The Statistical Analysis Plan of the VALIDATE Study

This Statistical Analysis Plan (SAP) was written according to the guidelines proposed by Yuan et al. (1) and by Gamble et al. (2).

1. SAP versions, dates and reasons for revision

Version #1: April 22nd, 2024.

2. Trial registration number

NCT04950244

3. Study title

Diagnostic performances of the pre-hospital high-sensitivity troponin I (hs-cTnI) to rule-out or rule-in patients with chest pain: a prospective and multi-centric cohort study.

4. SAP contributors with roles and responsibilities

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5. Background and rationale of study

Chest pain (CP) is a difficult symptom to apprehend in the out-of-hospital setting, due to the complexity required to reliably assess patients over the phone. The current standard of care implies dispatching an out-of-hospital vector (HEMS) in the case of CP highly evocative of acute myocardial infarction (AMI). Risk stratification then requires the collection of a detailed medical history, a physical exam, before performing a 12 and 18-lead ECG.

In the absence of ST-segment deviation, single or trending cardiac troponin levels guide decision making. Few prospective trials evaluate the impact of point-of-care (POC) troponin on prehospital triage of CP and two prospective studies evaluating POC troponin as a clinical decision guide are currently underway.

High sensitivity assays improve patient outcomes by providing a better negative predictive value (NPV) and better rule-in for type 1 and 2 MI. High sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI) are now routinely available on POC devices. The Atellica VTLI device offers the possibility of analyzing venous whole blood or capillary samples and returning hs-cTnI values comparable to central laboratory testing. The added advantage of hs-cTnI as opposed to hs-cTnT or standard troponin (cTnT) is its better NPV in ruling out coronary causes of CP. This biomarker offers the added advantage of being highly specific to myocardial injury and is correlated to the degree of muscle necrosis.

The hypothesis of the study is that the use of the high-sensitivity troponin I (hs-cTnI) could be of interest to stratify chest pain patients in pre-hospital setting.

6. Study type

This is a prospective multi-center cohort-based study.

7. Objectives and related endpoints (units and calculations used to derive outcomes)

An adjudication committee oversees the validation of the events considered in the following objectives and outcomes.

The primary objective is to determine the thresholds of the pre-hospital capillary and venous hs-cTnI that maximizes the Youden J index. The event to predict is the non ST-elevation acute coronary syndrome at hospital discharge (NSTEMI). The sensitivity is the proportion of patients with an hs-cTnI level higher or equal to the threshold among the patients with the syndrome. The specificity is the proportion of patients with an hs-cTnI level lower than the threshold among the patients without the syndrome. We will also determine the ROC (receiver operating characteristic) curves and the related areas under the curve (AUC) related to the two methods.

