PROTOCOL OF CLINICAL TRIAL WITH HEALTH PRODUCTS

Title: CROSS-OVER CLINICAL TRIAL TO ASSESS THE EFFECTIVENESS OF APPLYING DRY LOCAL HEAT AND/ OR HIGH TOURNIQUET PRESSURE WITH CURRENT CLINICAL PRACTICE FOR VENIPUNCTURE, AND BLINDED FOR EVALUATING THEIR IMPACT ON HEMOLYSIS.

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List of abbreviatons

AA. Adverse Events.

AEMPS. Spanish Agency of Medicines and Health Products.

BMI. Body Mass Index.

BP. Blood Pressure.

BPM. Beats Per Minute.

CI. Cumulative Incidence.

CLSI. Clinical and Laboratory Standtards Institute.

CMIO. Council for international organizations of medical sciences.

CMYK. Cyan, Magenta, Yellow, Black or Key.

CRF. Case Report Form.

ECG. Electrocardiogram.

GCP. Good Clinical Practices.

HR. Heart Rate.

ISO. International Organization for Standardization.

MMHG. Milimeters of mercury.

NM. Nanometers.

OPP. Optimal point of puncture.

RGB. Red, Green and Blue.

RPM. Revolutions per minute.

SOP. Standard Operating Procedures.

T. Temperature.

UECHUP: Clinical Trial Unit of La Princesa Hospital.

VAS. Visual Analogue Scale.

VIA. Venous International Assessment.

WHO. World Health Organization.

Glossary of terms

ABSORBANCE. Measure of the attenuation of a radiation when crossing a substance, which is expressed as the logarithm of the relationship between the outgoing and incoming intensity.

CURRENT CLINICAL PRACTICE. Treatment that is usually followed to treat, prevent or diagnose a disease or health problem.

ERYTHEMA. Reddening of the skin or mucosa due to an increase in blood in the small capillaries

HEMOLYSIS. Release of hemoglobin in plasma by destruction of red blood cells

ICTERUS. yellow of the skin and mucous membranes, due to an increase biliary depigment in the blood.

LIPEMIA. Presence of lipids (cholesterol, triglycerides and phospholipids) in the blood.

LOW LEVEL INTERVENTION CLINICAL TRIAL. A clinical trial that meets all of the following conditions: 1. The investigational drugs are used in accordance with the terms of the marketing authorization, or 2. the use of the investigational drugs is based on evidence and is supported by data published scientists on the safety and efficacy of such investigational medicinal products in one of the Member States involved. 3.º The complementary diagnostic or monitoring procedures involve a risk or additional burden for the safety of the subjects that is minimal compared with that of the usual clinical practice in any of the Member States involved.

OPTIMAL POINT OF PUNCTURE. The one that presents adequate conditions in resistance to rupture, elasticity to palpation and free venous trajectory from distal to proximal in the case of puncture for intravenous injection.

PARESTHESIA. Numbness and tingling produced by pathology oppression in the nervous or circulatory system

PROTOCOL DEVIATION. Changes to the protocol without prior agreement

2. ABSTRACT

2.1. Clinical trial type

Low level intervention health products clinical trial.

2.2. Clinical trial title

An incomplete cross-over clinical trial, with three arms interventions which its control is current clinical practice. It is unicentric to assess the effectiveness of applying dry local heat and/ or high tourniquet pressure for venipuncture and it is blinded for those who evaluate their impact on hemolysis.

2.3. Clinical trial identification

Protocol code: ECYPVEN-H/17

2.4. Clinical trial phase

Foruth phase

2.5. Sponsor and principal researcher

Non-comercial research. Dissertation. Complutense University of Madrid.

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2.7. Location to address the clinical trial

Clinical trials Unit of Clinical Farmacology Ward. La princesa Hospital of Madrid.

2.8. Ethical Comittee to assess

Research Ethical Committee of La Princesa Hospital of Madrid.

2.9. Monitoring

Any person will monitor this clinical trial because the sponsor and principal researcher are the same person. Nevertheless, an adherence to this protocol will ensure by principal researcher and co-researcher.

2.10. Interventions and control

Interventions:

1. To Apply local dry heat.
2. To apply high tourniquet pressure.
3. To apply both of them. (Dry heat and high pressure)

Control: Current Clinical practice.

2.11. Hypothesis and main goal

Hypothesis. The number of attempts of success venipuncture at first time are influenced by any of the interventions applied before.
Main goal. To identify the most effective intervention of applying dry local heat and/or high tourniquet pressure in relation of number success venipuncture attempts, compared to current clinical practice.

2.12. Design

An experimental, randomized study which is controlled with current clinical practice to insert a peripheral vein catheter. It is an incomplete cross-over clinical trial, with three arms which are involved interventions and control therapy.

2.13. Subjects and sample size

Population. Adult healthy subjects.
Sample size. It is required to enroll 54 subjects with a 95% of level of confidence and 80% level of power.

2.14. Main and secondary variables

Main variable. Succeed peripheral vein catheter insertion at first attempt.
Secondary main variable. Visual intensity of hemolysis.

2.15. Effectiveness assessment

The optimal effectiveness is considered when venipuncture success at first attempt exceeds 74% applying any of the interventions.

2.16. Planned date to address

It is planned to carry out around June and/or July of 2017.

3. GENERAL INFORMATION

3.1. Clinical trial identification

Protocol code: ECYPVEN-H / 17

3.2. Type of clinical trial

Clinical trial with medical devices. It is considered a low level intervention phase clinical trial to evaluate the effectiveness and safety of a sanitary product marketed for a different use than the accredited one.

3.3. Descripction and clasifications of health products

Source of pressure. Anaeroid sphygmomanometer Brand QUIRUMED with European Conformity (CE) 0197.

Dry topical heat source. Thermal sack of carob seeds 10x10. Tusacootermic brand, without CE marking

The health products described are considered active medical devices, class IIa, the sphygmomanometers (12) and without class awarded for topical heat because they do not provide CE marking and, therefore, with justification for their clinical investigation (9).

3.4. Data relating to the promoter and principal investigator

Independent research Doctoral Thesis Complutense University of Madrid.

Tutor: Dr. Emilio Vargas Castrillón.

Thesis Directors: Dr. Ismael Ortuño Soriano, Dr. Dolores Ochoa Mazarro and Dr. Emilio Vargas Castrillón.

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3.6. Research Center

Clinical Trials Unit of the University Hospital of the Princess (UECHUP). Clinical Pharmacology Service.

3.7. Ethical Committee that evaluates

The present clinical trial will be evaluated, in the first instance, by the Ethical Committee of Clinical Investigation with medicines and sanitary products of the University Hospital of the Princess.

3.8. Expected duration of the trial

The overall duration of the study will depend on the total of the subjects recruited in the bioequivalence trial in which the present is carried out with medical devices. Approximately, it is estimated that it will last about 30 days, from the training of nurses to the cessation of possible adverse events.

4. INTRODUCTION AND JUSTIFICATION

4.1. INTRODUCTION

4.1.1 DEFINITION OF VENOPUNTION

Venipuncture is a technique especially understood within the nursing competence (13). It is one of the most common invasive techniques among all those that are carried out in hospital environments. Several studies highlight the inherent risks involved in this procedure (14).

According to the Royal Spanish Academy, venipuncture is defined as "the puncture made of a vein to draw blood or inject something" (1).

However, according to a book of the nurse competition, venipuncture is considered to "the installation of a needle or a catheter in the light of a vein through the skin". He does not understand the concept of injecting an infusion as part of it (15).

That nursing procedure carries a multifactorial influence. Among all the predisposing factors to influence the result and the process of the technique there are some that are non-modifiable and, most of them, modifiable. Table 1.

MODIFIABLES FACTORS	***NON-MODIFIABLE FACTORS***
Body-environmental temperature. Thermoregulation	*Background and demographic characteristics*
Pain and axiety	*Age*
Hemolysis	*Sex*
Usage and type of gloves	*Race*
Experience and skills	*Overweight and obsesity*
Anatomical venipuncutre zone, needle type and adverse events	*Skin, tattos and dermatological pathologies*
Tourniquet pressure for vein stagnation	*Venous system and venous system patologies*
Hydration	*Affected upper limbs by non-circulatory disease*

Table 1. Modifiable and non-modifiable factors. Own elaboration, from 13,15,16,17 and 19.

Venipuncture is, psychologically and physically, a traumatic technique since many individuals develop needle phobia as a result of bad experiences in venipuncture (16,17).

Pain is a subjective concept that occurs during venipuncture and anxiety usually occurs before it; both complement each other (18). In recent years pain has been considered as a vital sign and the concern to develop measures to reduce pain in various techniques has also reached the field of venipuncture.

4.1.2. Findings

Several authors propose the use of the local anesthetic cream composed of lidocaine and prilocaine, EMLA®, as a measure to alleviate pain. It involves waiting approximately one hour from topical application until it reaches its maximum action and is subject to allergen-type adverse reactions (19). However, a recent study has found that the application of EMLA produces vasoconstriction (20).

Pain reduction has been described with non-pharmacological techniques, such as the Valsalva maneuver, which is imminent and not subject to adverse effects. It has been shown that this is as effective as the use of EMLA in terms of pain, but in terms of venipuncture it is more effective than the use of the local anesthetic (21).

