, nonparametric tests will be used as the main approach; however, presence or absence of normality renders more plausible some assumptions, if only on adjusted means when fitting a GLM model.

*Homogeneity of data across participating centres.*

This should have been dealt with during the data management process. However one should check whether a centre factor should be included in all the forthcoming analyses.

Centre nonhomogeneity will be considered if there exist significant differences between centres. Fisher's exact test as modified by Mehta for *r×c* tables will be used for categorical variables. A nonparametric one−way analysis of variance (Kuskal−Wallis test with correction for ties) will be used for continuous parameters.

The parameters for which nonhomogeneity between centres is present will be reported to the Steering Committee. If nonhomogeneity is obvious, the Committee may require that a centre factor be added to the anticipated model

Such requirements will be considered as an amendments to the statistical analysis plan.

It is also anticipated that the Steering Committee may require specific hormonal ratios to be calculated and used in exploratory analyses.

*Descriptive analysis*

Each parameter will be summarized globally and per factor (arm) level.

For categorical parameters, counts and proportions will be displayed for each category.

For continuous variables, counts, medians and 25th and 75th percentiles will be reported.

Based on these descriptive data, the Steering Committee will confirm whether the following derived parameters : obesity (BMI > 30 kg/m2), overweight (BMI>25 kg/m2), hypoTT (TT<3.0 ng/mL) hypo BT (bioavailable testosterone < 0.8 ng/mL) are to be kept in the analysis.

In this the case for TT and bioavailable TT, it is anticipated that patients will be categorized into 4 groups :

eugonadic [TT+BT+] (testosterone > 3.0 ng/mL AND bioavailable testosterone > 0.8 ng/mL), and hypogonadic: [TT+BT−] (testoterone > 3.0 ng/mL AND bioavailable testosterone < 0.8 ng/mL), [TT−BT+] testosterone < 3.0 ng/mL AND bioavailable testosterone > 0.8 ng/mL), [TT−BT−] testosterone < 3.0 ng/mL AND bioavailable testosterone < 0.8 ng/mL).

It is also anticipated that the Steering Committee may wish to put some emphasis on the role of the metabolic syndrome. Should this happen, an amendment will be added to the present statistical analysis plan.

Since one of the participating centres (Hôpital Foch) has previously reported data on hormonology of prostate cancers, the parameters of the current dataset for this hospital will be compared to the previously published values so as to ascertain the temporal stability of hormonal characteristics in the population with prostate cancer. Means and standard deviation will be calculated for the new dataset from Hôpital Foch and compared to the same summary values by a randomised t−test (k=10000 iterations). Should significant changes be found, the Steering Committee may decide to add exploratory analysis to understand why such temporal changes occurred.

*Comparison according to the predominant grade*

Grades will be categorized as predominant Grade 3 (6 or less or 3+4) and predominating Grade 4 (4+3 and above 7).

All parameters will be analysed according to these categories. Categorical parameters will be compared using Fisher's exct test while a randomisation t−test (with k=10000 iterations) will be used for continuous parameters.

Results will be provided to the Steering Committee as soon as they are available to allow decision about exploratory analyses to be made.

*Comparison according to categorization of grades in 4 classes*

Grades will be categorized as follows:

Low: below 7, Medium−low: 3+4, Medium−high: 4+3 and High: above 7.

Comparisons will be made using:

− for categorical variables, Fisher's test as modified by Mehta for *r×c* tables; if this test is significant or shows a trend, pairwise comparison will be performed using Fisher's 2×2 exact test with the Bonferronni−Holms correction;

− for contiuous variables, the Kruskal−Wallis nonparametric one−way analysis of variance corrected for ties will be used. If this test is significant or shows a trend, pairwise comparisons will be done using the Dunn's test and Bonferronni−Holms' correction.

*Assessment of the role of exgenous variables*

As they may play a role in the betwee−group differences, the following correlations with each hormonal parameter will be assessed using the Pearson's correlation coefficient.

BMI, Age, PSA, Total cholesterol, HDL cholesterol, Triglycerides, Blood sugar, which will be correlated with :

 TT, BT, SHBG, DHT, DHEA, Δ5, Δ4, E1 and E2:

To be considered as a covariable that may be included in a model, a variable should yield a correlation coefficicient above 0.7 for at least 4 hormonal levels as with such a large correlation coefficient, roughly 50 % of the bivariate variability will be accounted for.

*Covariable modeling*

If one of the above mentioned varible is found to be significantly correlated to hormonal levels, it will be included as a covariate in the models testing predominant grade, grading in 4 categories and these factor crossed with hypogonadism.

To this end a nonparametric model for partitioning dissimilarity matrices will be used (Permanova) with test of the main factor in a pseudo−analysis of covariance model.

Probability values will then be obtained by a permutation method.

Since this approach on dissimalarity matrix, which is based on centroids, does not provide adjusted means, a GLM model with the same main factor and the same covariable will be fitted to obtain approximate adjusted values for means.

*Assessment of the impact of different types of hypogonadism*

Hypogonadism is to be considered as a random factor that is crossed with the main factor (Grade).

An interaction grade × hypogonadism is of utmost interest to confirm our main hypothesis.

As nonmormality is postulated, a Permanova type of approach will be used on the dissimilarity matrix for samples on each hormone level. In addition, such an approach allows for single or multiple covariables.

Probabilites will be obtained using permutation of residuals.

Since this approach deals with centroids, it does not provide (adjusted) means; approximate values will be obtained by fitting a GLM model with the same factors, interaction term and covariable..