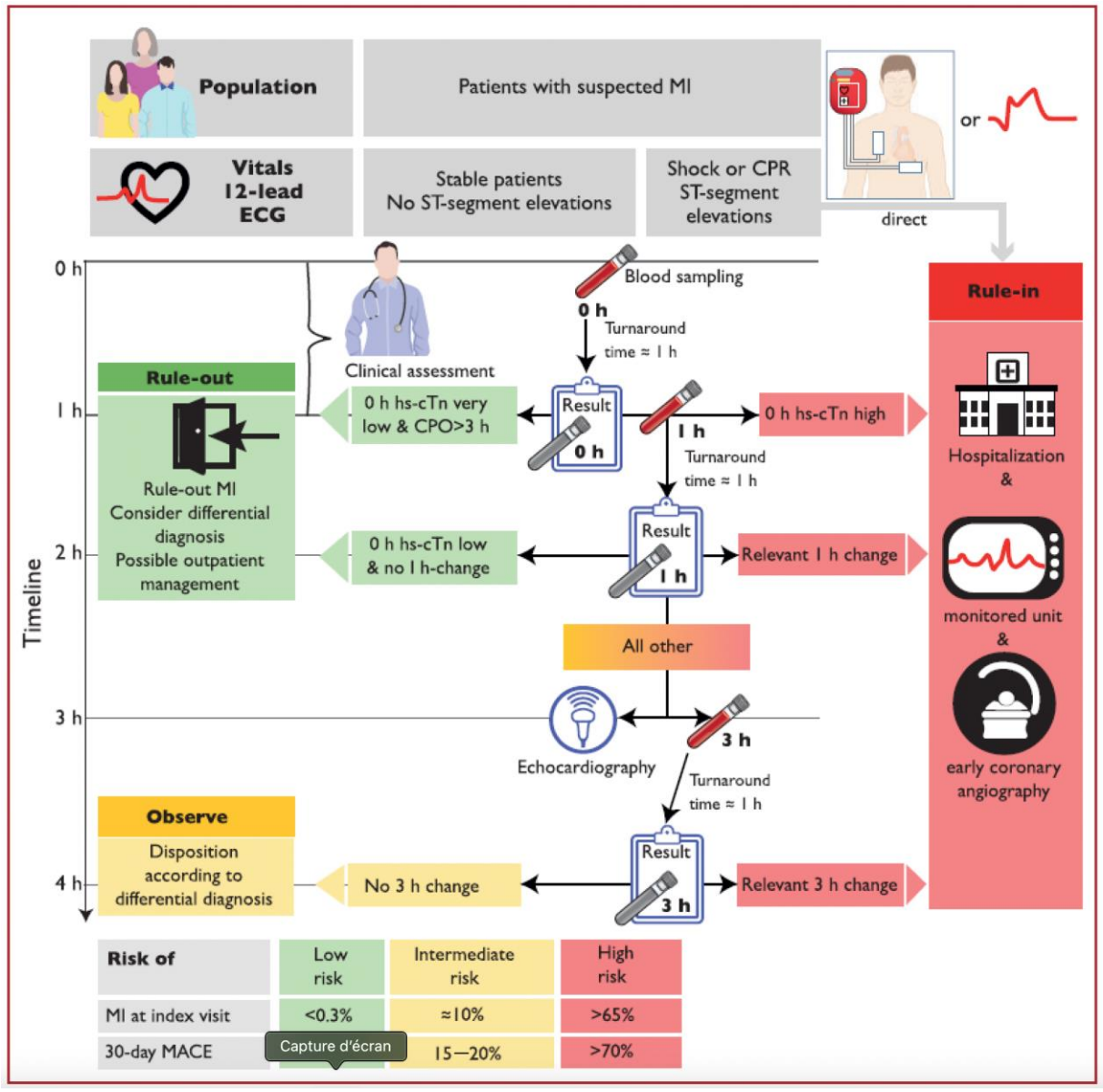

Figure 1. Decision tree for ruling-out ruling-in the patients with a suspicious myocardial infarction after their emergency admission.

The secondary objectives of the VALIDATE project are to study the discriminative capacities of the pre-hospital hs-cTnI measured by two different testing methods (capillary and veinous whole blood), and to select the method with the best performances. For that purpose, we aim:

- To evaluate the most relevant hs-cTnI measurement between the capillary- and the venous-based methods by i) reporting the concordance between the pre-hospital hs-cTnI measured by the capillary and venous methods, and ii) comparing the related AUCs for discriminating patients with NSTEMI.

- To estimate and compare the discriminative performances of the two methods by considering the Major Adverse Cardiac Events (MACE) occurrence at 30 days (ROC curves, AUCs, and Youden J index). Note that a NSTEMI systematically implies an outcome of MACE at 30 days.

Depending on the results at this stage, the rest of the analyses will be performed according to the venous or the capillary method, depending on their discriminative performances. In case of an equivalence, we will choose the capillary method because of its feasibility in a real-life setting. We will further investigate its usefulness to improve the ruling-in and ruling-out process described in Figure 1. More precisely, we aim:

- To propose a pre-hospital predictive tool of the probability of NSTEMI at hospital discharge. The candidate predictors will be the available demographic, clinical data, and pre-hospital hs-cTnI level.

- To estimate its discriminative capacities by estimating the AUC and comparing its value with the one of the in-hospital HEART score estimated at 0h.

- To estimate its ability to rule-out the patients instead of the in-hospital evaluation at 0h for relieving emergency services, i.e., to achieve a negative predictive value equals to 99% (3) for at least 5% of the patients.

- To estimate its ability to rule-in the patients in the cardiology department instead of the in-hospital evaluation at 0h for reducing the time to treatment of the MI, i.e., to achieve a positive predictive value equals to 65.0% (Figure 1) for at least 10% of the patients.

- To evaluate its increase of capacities for discriminating patients with NSTEMI by considering additionally the venous troponin measured in hospital (difference in the AUC).