Another non-pharmacological method developed in recent years is music therapy. Several studies have shown music therapy as anxiolytic, and indirectly as an analgesic, activating regions of the brain responsible for distraction and pleasure. Differences between music therapy and external noise have been observed, the latter being a stimulator of anxiety and music a depressant of pain and anxiety. Since it is a subjective component, it must be adapted to the patient's considerations and avoid melancholy memories (22). Music therapy, which should not exceed 60 minutes in each session, is physiologically translated into relaxation parameters in heart rate, breathing rate and blood pressure (23).

In the context of surgery without general anesthesia, the touch approach has been found, using an anti-stress ball, to channel all the feelings of each individual by crushing the ball, less innovative but easier to monitor (24).

There are studies that state that more than two thirds of altered results obtained from blood samples originate in the pre-analytical and even pre-analytic phase (25) of Lundberg's brain-to-brain turnaround time loop model; model that aims to provide laboratories with the economic budget related to the patient and the total analytical error (26,27). It is understood by the pre-analytical phase from the preparation of the patient to the preparation of blood samples for analysis, or storage; This is divided into pre-pre-analytical and pre-analytical, being the first from the preparation of the patient, staff and material involved until the blood extraction and the second preparation for the processing of samples (26,28). Several authors agree that the pre-analytical phase is responsible for 68.2% of the errors detected (29). The preanalytic phase focuses on phlebotomy, specifically in the process of venipuncture (28-30)

Lundberg et al emphasizes the following. Clinicians and laboratories should be concerned about the effects of that laboratory test and whether the performance of this was useful for the patient or for the public's health (26). ISO 15189: 2007 standard for laboratory accreditation as Oliviera et al. They demonstrate the importance of quality in the management and preparation of the patient in the first phases of the model previously stated. (25.28).

Additional effect is the time that the tourniquet remains for stagnation of blood, type of vascular access device, and the number of attempts can trigger hemolysis and, therefore, alter blood samples (27,31).

Lima-Oliveira et al. proposes a new mechanism, equipped with cold and vibration, applicable to the arm during the pre-pre-analytical and pre-analytical process, reducing pain, none of the samples extracted with this device obtained a sign of hemolysis; by visual inspection (25,32).

The most recent research in this area is based on intravenous cannulation in an ultrasound-guided manner, in order to reduce the number of attempts to achieve a vein catheterization. There is evidence, through published studies, that a reduced number of attempts safeguards brachial nerve damage in greater probability; adverse effect of venipuncture. However, ultrasound ecoguided cannulation does not guarantee the reduction of accidental damage to said nerve. Even when introducing the catheter in deeper planes, it usually causes a series of complications, puncture of a nerve or artery, which rarely appear in superficial punctures. In addition, the ultrasound has a higher cost than other alternatives and the nurses would have to receive a training course to learn how to use the ultrasound correctly; as proposed by the authors of the investigation (33.34). Despite multiple investigations aimed at the field of ultrasound ecoguided venipuncture, some authors continue to pursue puncture in superficial veins with the use of auxiliary alternatives such as the one proposed by Kotaka T et al. in your investigation; with the use of a topical cream called Camphor, compared to menthol. He observed that the increase of the flow depended more on the time of the local heat than on the high temperature of the same since it could produce burns (35).

A study has been developed in which clinically and statistically significant differences have been found in the antebrachial venous perception (p-value <0.01), with the use of dry topical heat and high pressure with the use of the sphygmomanometer, through a previously validated scale, Venous International Assessment (VIA) scale (10.36).

4.2. Justification

This study, which precedes the present one, concludes that: the application of this intervention is effective for the exacerbation of antebrachial venous perception, it denotes a characteristic of protection against the consequences that a difficult venous perception entails, thus reflecting a high impact avoidable potential of difficult venous perceptions, and all that this entails. Finally, it reveals that it is possible to benefit from this intervention to the entire population, but it is more fruitful if it is intended for subjects with a compromised venous perception, or with a low stage according to the VIA scale (10,36).

Likewise, the study ends with the need to which it is intended to respond in this protocol: clarify the cause of the finding found; that is, if the increase in antebrachial venous perception is due to exposure to dry heat and / or high stagnation pressure (37).

In the same way, it has been considered that the evaluation of the quality of the blood samples could be justified, in what refers to the intensity of hemolysis. No study has been found that proves, empirically and validly, what both Lundbeg et al. like other authors in the pre-pre-analytical phase; number of attempts in intravenous cannulation, pain and tourniquet aspects with respect to the intensity of hemolysis (25, 26, 28,30).

As a consequence, it has also been considered that the need to design and validate a visual scale of hemolysis could be justified; Detecting the intensity of hemolysis in a precise and valid way.

5. HYPOTHESES AND OBJECTIVES

5.1. Hypothesis

The frequency of cases in successful antebrachial venepuncture at the first attempt is affected by the applied method.

5.2. OBJETIVES

5.2.1. Main objective

To identify the best effectiveness of the application of topical heat and / or high stagnation pressure in successful antebrachial venipuncture at the first attempt; Regarding the usual practice so far.

5.2.2. Secondary objectives

o Identify the effectiveness of all the methods used in antebrachial venipuncture, in what refers to venipuncture at the first attempt.

o Design and validate a visual scale of hemolysis.

o Evaluate the intensity of hemolysis of blood samples, in what refers to the method applied, respectively.

o Identify the frequency of successful venipuncture at the first attempt, and analyze whether there is an association between it and the demographic characteristics of the individuals.

o Analyze the correlation between methods used, intensity of hemolysis and pain.

o Identify and quantify adverse events, as well as their relationship with skin types.

6. TYPE OF TRIAL AND DESIGN

6.1. Phase of the clinical trial

It is considered a low level intervention phase clinical trial to evaluate the effectiveness and safety of a sanitary product marketed for a different use than the accredited one.

6.2. Design and comparator

Experimental, randomized and controlled study with the usual technique of inserting a venipuncture catheter until now. Incomplete clinical trial, blind to third parties, in healthy volunteers, which consists of three arms, in which they are intervened and the comparator is applied.

Three interventions are established and a common comparator for all of them. Interventions:

1. Application of dry topical heat.

2. Application of high stagnation pressure.

3. Application of dry topical heat and high stagnation pressure. That is, the combination of the previous two.

Comparator:

1. Usual clinical practice, until now

Therefore, all individuals will be subjected to one of the three interventions and the comparator, whose sequence corresponds to the two periods established by the clinical trial of bioequivalence.

Therefore, all individuals will be subjected to one of the three interventions and the comparator, whose sequence corresponds to the two periods established by the clinical trial of bioequivalence.

It is understood that it is a crossover clinical trial because each subject is exposed to an intervention, and it is their own control; it is exposed to the comparator, whose purpose is to reduce the intraindividual variability and accentuate the effectiveness of the intervention per se. It is an incomplete crossing because it is not possible to subject each individual to the three interventions and it must be only one per random assignment. This impossibility could be supported by the low frequency of clinical trials of four periods carried out in the accessible environment.

In this way, any of the interventions assigned to it can be compared with the current procedure for antebrachial venous cannulation

6.3. Random assignment of interventions.

The intervention and the sequence of application of the intervention with the comparator will be randomly assigned.

They will be assigned with sealed envelopes where inside the corresponding intervention to be applied, the comparator and the corresponding sequence will be detailed. Emerging the following options:

1. CA-> C. Topical first period heat. Comparator in the second period.
2. C-> CA. Comparator in the first period. Topical heat in the second period.
3. P-> C. High pressure in the first period. Comparator in the second period.
4. C-> P. Comparator in the first period. High pressure in the second period.

5. CA + P -> C. Combination of dry topical heat and high stagnation pressure in the first period. Comparator in the second period.
6. C-> PA + C. Comparator in the first period. Combination of dry topical heat and high stagnation pressure in the second period.
As a result of these options and the coverage of the Clinical Trials Unit of the University Hospital of La Princesa is twelve individuals in each group, the following sealed envelopes for each group are established

- Four contain topical heat and comparator intervention
- Two will be topical first period heat. Comparator in the second -> [CA-> C]
- Two will be comparator in the first period. Topical heat in the second -> [C-> CA]

- Four intervention of high pressure and comparator
- Two will be high pressure in the first period. Comparator in the second -> [P-> C]
- Two will be comparator in the first period. High pressure in the second -> [C-> P]

- Four combined (topical heat and high pressure) and the comparator
- Two will be combination in the first period. Comparator in the second -> [CA + P-> C]
- Two will be comparator in the first period. Combination in the second -> [C-> CA + P]

This scheme of twelve envelopes will be carried out five times to address a sufficient sample size, of sixty subjects, to assume the estimated losses and, finally, to obtain the number of subjects, who have completed both periods, calculated to obtain a statistical significance of the results.

Due to the inclusion / exclusion criteria established, and which will result from the first evaluation VIA (36), an equivalent number of individuals in each arm will be sought.

In each envelope, the chronological order of application of the intervention-comparator will be included, by default and without previous knowledge.