*Predicive factors of cancer aggressivenss*

Loigsitic regression will be used with all available parameters as predictive factors or limiitng predictive factors to those that might be easily available to surgeons (weight, height, ehtnic group, age, waistline, history of cancer, biopsy staging, biopsy grade, Gleason score of biopsy, PSA, presence of diabetes, presence of hypertension, presence of other cardiovascular disease, any treatment, blood sugar, total cholesterol, HDL cholesterol, testosterone) prior to surgery.

The predominant grade found on radical prostatectomy specimens will be used as the dependent variable.

The heuristically better model (at most 10 variables, each predicitve factor being individually significant in terms of Wald probability) is to be sought starting with all selected variables and suppressing at each iteration the variable that has the lowest predictive value (highest Wald probability); iterations are stopped when both the above−mentioned criteria are met. A random sambple with two thirds of the cases will be used to construct the model while the remaining third will be used to validate it.

The process is repeated 20 times with diffrent samples selected at random to construct the model so as to elimiante predicitive factor that woul be chance findings.

Two runs will be conducted for each modeling session: one with all the cases and one in which the cases that had a Predominant Grade 4 biopsy have been removed, since there is no question that active surveillance is not suitable for such patients.

**Statistic process and study objectives**

*Primary objective*

Cancer agressivenss is to be graded either in two categories or in 4 categories according to the predominant Grade of the prostate specimen and the Gleason score, after slides have been reviewed centrally. Hypogonadism is to be categorized according to the serum levels of TT.

However, as it is hypothetized that BT levels are parit independent of TT levels, hypogonadism may be more accurately categorized using of the descriptive statistics for BT, which leads to 4 different groups.

Secondary objective №1: Categorization as eugonadic or hypogonadic and the category of hypogonadism will be determined from the descriptive analysis.

Secondary objective №2: once the relationships between hormonal profiles and cancer aggressiveness have been clarified, the Steering Committee will meet and provide guidance as to the way questionnaire items should be used to define clinical symptoms that are correlated to each type of hypogonadism; furthermore, clinical symptoms, as recorded during the presurgery visit or that explain why some medications are prescribed may be added to the lsit of symptoms correlated to hypogonadism categories.

Secondary objective №3 (to assess the impact of prostatectomy on the hormonal profile one year after surgery), №5 (clarifying the relationships between hormonal profile and biological recurrence assessed by PSA at follow−up) and №6 (uncovering relationships between hormonal profiles and patients’ survival): the statistical process to be used will be detailed in a forthcoming Statistical Analysis Plan that will be geared on the reactions perceived by the Steering Committee to the publication of the reults on preoperative data.

Secondary objective №4: this has been recognized as the main objective of the SterKPro study for which a specific Statistical Analysis Plan has been established.

**Exploratory analyses**

Once the statistical analysis is completed, the statistician of his own accord, or the Steering Committeemay decide to conduct exploratory analyses to clarify some of the results. Even if not formally recorded as such, this decision will be considered an amendment to the present plan. Analyses performed in this realm, if reported in the statistical analysis report will be clearly indicated as exploratory, post−hoc analyses.

**Software**

Analyses will be performed using up−to−date versions of the followign software NCSS (NCSS Ltd, Kaysville, Utah, USA), R (The R Foundation, Wien, Austria) and Primer (e−Primer, Auckland, New Zealand).

**Changes to the Statistical Analysis Plan**

Any change to the present plan will be documented in writing and added to the present plan as an amendment.

The modified plan is passed to the Steering Committee for approval and to the sponsor of the research for filing.

**Communication of analysis results**

Written reports will be prepared by the statistician and passed to the Steering Committee. Every report that is considered as final will be electronically signe by the statistician prior to its despatch to the Steering Committee and study sponsor.

Electronic files that were created during the analysis will be burnt on a non rerecordable CD that will be dated and signed by the statistician in charge. One copy will be prepared for the sponsor, one dispatche to the Steering Committee and one archived by the statistician.

# Abbreviations

CI Confidence interval

CRF Case Report Form

KWR Kruskal−Wallis−Rijkoort (test)

SAP Statistical Analysis Plan

SOP Standard Operating Procedure

# Modification № 1

Date: 11 april 2017

Author: JF DREYFUS

Since Modification №1 of the the 17∩03 version of the SAP was still present in the document although its content had been integrated to the plan, it has been erased from Version 17∩06.

Following a cursory review of the results already available, the Steering Committee has demanded that several hormonal ratios, namely BT/TT, TT/PSA, BT/PSA, SHBG/TT, SHBT/BT be introduced as derived variables to be included in all analyses.

# Modification № 2

Date: 12 july 2017

Author: JF DREYFUS

Since the logistic regreossion model does not provide a sufficient protection agains falsely negative Predominant Grade 4, the Steering Committee demands that a ROC curve be added to the logistic regression model with penalisation of the falsely negative predicted Predominant Grade 3 as such error lead to suggestiing that active surveillance be recommended to patients likely to present a Predominant Grade 4.

The Steering Committee sugges no penalty for rightly categorized patients, a 1 point penalty for falsely positive Grade 4 and a penalty of 20 points for false negative Grade 4.

# Modification №3

Date: 6 August 2017

Author: JF DREYFUS

Considering that logistic regression performs poorly to predict agressiveness, the Steering Committee agrees to the suggestion of the statistician that another classification scheme should be tried.

Consequently, the C5.0 algorithm will be tried as a first candidate.

1. Commission Nationale Informatique et Liberté. National Commission for Computing and Liberties [↑](#footnote-ref-1)
2. c: clinical preoperative, b: biology, h: hormonology, p: pathology, q: questonnaire, d:derived [↑](#footnote-ref-2)
3. CAT: categorical, NUM: continous, ALP: alphanumeric, ORD: ordinal [↑](#footnote-ref-3)