8. Inclusion criteria

- Age ≥ 18 years.
- Chest pain for which a mobile emergency unit (HEMS) is dispatched for pre-hospital assessment.
- Chest pain evocative of AMI; medio- and retro-sternal, constrictive, radiating into the jaw, neck or left arm.
- Non-qualifying ECG or non-ST elevated ECG
- Non-opposition given by the patient after clear and fair information.

9. Non-inclusion criteria

- Qualifying ECG with significant ST-segment elevation.
- Diagnosis of acute coronary syndrome has been ruled out by the HEMS physician.
- Patient care within 20 minutes of pain onset.
- Pregnant and/or breastfeeding women, women of childbearing age without contraception.
- Patients under guardianship, curatorship, or subordination.
- Patients benefiting from reinforced protection.

10. Sample size

For the primary objective, we expect a sensitivity of 95%. We aim to include 73 patients for a maximum length of the 95% confidence interval equals 10% (+/-5%). Assuming a NSTEMI prevalence of 10%, 730 patients must be analyzed to achieve this precision. By anticipating a 10% rate of non-exploitable data (missing value, lost to follow-up, etc.), 800 inclusions are necessary.

11. Interim analysis (timing, person, adjustment of the significance level)

No interim analysis was planned.

12. Reporting the representativity of the studied population

A flowchart will be decomposed according to the inclusion, non-inclusion criteria, and exclusions because of missing data for the disease diagnosis (NSTEMI, unstable angina, or major cardiac event at 30 days). The excluded patients will be compared to the others for identifying a potential selection bias.

13. Level of statistical significance (p-values, confidence intervals, one- or two-sided)

Statistical significances will be achieved for p-values lower the 5% by using two-sided alternative hypotheses and two-sided 95% confidence intervals. The 95%CI will be estimated by non-parametric bootstrap.

14. Plan and rationale for controlling the type I error (multiplicity of tests)

We will not consider the inflation of the type I error.

15. Sub-group analyses

Because hs-cTnI increases during the first hours post-chest pain, its value may be more informative after few hours. Therefore, we plan a subgroup analysis in patients with less than three hours between chest pain onset and hs-cTnI measurement.

16. Description of baseline patient characteristics

The baseline characteristics will be summarized for the full cohort and according to the main and second outcomes, respectively: presence NSTEMI versus no NSTEMI and presence of MACE at 30 days versus no MACE at 30 days. The baseline is the time of the patient assessment by the HEMS.

The means, the standard deviations and the number of missing values will be presented for the following continuous variables:

- age (years)
- body mass index (kg/m²)

The proportion of patients will be presented for the following categorial variables:

- Gender
- Risk factors of coronary artery disease (CAD):
    - HBP
    - Hypercholesterolemia
    - Diabetes mellitus
    - Family history of coronary artery disease
    - Active smoking
    - Past smoking (weaned > 5 years prior to recruitment)
- Cardiovascular history:

    - Stented AMI
    - Coronary by-pass
    - Peripheral vascular disease
    - Chronic kidney disease
    - Stroke/ TIA
- Current medication:
    - ACE/ ARA2
    - Calcium inhibitors
    - Beta blockers
    - Anti platelet therapy
    - VKAs
    - Statins

We will also describe characteristics linked to the initial HEMS evaluation:

- Clinical presentation evocative of AMI on a 3-point scale (highly unlikely, moderately likely, highly likely)
- Delay between chest pain onset and HEMS evaluation (≤ 3 hours versus > 3 hours)
- HEMS ECG findings (normal, ST-segment depression, localized negative T-waves, left bundle branch block, other findings)

The frequency and percentage of patients in each category and the number of missing values will be reported for categorical variables: gender (male / female), risk factors (HBP, Hypercholesterolemia, Diabetes mellitus Family history of coronary artery disease, Active or past smoking), cardiovascular history and medication. For characteristics with multiple modalities, some may be merged in the case of small frequencies.

P-values will be reported with no correction for multiplicity. Categorial variables will be compared using a Chi-2 test (or Fisher exact test if the expected number of participants is less than 5), while continuous variables will be compared using a Student test (or Wilcoxon test if the minimum number of participants between the two groups is less than 30).

17. Description of the outcomes

NSTEMI and MACE at 30 days will be reported with the related frequency and proportion, knowing that a NSTEMI involve systematically a MACE at 30 days.