It will be randomized only once, in the first period. In the second period, the remaining sequence will be applied. Figure 1

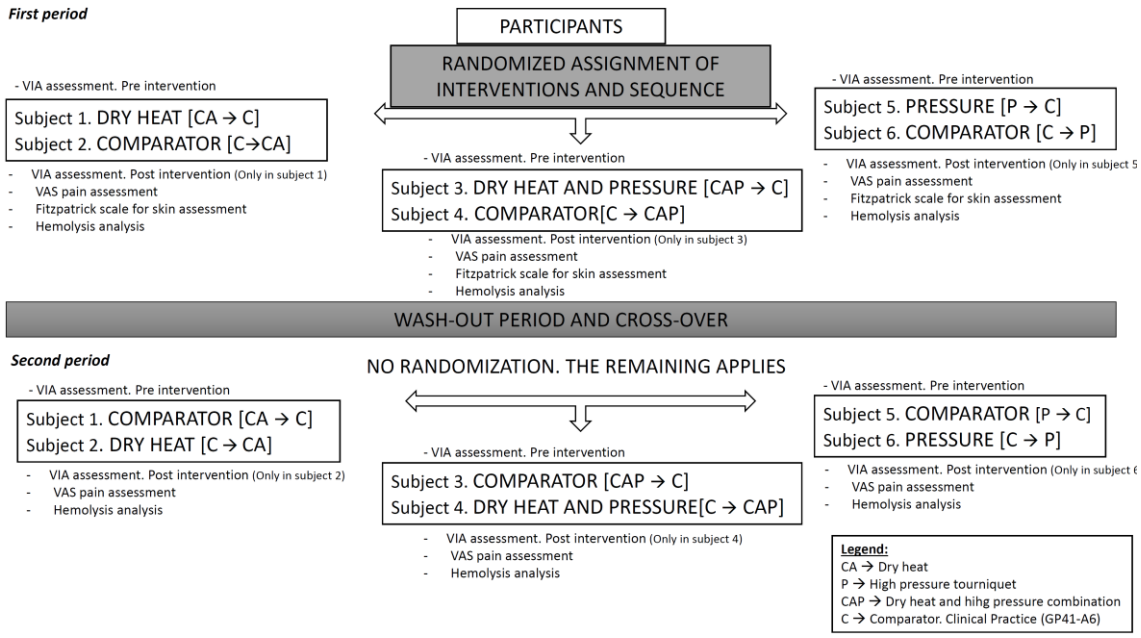

Figure 1. Design flow diagram. Own elaboration.

6.4. Masking

The present study is considered blind to third parties; simple blinding due to logistics problems is not considered. Both the principal investigator and the nurses and subjects participating in the study know the method that will be applied to them. However, if it is considered blind to third parties, since the assessment of hemolysis is carried out by team personnel who are unaware of the method used in antebrachial venous cannulation for each sample analyzed.

6.5. Period of washing and follow-up

A washout period is foreseen between the intervention and the randomly assigned comparator for approximately one week. It will depend on the washout period of the clinical trial of bioequivalence. As a consequence, sufficient time is considered to eliminate the possible residual effect of topical heat and high pressure of the sphygmomanometer. Figure 1.

In the bibliography there is no indication of the time of the antegrade reflux in the upper limbs, so it has been adapted to what is indicated for the lower limbs in retrograde reflux, which is 0.5 seconds without muscle action (38). It has been estimated approximately 0.2 seconds since it is anterograde reflux with muscle action.

In the first instance, no follow-up period is foreseen, unless there is a secondary effect, which will continue until its cessation.

6.6. Justification of the design

It is considered that the methodology reflected in the present study could be justified under the following assumption well designed and correctly executed randomized clinical trials (RCTs) provide the best evidence on the effect of health interventions; according to the CONSORT guide (39,40).

All the "critical points" that this guide reflects throughout the present work have been addressed; in order to ensure that the information provided is sufficient and accurate (39,40).

Likewise, the study is supported by the extension of the guide that collects information adapted to clinical trials with non-pharmacological interventions; as is this case (40,41). To address the disparity of experience and skill of nurses is supported by the randomization of both interventions and the indistinct eligibility of the nurses participating in the present clinical trial (41).

Open study is justified by the difficulty of masking interventions. In the extension CONSORT for Trials Assessing Nonpharmacologic Treatments, it highlights the change in its item 11, masking, in what refers to semantics. In such a way that it introduces the possibility of directing the masking towards those collaborators who apply the interventions, instead of masking the interventions towards the subjects and in the evaluation of results (41).

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1. Sampling and type of population

It is considered a non-probabilistic sample study, for the convenience of healthy volunteers. The existence of the bioequivalence test is communicated and it is the volunteers who come to register their participation for particular reasons, they are easily accessible groups with specific characteristics. In addition, all of them maintain a fast of, at least, 6-8 hours prior to peripheral intravenous cannulation.

All individuals recruited to perform the fieldwork must have met all the inclusion criteria, and none of exclusion.

7.2. Inclusion criteria.

Subjects that meet the following requirements will be included:

o Age 18 to 55 years old (42).

o Have signed the informed consent of this essay.

o Participate in a clinical bioequivalence trial of the Clinical Trials Unit of the University Hospital of La Princesa.

o Do not reach stage I on the VIA scale, in the first assessment.

o Fluid intake equal to or less than 500 ml, in the last 6-8 hours before venous drainage (15,38).

The exclusion criterion of VIA stage I scale could be justified as a consequence of the conclusions of the research study that precedes the present one (37).

7.3. Exclusion criteria

The default exclusion criteria:

o Carrier of a recognized or diagnosed disease. o Reach stage I on the VIA scale, in the first assessment.

o Ingestion greater than 500 ml of fluids in the last 6-8 hours before venous drainage (15,38).

o Not demonstrate the ability to understand the information sheet, and adhere to the instructions described.

7.4. Criterion of withdrawal and final data matrix

The data will be included in the final matrix for the analysis to those who have complied with the guidelines throughout the field work; in accordance with exclusion criteria. The following lost data are considered:

- No possibility of intravenous catheterization in the first period of the bioequivalence study.
- Subject that does not attend the second period.
-

It is not considered lost data when in the second period an intravenous cannulation is not possible but it has been tried at least once. It is possible to collect all the data required by this study for analysis of the primary variable; which is for which the individuals calculated in the sample size are required.

That is, only the data not lost or deviations from the protocol will be included; analysis for protocol compliance

8. **DESCRIPTION OF THE INTERVENTIONS AND THE COMPARATOR**

8.1. Description of interventions

8.1.1. Application of dry topical heat:

The application will be carried out with two sacks of algarrobo seeds, joint previously heated in the microwave for 0.30 seconds at 850 W of power; 0-1 minute according to instructions for use. Both sacs are deposited in an aligned and consecutive manner in the anatomical area that is a

candidate for venipuncture, usually antebrachial. The first sac will be located in the most caudal part, usually the wrist, and the second in a more cranial area. This is intended to enhance the venous flow from distal to proximal. Dry heat is effective at a temperature of 94 degrees Fahrenheit; an approximation to degrees centigrade has been made, 34.47 ºC, put into practice, it could be set as 34-35ºC (38,43).

The application of the bags should be carried out for 7 minutes in the approximate area to be punctured before applying pressure to stagnate the blood and thus collect the largest amount of venous blood in the area of interest (43).

The bags do not include any CE whose trademark is Tusacotermico ® purchased through its online website. This method is used to adapt perfectly to the anatomy of the body area in which it is superimposed, to be of low cost, approximately eight euros, and covered by cotton cloth which is a thermally conductive material.

In the dry topical heat intervention arm, stagnation pressure will be applied with the elastic tourniquet, whose pressure can not be monitored.

8.1.2. Intervention of high pressure tourniquet application:

It will be carried out with the use of the manual anaeroid sphygmomanometer. Brand QUIRUMED with CE Marking 0197. A pressure lower than systolic blood pressure will be applied, measured in millimeters of mercury (mmHg), or what is the same, although the radial pulse of each patient is still palpable (16). It should be placed 10 cm above the level to be punctured if it is in the ulnar zone; or in the lower area of the arm without exceeding the elbow in case of puncture on the back of the hand (15).

This material resource has been chosen because of its easy access in any nursing work environment and its acquisition would not have an additional cost; It would even be making its use profitable. In addition, it does not involve any teaching prior to its use because the nurses are acquainted.

8.1.3. Application intervention of both:

Heat will first be applied, as outlined above. After seven minutes, the pressure is applied with the sphygmomanometer throughout the venipuncture process until the vein that has been marked as a priori target is catheterized. As a result, the effect is simultaneous.

8.2. Temporality inherent in interventions

It has been recorded that the third method describes the duration of the minutes approximately (37). The rest is considered to have a shorter duration.

8.3. Description of the comparator

Usual practice, up to now: The steps established in the standardized and approved venipuncture guide CLSI GP41-A6 (44) have been followed. It has been taken into account, the modification of said guide by Lima-Oliveira et al. in the order of steps to follow to put on the gloves; in such a way that it adapts to the usual clinical practice in Spain (27).

Therefore, the steps of the latter, CLSI GP41-A6 (44), will be followed, allowing the gloves to be placed in the order considered by the nurses. The irrevocable condition will be to comply with this step, as well as to comply before the step corresponding to the venipuncture itself.

The usual method does not involve the application of topical heat previously, and the tourniquet corresponds to latex.

In this protocol, venipuncture will refer to the insertion of peripheral intravenous catheter, from the caudal to the cranial part of the forearm. It will be considered successful, after the insertion of the catheter, venous reflux occurs and the extraction of a basal blood sample is possible.

8.4. Justification of the comparator

The comparator corresponds to the usual clinical practice up to now, arises from the need to know new alternatives to the work method harmonized in our environment.

In particular, both the approved and standardized guide (44) and an unstandardized guide have been considered, which, based on the first one, undergo minor modifications (27). While the standardized guide comes from the United States, so the work dynamics is different, the non-standardized one emerges at the Complutense University of Madrid, at the national level; It addresses the necessary adaptation to the Spanish work dynamic (27,44).

8.5. Adherence to interventions and protocol deviations

Adherence to the protocol will be ensured with the following resources:

- The time and the power of the heat source emitted to the carob bean sacks will be carried out by means of a microwave, whose power and time will be pre-set.

- The application time of the seed bags, seven minutes, will be controlled through a portable countdown timer. There will be a countdown and ascending timer with CE marking, for each subject. The nurse will program the device so that, after seven minutes from the application of the bags, an alarm is issued to remove them. Likewise, when the alarm sounds, an ascending count begins and the time elapsed in excess can be known; in which case.

Deviations from the protocol and data retrieved will be considered in the following cases:

Application of dry topical heat

1. If the two sacks of seeds are not heated sufficiently -> It will proceed to heat two more again. There will always be two more sacks of reserve; so reheating sacks is not recommended because the temperature that could reach them is unknown and could generate "noise" to assess the effectiveness of the intervention.
2. If 7 minutes are not reached -> Protocol deviation
3. If the bags are left in excess of 7 minutes -> Protocol deviation
4. If a sack of seeds is released, or both, and recovers to its previous position in less than 1 minute (Flexibility time). No need to let act one more minute -> Data recovered. 5. If a sack of seeds, or both, comes off and does not recover to its previous position in less than 1 minute (Flexibility time) -> Protocol deviation
6. If the two sacks of seeds were detached, without the possibility of estimating the time that has elapsed approximately -> It is recovered and the process is started again.

There will be another pair of bags for the possibility of loss of them.

Application of high tourniquet pressure

1. Pressure exerted with the sphygmomanometer less than 100 mmHg is considered insufficient -> Protocol deviation
2. If the pressure of the sphygmomanometer gradually decreases due to forgetfulness of the valve fixing that controls the air output before performing venipuncture-> fix the valve. Data recovered.
3. If the pressure of the sphygmomanometer gradually decreases due to forgetfulness of the valve fixing that controls the air output after performing venipuncture-> Protocol deviation

9. STUDY DEVELOPMENT

9.1. Coverage of Clinical Trial Unit

Clinical bioequivalence trials are conducted in the Phase I room. This one has twelve chairs, one for each subject. It is possible to realize groups of twelve people until reaching the totality of the subjects recruited.

It consists of three consultations, two carts where the most frequently used material is stored by the nurses and in the most accessible way, as well as a stop cart. It is strategically located, in front of the Resuscitation Unit in case emergency assistance is necessary. It consists of four electrocardiographs, three portable tensiometers and one fixed one. Four tympanic thermometers, two anaeroid sphygmomanometers, two phonendoscopes, a stadiometer and a scale. Two toilets for use by volunteers with bell, in case of assistance, as well as security cameras and a room of common use for the personnel involved.

9.2. Workflow of Clinical Trials Unit for bioequivalence studies

In any of the bioequivalence trials, subjects are recruited who meet the inclusion and exclusion criteria added to those described above.

It supposes an income around 24 h, according to the design of each trial, and the intravenous cannulation to each subject to perform the pharmacokinetics of each drug. A recruiting visit is made to inform about the clinical trial in question, the Patient Information Sheet and the Informed Consent are delivered.

It is ensured that they do not carry any disease; the following is done:

- Blood and urine extraction. Biochemistry, Hematology, Serology and urinary sedimentation. - Vital signs. Blood Pressure (BP), Heart Rate (HR), Temperature (T).
- Anthropometry Weight, Size, Body Mass Index (BMI)
- Electrocardiogram (ECG).
- Physical exam. Concomitant treatment.

In this visit will be carried out, only the selection of subjects according to the criteria of inclusion / exclusion of the trial that takes place in the unit. Subjects enter the night before the prescribed day to perform pharmacokinetics. Next day, at 08:00 h, intravenous cannulation is started by the nursing team. The subjects remain fasting from dinner on the day of admission. The medication is always administered after the canalization and once a basal blood sample has been extracted in tubes whose additive is dipotassium ethylenediaminetetraacetic acid (K2EDTA).

9.3. Workflow between Clinical Trial Unit and ECYPVEN-H/17

It is the day of the pharmacokinetics or, day two of entrance of the essay of bioequivalencia, when it will carry out the application of the three interventions and the comparator.

9.4. Development of fieldwork of the trial

The first evaluation of the VIA scale (36) will be carried out, according to the number of optimal puncture points (PPO), before the application of the arm assigned by the main researcher and will correspond to the inclusion and exclusion criteria; pre-intervention assessment. This will be transcribed as the first VIA scale assessment (36).

In the interventions, the VIA scale will be evaluated twice (36). The second assessment will be carried out after the application of the intervention or randomly assigned comparator; and before performing venipuncture.

In the case of the comparator, it will only be evaluated once; It will not be assessed if it denotes a change of stage, since no intervention is applied. If this corresponds to the first period, the registration stage of the inclusion / exclusion criteria will be transcribed, if it corresponds to the second period, the corresponding nurse will assess it.

In both cases, the intervention or comparator will be applied, the success or failure in successful venipuncture will be recorded on the first attempt. In the case of failure, the attempt number to which the catheterization was possible will be recorded. Table 2

ORGANIZATION CHART ACCORDING TO VISITS		
ENROLLMENT VISIT	FIRST DAY ENTRY	SECOND DAY ENTRY
1. Information Sheet 2. Informed consent form. 3. Training for the nurses listed on the form.	X	1. Subjects inclusión according to inclusión and exclusión criteria VIA assessment 2. Randomized assignment 3. Intervention/comparator asigned application. 4. Post intervention VIA assessment. 5. VAS pain assessment. 6. FITZPATRICK scale skin assessment 7. Hemolysis analysis.

Table 2. Organization chart according to visits. Own elaboration.

This organization chart, Table 2, will be specific to the first period of the clinical trial. Another detailed organization chart has been prepared for the two study periods in section 9.7.3.

9.5. Quality control and monitoring

The present work does not work with a monitor since the promoter and main researcher coincide in the same person. In addition, when considering a low-level intervention study, the following is established:

The concept of "low-level intervention clinical trial", which calls for adopting less rigorous standards in aspects such as monitoring, the content of the master file or traceability, without impairing the safety of the individuals participating in them (2).

However, the principal investigator of this trial and the principal investigator of the bioequivalence clinical trial and collaborating researcher of this one, will be in charge of monitoring the course of the clinical trial according to what is established in the protocol. Likewise, they are responsible for the good progress of the study, for carrying out quality controls and the degree of cooperation of different collaborators not mentioned in this document.

9.6. Thermoregulation monitoring

The temperature and humidity will be monitored with an environmental clock, oh! Haus & co.®, with CE marking. It will collect the temperature of the whole phase I; between 23 and 25 º C with a humidity of 27 and 31% approximately. Likewise, light will always be artificial.

The clothing of the subjects is harmonized by a hospital cotton pajama. Therefore the thermoregulation is considered to be roughly harmonized and that the intersubject variability in that aspect is not high.

9.7. CLINICAL FINDINGS AND MATERIAL RESOURCES INVOLVED

9.7.1. Pain assessment

The evaluation of the pain will be carried out through the scale validated Visual Analog Scale (VAS) / numerical (45). Linear scale from zero to ten, from minimum pain to maximum perceived pain, escalating with integers in increasing order. The use of this scale is considered pertinent because it has been validated for the detection of both chronic and acute pain; through changes in temperature and for groups of individuals (45,46).

The individual will be provided with this scale, in paper format, so that he self-expresses the pain perceived in the venous cannulation with the randomly assigned method. It is provided immediately after the fixation of the vein catheter.

9.7.2. Evaluation of skin types

An evaluation of the skin type will be carried out according to Fitzpatrick's validated scale. Scale commonly used in the field of dermatology, which consists of six skin types according to their visual characteristics and the frequency of burning before sun exposure (47). The phototype one refers to white skin that always burns with sunlight and, phototype six refers to dark skin that never burns. The identification of these phototypes is self-expressed by the subject, valuing the medial anatomical region of the arm; part considered by experts as less exposed to sunlight (47,48).