18. Description of the hs-cTnI discriminative capacities

The primary objective is to determine the pre-hospital capillary hs-cTnI threshold that maximizes the Youden index. The Euclidean distance (also called the upper-left index) will also be estimated. For the corresponding thresholds, the results will be reported on the related ROC curve. The related effectives (true positives and negatives, false positives and negatives) will be reported. The accuracy will also be computed (proportion of true results). On the same figure, the results related to the pre-hospital venous-based hs-cTnI will be reported. The difference between the two curves will be estimated and the corresponding bilateral test to evaluate the significance will be performed from the 95%CI (confidence interval).

The intra-class correlation coefficient (ICC) will additionally provide a single measure of the agreement between the capillary- and venous-based methods. Two scatter plots will be proposed to represent the degree of concordance: a plot with the pre-hospital venous-based hs-cTnI (y-axis) against the capillary-based measurements (x-axis), and a Bland–Altman plot with the difference between the two measurements (y-axis) against the average of the two measurements (x-axis).

The discriminative performance of the pre-hospital hs-cTnI will then be estimated for the MACE at 30 days as outcome. For that purpose, two ROC curves on the same graph will be plotted for the capillary- and venous-based measurements. The related AUCs and differences will be reported.

Depending on the results at this stage, the rest of the analyses will be performed according to the venous or the capillary method. In case of an equivalence, we will choose the capillary method because of its feasibility in a real-life setting.

19. Proposition and validation of a predictive tool for pre-hospital rule-out and rule-in

The cohort will be divided into two parts: two thirds for learning and one third for validation. The description of the two samples will be performed with the same baseline variables listed in the section 16. No p-value will be computed because of the random allocation of the patients. Instead, standardized mean differences will be estimated to evaluate the comparability of the two samples.

From the learning sample, a logistic model will be estimated with LASSO penalization to select the subset of predictors among the following list of candidates: age, gender, body mass index, active smoking, stented heart disease, bypassed heart disease, compatible clinical history, delay after onset of pain lower than 3h; normal ECG evaluated by the HEMS, and the following risk factors: hypertension, diabetes, hypercholesterolemia, family history and hs-cTnI. The outcome will be the diagnosis of NSTEMI. We will perform a 20-fold deviance-based cross-validation to choose the best tunning parameter.

A particular attention will be paid to the interaction between hs-cTnI and the delay after onset of chest pain because the level of troponin may continue to grow up to three hours after the chest pain. This may lead to increase the proportion of false negatives.

From the validation sample, we will estimate its discriminative capacities by the ROC curve and the related AUC. Its goodness-of-fit will be evaluated by a calibration plot of the observed probabilities against the mean predicted probabilities for five subgroups of equaled size. The corresponding Hosmer and Lemeshow statistic will be estimated.

Always from the validation sample, we will compute the in-hospital HEART score at 0h, the related ROC curve and AUC, and its difference with the pre-hospital predictive model.

20. Investigation of the utility of the prediction model

From the validation sample, we will estimate its ability to rule-out the patients instead of the hospitalization. For that purpose, we will estimate the percentage of event-free patients at hospital discharge (no NSTEMI) among the ones with a predicted probability of event less than 0.3. We will also estimate the percentage of patients with a predicted probability of event less than 0.3.

From the validation sample, we will estimate its ability to rule-in the patients instead of the in-hospital evaluation at 0h by the percentage of patient with the event (NSTEMI) among the ones with a predicted probability of event higher than 0.65. We will also estimate the percentage of patients with a predicted probability of event higher than 0.65.

21. Handling missing data

We will perform complete case analyses. Excluded patients will be described against the others to evaluate a possible selection bias. The methodology described in the section 16 will be applied.

22. The assumptions of the statistical methods, and alternative methods if they are false

The main assumption is the good specification of the predictive model. In case of a poor goodness-of-fit, we will use B-splines to evaluate the better respect of the log-linearity assumption.

23. References

1. Yuan I, Topjian AA, Kurth CD, Kirschen MP, Ward CG, Zhang B, et al. Guide to the statistical analysis plan. Paediatr Anaesth. mars 2019;29(3):237‑42.

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3. Ashburn NP, McCord JK, Snavely AC, Christenson RH, Apple FS, Nowak RM, et al. Navigating the Observation Zone: Do Risk Scores Help Stratify Patients With Indeterminate High-Sensitivity Cardiac Troponins? Circulation. 2 janv 2024;149(1):70‑2.