The concordance of the self-expressed skin type according to this scale and the melanin concentration with spectrophotometry have been evaluated. Some studies agree on the finding of concordances in some phototypes and in others, but they point out that it could be due to the self-evaluation of the subject; even so all of them consider that it is a good estimator of the skin type. However, other studies have validated the scale for all phototypes only in women (49), and another study has validated five of the six phototypes, with the exception of the first one, due to the characteristics of their mixed accessible sample (50). All the findings consider appropriate the scale to assess the type of skin according to the color and frequency of tanning / burning, and not so to identify the genetic disposition. Therefore, it could be considered appropriate to use the Fitzpatrick scale to identify the type of skin and the frequency of possible erythemas caused by topical heat in this study (47).

Individuals will be provided with said scale, in paper format, and they will themselves indicate the item in which they are identified. They will be reminded to look at the medial region of the arm.

9.7.3. Hemolysis evaluation

After bibliographic search, no validated methodology was found to evaluate said construct, which is why it is intended to design a scale, in spectrophotometric terms of absorbances and colorimetry; through images and / or photographs, and subsequently validate this scale throughout the fieldwork. It will be carried out in the extraction of blood samples at different times throughout the admission of healthy subjects in UECHUP.

Different cross-interferences for hemolysis have been described, which could bias the identification of their intensity, both in absorbance and in visual detection (51, 52,53) and, therefore, interfere in some analytical determinations. Cross-linked interferences such as

bilirubin and lipemia are identified, which may cause a "yellowish" color and / or "turbidity" in the plasma, respectively (53). In general it is known that lipemia acts by overestimating the intensity of hemolysis and indirectly the accuracy in the determination of blood constituents and the bilirubin denoted by an underestimation effect in the intensity of hemolysis and accuracy of the determinations of other blood constituents in concentrations, measured in milligrams / deciliter (mg / dl) (30). In addition, Saldaña IM et al has shown, in its interferogram, that there is a non-linearity relationship between the concentration of free hemoglobin in plasma and the determination of total bilirubin, denoting an underestimation of it in small concentrations of extracellular hemoglobin and subsequently producing a overestimation In the same way, it states that not only depends on the amount of hemoglobin, but also the concentration of bilirubin. (54).

Hemolysis, without cross-interference, alters the accuracy of some analytical determinations, generally increases the concentration of those determinations that occur in large concentrations at the intracellular level, and decreases those that are found at low concentrations (53). Currently, the serum indices are being used as parameters to evaluate the intensity of hemolysis. In a semiquantitative term, they defend the use of absorbance for their measurement; However, no manufacturer provides calibrators and controls for these indices, which prevents the evaluation of their analytical performance (30, 51,53).

It is denoted that at 414 nanometers (nm), where the maximum intensity of free hemoglobin in plasma is generally found, it is a specific point of measurement in which the absorbance curves of bilirubin and lipemia overlap; between 400 nm and 540 nm for the detection of jaundice whose maximum expression is evidenced at 460 nm, and between 300 nm and 700 nm for lipemia without maximum peak expression but optimal absorbance at 300 nm. Farrell CJ et al proposes a correction factor to eliminate these interferences which does not contribute. As a consequence, a possible cross-interference of hemolysis will be eliminated by monitoring the bilirubin of the subjects through blood analysis in the screening visit of the bioequivalence study. For lipemia, the analysis of those blood samples that are as remote as possible, in time, from the food intake of the volunteers; It is evident that the most common cause of lipemia is the recent intake of a meal high in saturated fats (53). Therefore it is not considered necessary to perform any correction factor for the cross-linking of bilirubin, however, if it could be useful to subject those blood samples in which lipemia is suspected to an absorbance of 650 nm, according to the schedule established for the extraction and its postparallel temporal relationship. Although at 300 nm the absorbance of lipemia is higher, it interferes with extracellular hemoglobin; not so at 650 nm (53). If there is present lipemia, in spite of its avoidance, it would be representing the

target population and the reality as it manifests an increase in blood samples without having respected a fast and these conditions are even allowed in some guidelines for the evaluation of cardiovascular risk (53); therefore, it is considered that the adjustment method could be justified to what the authors propose.

The evaluation of hemolysis will be carried out by absorbances of extracellular hemoglobin after centrifugation of these. The material resources involved and their use are the following:

1. NanoDrop ™ 2000 Spectrophotometer Thermo Scientific

Device, of small size, approximately 20 cm wide, 14 cm deep and 20 cm high, equipped with a broad spectrum of Ultraviolet-Visible (190-840 nm) for micro volumes of 0.5 to 2 microliters of sample initial through fiber optics. It has the measurement of very concentrated samples without the need of previous dilutions. The quantification of the absorbance of oxyhemoglobin at 414 nm will be measured by dropping one to two microliters of the substance to be analyzed. On this substance the device emits a beam of light that crosses the sample. Depending on the beam of light traversed and, therefore, received, it is considered transmittance. Although the transmittance leads to losses due to dispersion in the solution, it is considered, according to the Lambert-Beer Law, that the transmittance and absorbances are inversely related. Absorbance is the intensity of light that the chemical component of the sample retains and, therefore, is the opposite of the received light. As a consequence, the absorbance is measured with the opposite of the logarithm of the transmittance, expressed as follows: A = -log T

It will be necessary to use the centrifuge Hermle z326K, centrifuge with possibility of cooling. Automatic pipettes of different microliter ranges from the Eppendorf Research Plus brand.

Tubes whose additive is dipotassium ethylenediaminetetraacetic acid (K2-EDTA) that will be centrifuged at 3500 revolutions per minute (rpm) for 10 minutes at 4 ° C will be used.

The plasma of the blood samples will be transferred to aliquots with a capacity of approximately 1.4 ml. The biological material to be transferred will be approximately 1 ml, regardless of the one obtained. The remaining plasma will be discarded to a biological container and destroyed according to the usual procedure and marked by the clinical trial with drugs.

The pertinence of this material resource could be justified by the following:

1. Coverage in the Clinical Trials Unit and Collaborating Researchers.

2. Acceptable capital, compared to other methods.

3. Objective method and sensitive to changes supported by Janish S et al (52).

2. Photoshop CC.

Application intended for the identification of the color of photographs. The program outputs the values in RGB and CMYK automatically.

3. Photographic camera

The photographs will be taken through the SONY® SLT-α58 camera with HD CMOS sensor and RGB filter with CE marking.

The design and validation of the scale are collected, in detail, in section 14.2.3.

ORGANIZATION CHART OF CLINICAL FINDING ACCORDING TO VISITS		
FIRST PERIOD	WASH-OUT PERIOD	SECOND PERIOD
VAS/NUMERIC scale for pain assessment FITZPATRICK scale for skin assessment Hemolysis analysis ▪ Baseline blood sample ▪ Afternoon blood sample ▪ Evening blood sample	**X**	VAS/NUMERIC scale for pain assessment Hemolysis analysis ▪ Baseline blood sample ▪ Afternoon blood sample ▪ Evening blood sample

Table 3. Organization chart of clinical findings according to visits. Own elaboration.

9.8. RISKS-BENEFITS

A. RISKS

Derived from interventions

Possible local erythema due to the application of dry topical heat. They have only been registered in those individuals that denote "sensitive" skins; without being visible in "non-sensitive" skins. In addition, it has been documented that it remits after a few minutes without the need for treatment (37).

Possible transient paresthesia in the arm applied the high pressure with the sphygmomanometer because of overcoming an average blood pressure of 100 mmHg. No case has been documented

(37) although the possibility of it is considered. It is solved assuring the presence of radial pulse in mentioned upper limb.

Derived from venipuncture

Possible hematoma and pain due to the venipuncture procedure itself. As well as a vasovagal syncope. The sensation of pain due to the pressure exerted by the elastic compressor is also contemplated, whose origin is attributed to the type of material rather than the pressure exerted.

Derived from the evaluation of blood samples

No risk has been registered, neither direct nor indirect, for the observation of plasma in blood samples.

B. BENEFITS

Beneficial effects have been registered in the increase of the antebrachial venous perception, previous step to the venipuncture, with the combined application of pressure and heat (37). Therefore, it suggests a possible beneficial effect in successful intravenous cannulation in a smaller number of attempts.

No direct benefit has been recorded on the patient in observing the plasma of the blood samples. However, it could be considered that the indirect benefit would be directly related to the transcendence of the clinical findings.

A. EVALUATION OF THE RISK-BENEFIT BALANCE

As a consequence, the local erythema that remits in minutes would reflect greater benefit than the appearance of hematoma of prolonged duration to days; produced by failed intravenous lines. The transient paresthesia, with easy solution, could reduce the pain perceived by the subject, whose impact would be beneficial. Both the pain of venipuncture and vasovagal syncope occur with reduction thereof, the lower the number of attempts at venipuncture.

It implies an increase in resources for a second or third intravenous cannulation, both of material resources involved in the technique of venipuncture as well as that for the treatment of hematoma and vasovagal syncope, as well as the time of the nurses. To this is added the opportunity time; the lack of investment in other objectives or activities, and the discomfort of the subject.

The transcendence of the clinical findings of hemolysis would exceed the possible discomfort generated by the observation of one-two microliters of plasma.

As a result of what has been described above, it could be concluded that the attributed benefits outweigh the acceptable risks.

9.9. FINISHING OF FIELD WORK

It is considered that the fieldwork has ended when the randomly assigned arm and venipuncture have been applied on the first attempt to the subject number 54. In the case in which any of the subjects had adverse reactions, the completion of the fieldwork of the patient will be prolonged until the reaction ceases. At that moment the data entry begins in the SPSS v23 program.

10. EVALUATION OF EFFECTIVENESS

10.1. Scope of the effectiveness of the clinical trial

The effectiveness will be considered reached when a clinically relevant parameter in venipuncture is reached, section 10.2.1, and, whenever possible, an attempt will be made to reach the global effectiveness, section 10.2.

10.2. Global effectiveness reach

10.2. 1. Clinically relevant parameter in venipuncture

An optimal effectiveness for venipuncture at the first attempt will be considered reached when one of the three interventions manages to overcome the success rate at the first attempt through usual clinical practice. That is, when a success rate of more than 74% is achieved (55).

10.2.2. Clinically relevant parameter in pain

In the totality of the intravenous cannulations at the first attempt with any of the interventions, cases with moderate-moderate severe pain scores should be higher in cases of severe pain. That is, the scores from zero to six, included, must be greater than the scores from seven to ten, including (46).

10. 2. 3. Clinically relevant parameter in hemolysis.

In the basal samples, taken immediately after the application of any of the test arms, they should be less than 25 mg / dl (54). Concentrations will not be measured, but they must carry out a visual inspection and / or absorbance less than what corresponds, by means of bibliography, said cut-off point.

11. SAFETY ASSESSMENT

11.2. Safety monitoring

11.2.1. Record adverse events

A record of the secondary events and the symptomatic and specific adverse reactions will be carried out, which will be assessed by visual inspection. It will be the nurses, who apply each arm, those responsible for the visual inspection and the communication of the adverse reaction, if any, to the principal investigator and / or collaborating researcher. Both the principal investigator, the collaborating researcher and the collaborating nurses will be able to record any secondary effect (9).

The definition of adverse reactions requires adaptation to medical devices. Its calving is considered from the application of any intervention and / or comparator. There will be an approximation of the causality between the effect and the applied method; according to what the World Health Organization establishes (WHO) (56):

1. Certain
2. Probable.
3. Possible.
4. Unlikely
5. Conditional / Not classified
6. Not evaluable / Unclassifiable

Likewise, the severity will be recorded; according to the WHO (57), with modification:

1. Serious
2. Moderate.
3. Mild.
4. Incidental.

The frequency of appearance thereof will be calculated according to what is established by the Council for International Organizations of Medical Sciences (CIOMS) (58):

1. Very common: They occur with a frequency greater than or equal to 1 case every 10 patients.
2. Frequent: They occur with a frequency greater than or equal to 1/100 but less than 1/10.
3. Uncommon: They occur with a frequency greater than or equal to 1 / 1,000 but less than 1/100.
4. Rare: They occur with a frequency greater than or equal to 1 / 10,000 but less than 1/1000.
5. Very rare: They occur with a frequency less than 1 / 10,000.

Each case will be followed until it ceases, and the method used to remit said effect will be registered.

The appearance of local erythema has been registered as an adverse effect of dry topical heat (37). Likewise, the appearance of a hematoma due to traumatic puncture is expected.

It is considered that the registration of side effects in this trial could be justified as one of the objectives of clinical research with medical devices, both under the conditions of use for which they have been accredited and for those that have not yet been . In this way, it is intended to increase the knowledge about the possible risks, and that these do not exceed the benefits, as well as the feasibility of the implementation thereof for the intended use in the present clinical trial (9).

11.2.2. Serious and unexpected adverse events

No serious and unexpected adverse reactions are anticipated with the medical devices used in this trial. However, if they were produced, they would be registered in the same manner as for the adverse events and their notification would proceed; Section 11.2.3.

11.2.3. Serious and unexpected adverse events notification

The notification will be carried out early and in the shortest possible time from the appearance of said reaction. The responsibility for the notification rests with the principal investigator. The principal investigator of the clinical trial of bioequivalence, the Clinical Research Ethics Committee with medicines and medical devices (CEIm) involved, and the Spanish Agency for Medicines and Health Products (AEMPS), the department of health products involved, will be notified

The notification to the AEMPS of the Serious and unexpected adverse events will be carried out as established by the AEMPS in the national environment. It will be done in Spanish, notifying through the FEDRA portal; when you get access to it.

In case of impediment in accessing said portal, it will be sent by fax, to the number (+34) 91.822.50.76, or, through CD / DVD with postal mail, enclosing the accompanying letter and validated forms destined for such use ; available in your guide (59).

In addition, it is intended to notify the Serious and unexpected adverse events in Eudravigilance-CT.

11.2.4. Monitoring of adverse events and / or serious and unexpected events.

The follow-up will be carried out in person and, if this is not possible, by telephone or via e-mail. Likewise, the time of follow-up is not established and will be the time necessary until it ceases, it will be the effect itself that marks the temporality.

12. ETHICAL AND ECONOMICAL ASPECTS

12.1. Ethical aspects

12.1.1. Legal framework of the trial

The ethical, methodological and protection principles of the subjects of this study are governed by the provisions of307 Royal Decree 1090/2015, of December 4, which regulates clinical trials with medicines, the Ethics Committees of the research with medicines and the Spanish Registry of Clinical Studies through which the rights of respect, beneficence, non-malfeasance and justice prevail over any other interest (2). In the same way, all the principles contemplated in the Declaration of Helsinki (60), applicable to nursing research and competence, will be safeguarded.

The present clinical trial could be considered as a low intervention level, according to the definition established for this type of tests by Royal Decree 1090/2015 (2).

Likewise, the medical devices used in this study are considered active according to Royal Decree 1616/2009, of October 26, which regulates active implantable medical devices. (9).

12.1.2. Notification to entities and committees

According to the provisions of the AEMPS and current legislation, the need for notification of the existence and intention to carry out the present clinical trial to the AEMPS is foreseen, provided that the favorable opinion of a CEIm has been previously obtained (2 , 9,12). Likewise, the request for exemption from fees is envisaged, since it is an independent investigation of an academic nature.

The non-issuance of an AEMPS approval is considered justified because this study is considered a low-level intervention clinical trial, in which all medical devices are commercialized. Both are considered class IIa according to the classification of the AEMPS and, although in the case of seed bags do not show the CE mark which could justify their clinical research, they will be used for a different purpose than the accredited one. However, its application will be carried out according to the instructions for use described for the purposes that are accredited for each medical device involved in the study.

12.1.3. General rules for collaborating researchers

All the personnel involved in the collaboration of this clinical trial have a certificate of Good Clinical Practices (GCP) in force; less than two years old. In this way, the acceptance of the data presented is facilitated, derived from the compliance of this protocol. This is how the quality standards and the guidelines of the International Conference on Harmonization (ICH) on good clinical practice are assumed (2). In addition, it is ensured, by means of a register, that all the components of the equipment work in a harmonized manner according to the Standardized Work Procedures of the UECHUP.

12.1.4. Information sheet and informed consent

First, the individual will be informed of the intervention as verbal communication as written through the information sheet. You will need to write your name, date of birth and the name of the professional who has explained the information in the informed consent in your handwriting. Subsequently, the signature and date of the agreement of the subject, as well as of the principal investigator before it, will be collected and an original copy of both the information sheet and the informed consent will be provided; APPENDIX I.

It was considered appropriate to draw up a box corresponding to the evaluation of hemolysis. It is considered that it could be justified because blood extractions are not required in addition to those already stipulated, manipulation of the peripheral venous accesses is not affected more than expected, two microliters would be collected in at most in each sample; Finally, his analysis has no connection with genetic data or any other personal data. In addition, your evaluation will be blind (61).

The subject will be able to ask all the questions that he considers pertinent and will be answered, likewise, he can revoke his agreement at any time, without negative consequences and without the need to provide a justified reason. No procedure will be carried out if the compliance duly completed by each subject has not been collected.

12.1.5. Confidentiality access to information.

The protection of the data of the participants, whether personal or health related, will be kept coded, with two digits, in a reversible manner, by means of the document prepared which will only be accessed by the sponsor, principal investigator and collaborating researcher in the trial. Said file will be confidential and will not leave the institution where it has been collected,

UECHUP. In this way it is intended to address the security of data as established in Organic Law 15/1999, of December 13, on the Protection of Personal Data (62).

The data will be collected in paper format and a collection record form (CRF) will be created. The code will be dissociated with the personal data of each individual in a reversible manner until the end of his admission or refer the adverse effect; in which case.

The coded and, later, anonymized data will only be used to check the sample count and that no subject is lost in the transcription of the information to the statistical database SPSS version 23 for further statistical analysis. Only the principal investigator will have access, in the first instance, to avoid altering the data processing and controlling access to it (62).

All collected data are subject to professional secrecy during and after the investigation, as well as to its storage, safeguarding the integrity and readability of the data during, at least twenty-five years after the end of the study (2.62). If there is an inspection in some cases, they will have access to the data, as long as they maintain professional secrecy, as established in Organic Law 15/1999, of December 13, on the Protection of Personal Data (62).

12.2. Economical concerns

12.2.1. Insurance

As a result of being a low-level intervention clinical trial, the attributable risks are considered minimal compared with those of the usual clinical practice and according to what is established for them by Royal Decree 1090/2015 (2) in Chapter III Article nine of compensation for damages, it is considered that the specific insurance contract for the present trial is not necessary and that it could be supported by the clinical bioequivalence trial insurance in which this protocol is applied.

12.2.2. Economic report of the trial

All the necessary additional material is paid by the promoter and principal investigator of this clinical trial. That is, the coverage of the sphygmomanometers, seed sacks, temperature thermometer, portable countdown timer, camera, stationery and toner.

As well as the training of nurses, collaborators / observers and the acquisition of the Photoshop program and statistical package SPSS version 23.

No economic goods or economic budget for the material resources by external to the investigation are contemplated.

13. PRACTICAL CONSIDERATIONS

13.1. Training of the nursing team and collaborators / observers

It is considered necessary to train the nursing team with certain scales that, except for the VAS / Numeric scale, are not commonly used in routine clinical practice. Likewise, interventions require specific knowledge a priori.

No training is contemplated, because the nurses are familiar with the material resources they will use, only a training is planned with the possibility of asking questions and these will be solved and / or clarified. The main researcher will belong to the collaborating nursing team and will support them in the fieldwork.

Similar situation is that of collaborators / observers of the intensity of hemolysis. Those involved in the evaluation of blood samples will be trained, although they are trained in the use of the material involved.

13.2. File of the documentation

Throughout the study, a file is created with all the documents involved throughout this essay. At least the following will be included:

- CEIm report
- Information sheet and informed consent.
- This protocol, as well as its amendments; in which case.
- Any correspondence with the CEIM.
- Registration of signatures of the collaborating nursing team.
- Any document related to the acceptance of the viability of the project in the institution.
- Serious adverse events notification form
- Registration of the reversible coding of subjects.
- CRF copies

The results of the CRF are considered complete at the time of venipuncture. It has been considered appropriate to carry out two CRFs, with the completion of workfield of fusion, due to the need for the evaluation of hemolysis. In this way, it is intended to complete the CRFs in two different work rooms: CRF for hemolysis in the laboratory and those corresponding to venipuncture in the phase I room; Avoiding the violation of blinding.

It is estimated that once the study was completed, a place where the personal data of the subjects were recorded was saved and saved, under the same conditions as those reflected in section 12.1.5.

13.3. Terms of results publication

Once the fieldwork is completed, the data will be irreversibly dissociated and therefore anonymized data will be obtained. It is considered irreversible when establishing the link between a data and the subject to which it refers is no longer possible by reasonable means (2,62).

Only information that is anonymous can be transferred to third parties (62).

13.4. Amendments to the protocol

When amendments to the protocol are made, they will be notified their evaluation and subsequent opinions.

14. STATISTICAL ANALYSIS PLAN

14.1. Main analysis

14.1. 1. Definition of variables

Primary variable

Successful venipuncture at the first attempt. Dichotomous qualitative variable. Success / Failure

Secondary variables

Successful venipuncture frequency. Discrete quantitative variable. (1,2,3,4)

Visual intensity of hemolysis. Discrete quantitative variable. It is measured in stadiums.

Scale of expressed pain intensity (VAS). Discrete quantitative variable.

Scale skin types Fitzpatrick. Discrete quantitative variable.

Ingestion of liquids (less than 500 ml) in the last 6-8h prior to cannulation. Discrete quantitative variable (measured in glasses); it will be transcribed continuously for statistical analysis.

Affiliation of individuals

Respected fasting of 6-8 hours. Dichotomous qualitative variable.

Age. Quantitative variable continues.

Sex. Dichotomous qualitative variable.

Race. Discrete quantitative variable. 1 -> Caucasian 2 -> Black 3 -> Asian 4 -> Latin

BP. Continuous quantitative variable.

HR. Continuous quantitative variable.

T. Continuous quantitative variable.

BMI Continuous quantitative variable.

14.1. 2.Sample size calculation

The sample calculation is performed with the GRANMO calculator (63). It assumes a random risk (alpha) of 5% and a statistical power (beta) of 80%; for which it is tried to prove the veracity of the null hypothesis, of bilateral type.

That is, the risk of rejecting the null hypothesis when this is true is 5%, and the probability of not rejecting it being false is 20%.

In the literature, the success of peripheral intravenous canals was recorded in the first attempt, using the usual technique, in 74% of the cases (55). Currently, a proportion of peripheral intravenous cannulation has been registered at the first attempt of 73%, in an ultrasound ecoguided manner (33). In relation to the second successful attempt, in the usual clinical practice it amounts to 92% of the cases and, in the ultrasound ecoguided to 91% (33.55).

For the present work we intend to achieve a 95% success rate in antebrachial venous cannulation at the first attempt. Thus, overcome with one of the alternative methods proposed in this study the cumulative proportion of the second attempt of the usual and eco guided clinical practice.

It is considered a clinically relevant difference the successful venous cannulation at the first attempt when, not only allows the cannulation at the first attempt of those cases that hypothetically would be observed, but accumulate to those cases that, in a way observed in the literature, the cannulation has been achieved at the second attempt, and hypothetically, that success can be achieved in the first attempt.

The comparative indicators are based on the comparison method and the reduction of intraindividual variability. It is considered a root of the experience, one of them is lost; An average of both percentages has been made, thus obtaining a 12% loss approximately.

Therefore, the calculation of the sample size should be followed through the modality of proportions, paired measures (repeated in a group). Fix in the proportion with pre-intervention event 0.74, in the proportion with post-intervention event 0.95, and the loss ratio 0.12.

Finally, the estimated sample size is 60 subjects.

14.1.3. Methodology of data analysis

14.1.3.1. Statistical methods

It is assumed that of the primary variable, being a dichotomous qualitative, there is not enough data available regarding its distribution and, therefore, non-parametric statistical techniques will be carried out.

14.1.3.1.1. UNIVARIATE STATISTICS

In order to prove the veracity of the following null hypothesis, in the previously calculated sample it will be necessary to use a univariate nonparametric test for related samples; Mc Nemar test.

H0: Successful antebrachial venipuncture at the first attempt is not affected by the use of alternative methods.
H1: Successful antebrachial venipuncture at the first attempt if it is affected by the use of alternative methods.

The alternative methods refer to the interventions mentioned above.
If Mc Nemar's test results in a p-value <0.05, the null hypothesis can be rejected and the alternative approved. As a consequence, there will be a need to know which alternative method of the three tested is the most effective.

14.1.3.1.2. MULTIVARIATE STATISTICS

Therefore, it is considered pertinent to perform a logistic regression, multivariate statistics. The primary variable is assumed as the dependent variable and the secondary variables as returnable or explanatory. The primary variable fulfills the requirement of being dichotomous and the return variables assume any type of variable. It is assumed the need to create dummy variables with alternative methods, whose reference will be the comparator.

14.1.3.2. Epidemiological methods

14.1.3.2.1. FREQUENCY MEASUREMENT

It is considered pertinent to carry out measures of frequency of the event of success and failure. It is intended to be addressed through the calculation of cumulative incidence (CI); all the subjects are measured in the same approximate period of time both in the exposure to the methods and if adverse reactions appear. Thus it is possible to know the probability of developing the successful event in a period of time. It will be calculated as follows: CI = No new

cases / population at risk This methodology could be justified because it is a prospective study, in which the time variable is not the main one, however, it is found throughout the field work.

14.1.3.2.2. ASSOCIATION MEASURE

To measure the effect of the exposure of each intervention, a measurement will be carried out through Odds-Ratio (OR).

14.2. Secondary principal analysis

14.2.1. Definition of variables

The main secondary variable is established:

Intensity of visual hemolysis. Discrete quantitative variable. It is measured in stadiums.

It aims to carry out the following secondary objective: Design and validate a visual scale of hemolysis.

To carry out its approach, through statistical methods, the following secondary variables are defined:

o Absorbance of free hemoglobin in plasma. Continuous quantitative variable. It is measured in decimals.

o Stadiums. Continuous quantitative variable. It is measured in absorbance ranges.

or RGB colorimetry. Discrete quantitative variable. It is measured with whole numerical codes.

o CMYK colorimetry. Continuous qualitative variable. It is measured in continuous percentage.

o Blood samples. Discrete quantitative variable.

o Observers Discrete quantitative variable.

The following variable is defined independently, since it will not be measured or used in the statistical analysis:

• Concentration of free hemoglobin in plasma. Continuous quantitative variable. It is measured in mg / dl.

However, it is considered an implicit variable in the scale on which the project is based (29) to carry out the modification of this and, therefore, the design of a new scale.

14.2.2. Statistical power and calculation of sample size

The choice of this secondary variable could be justified as the main one due to its statistical power, in relation to the sample size calculation, in order to conclude with a statistical

significance. That is, the sample size calculated for this variable could be included in the sample size calculated for the main variable; using the same fieldwork

According to a study (52) at a cutoff point for evaluation of free hemoglobin in plasma through absorbances and visual detection, the following is stated: of fifteen samples detected with absorbances, eight of them were not detected visually. That is, the visual inspection shows a predictive positive values (PPV) of 46.6%. It is tried to prove the veracity of this assumption and for it the following verification of hypothesis, of bilateral type is proposed:
H0: The positive prediction of intensity of hemolysis through visual stages is not optimal.
H1: The positive prediction of intensity of hemolysis through visual stages is optimal.

A calculation of GRANMO samples (63) has been carried out in the modality of proportions observed with respect to a reference. With an alpha error of 5% and a power of 1%, bilateral test. The reference proportion is 0.466 (52) and the objective is to reach a positive prediction of at least 0.80; It is considered optimal to detect the intensity of hemolysis in each stage. Therefore, the minimum difference to be detected is obtained through the objective of 0.80 - 0.466, that is, 0.334. Finally, it is obtained that at least 41 individuals are needed or, what is the same, 49 blood samples with a predicted proportion of losses of 20%; corresponds to possible samples with insufficient blood content for inspection.

14.2.3. Scale design

14.2.3.1. Description

1. It will be based on a scale previously prepared (29). This will give support to the reference colorimetry and the cut points on which establish stages. The cut-off points, measured in concentrations (mg / dl), are considered as clinical relevance valued by a panel of experts(30,54).

2. The EDTA sample will be processed. Subsequently, 1 ml of the plasma of each sample is transferred to aliquots. The photograph of said aliquot will be made.

3. The absorbance of hemolysis will be analyzed. Six microliters of the plasma will be collected; because of the need for three repeated measurements.
4. The specific color of each sample (29) will be identified through the Photoshop software.

At least two design versions will be developed, based on visual colorimetry aspects.

It excludes jaundice for being healthy subjects, and try to reduce lipemia through strategically programmed blood extractions, as it appears in section 9.7.3.

In the first instance, the samples of the first period will be used for the design of the scale, and those of the second period for the validation process.

14.2.3.2. Justification of the design of the scale

The scale or table of hemolysis for serum or plasma is used; proposed by MAYO Medical Laboratories (29) because it is the most used by other research studies and, the extrapolation of results is less difficult (64,65). Each cut point collects a color and is associated with a different concentration (29). It is considered necessary to clarify that the inference or association of colorimetry to concentrations in the new scale is not foreseen.

It is estimated that the use of these resources could be justified in the following cases:

It is considered relevant to start from this base scale (29) by the information it provides, in terms of colorimetry it is useful to decipher its coding, through the Photoshop software. It serves as a reference for the association of the information of colors that are found in the field work, and indirectly of its visual intensities of hemolysis. Thus, as of the points of cut that appear, to be able to establish stadiums.

It is foreseen the need to harmonize the structure of the support in which the photograph will be made, in this case in aliquots of the same capacity. Each clinical trial of bioequivalence requires different amounts of plasma, which is considered to alter the colorimetry of the photograph. In this way, possible fluctuations in capacities and quantities of biological material that could result in the case of the present protocol being carried out in different clinical trials due to the required sample size, would be avoided.

Although Photoshop is not the most accurate technique for measuring colors, difficulties have been encountered; because the correlation of colors in liquid and solid media does not adjust. In addition, the measurement of hemoglobin requires a fixed cut-off point of nanometers, so that the correlation of wavelength and its corresponding color would be biased and the information obtained would not be useful.

In addition, colorimetry is compromised in different liquid and solid media such as the computer screen format.

For the above reasons, the elaboration of versions in the design process of the new scale is considered justified. A priori it is not known if, the estimation of the colorimetry for the adjustment between the means, could cause biases or, on the contrary, it would not be significant enough. It has been considered that, in the validation stage, will reveal how much each of them fits.

The use of spectrophotometry is considered pertinent, although it is not the most discriminative technique currently used for the quantification of absorbances, if it is identified as discriminative and widely used for its price-quality ratio. In spite of having a sensitivity of 48% according to Shash JS et al., The rest of the precision values of this diagnostic technique are within the ranges considered acceptable (52). Their positive / negative likelihood ratios are unknown in order to extrapolate these results and, therefore, those that they expose would be distorted by the sample used in their research.

Shah JS et al. and other authors, in the last years of research in this field, affirm the relevance of the absorbance technique for the detection of hemolysis as greater precision than the concentrations, as it was done (51,52,53).

In addition, in the institution where the fieldwork is planned, it has access to said device and procedure, available and economically feasible, through a collaborating researcher of the present study.

The first samples obtained from the first volunteers included in this study will not be possible to subject them to visual inspection of the observers; it would still be in the design phase. However, these will be analyzed with spectrophotometry and photographed, so they would be measured equally but objectively.

14.2.3.3. Unidimensional scale validation

14.2.3.3.1. Precision analysis

A. RELIABILITY / REPRODUCTIBILITY OF THE SCALE.

It reports the degree to which an instrument measures without error and whose variability of data is due to the real values that a variable takes, without being a product of bias or random error. It requires repetition of measurement of absorbances of the plasma of the subjects.

The instrument is calibrated and the hemolysis tubes will be harmonized, always being the same additive. The nurses have experience in the extraction of samples and the collaborator in the handling of the device correctly.

The variability between the samples will not be very large due to screening of inclusion and exclusion criteria of the clinical trial with drugs.

For all this, the systematic error decreases, although the total variance must be studied, with greater weight of said variance for the intraindividual variability (true differences and not due to biases or random).

B. INTERNAL CONSISTENCY.

A correlation could be made between absorbance ranges and stages to observe if both follow a linear association of increase.

C. DISCRIMINATING POWER.

It will not be evaluated from a statistical point of view. For the stages, what is studied in the literature as clinically relevant will be followed; it justifies that each stage discriminates objectively.

D. TEST-RETEST / INTRA-OBSERVER RELIABILITY.

The reproducibility of the instrument will be evaluated by evaluating the absorbance of the plasma of the blood samples in equivalent conditions, since time influences in which the conditions of the blood plasma change, repeating the measurement twice minimum. The technical error of measurement (ETM) can also be known by concordances (kappa index when appply) of measurements reported by observers. Same calculation will be carried out for all versions.

E. INTEROBSERVATORY RELIABILITY.

This item will be approached with the collaboration of several observers.

They will be provided with a training, and an independent CRR for blind evaluation, in which new versions will appear in different formats, and will ask them to score some blood samples, strategically selected. They will be required to score individually according to the stadiums. We will proceed to calculate the Kappa index for each stage and for each version.

14.2.3.3.2. Validity analysis

A. VALIDITY OF APPEARANCE.

To address the convenience of stages, it has been based on literature; They are considered experts in the subject. It will be reflected in section 14.2.3.

B. VALIDITY OF CONTENT.

We intend to evaluate if the items represent those that we want to measure, that is, increase in intensity of hemolysis. A correlation between visual stages and intensity scores could be used. assessed in 14.2.1.2. B.

B. VALIDITY OF CRITERIA.

We intend to evaluate the concordance between the measures by absorbances and those made by the observers. It is intended to calculate sensitivity, specificity, positives and negatives values of the visual inspection.

C. CONSTRUCT VALIDITY.

A possible convergence or divergence of versions one and two will be assessed. In the case of finding a convergence between two of scales, the one that higher value of concordances will be the one that will be used for the verification of hypothesis.

14.2.3.4. Methodology of the design and validation of the scale of hemolysis

It is considered that the methodology used to carry out the validation of the scale could be justified by the guide of studies of diagnostic precision STARD (66). This guide addresses studies in which what is intended to be studied is evaluated with a clinical or gold standard reference (64). The steps specified in the guide are followed to ensure that the results and conclusions are valid, as well as the consideration of their applicability.

14.3. Secondary analysis attached

It has been considered that the approach of non-main secondary objectives could be carried out through the following methodology:

To identify the effectiveness of all the methods used in antebrachial venipuncture, in what refers to venipuncture at the first attempt.

It is intended to be addressed by means of a logistic regression, at the same time as the multivariate statistics for the main variable.

To evaluate the intensity of hemolysis of the blood samples, in what refers to the applied method, respectively.

It is considered that there is enough information on this subject to use a parametric test. In this case, the intensity of hemolysis is the dependent variable, it is considered justified to use an ANOVA test of a block test.

Identify the frequency of successful venipuncture at the first attempt and analyze if there is an association between it and the demographic characteristics of the individuals.

It is intended to be addressed through descriptive statistics. To analyze the association, a logistic regression will be performed; multivariate technique.

Analyze the correlation between methods used, intensity of hemolysis and pain.

It could be considered that enough information is known about pain to use parametric techniques, it is supposed to be the dependent variable and it is discrete. Therefore, it is intended to be approached using the ANOVA statistical technique or two-way F-test without interaction.

Identify and quantify adverse events, as well as their relation to skin types.

For the identification and quantification, a descriptive statistic will be carried out. In order to assess the possible association, using the Fitzpatrcik scale, it is considered that there is not enough information about its distribution, so it is considered appropriate to use a nonparametric type test; the Kruskal-Wallis test.

14.4. Intermediate analysis.

Does not apply.

APPENDIX.

APPENDIX A. INFORMATION SHEET AND INFORMED CONSENT FORM

INFORMATION SHEET

INCOMPLETE CROSS CLINICAL TRIAL WITH THREE ARMS CONTROLLED WITH HABITUAL, UNICENERIC AND BLIND CLINICAL PRACTICE TO THIRD PARTIES TO EVALUATE THE EFFECTIVENESS OF HEAT AND / OR PRESSURE IN ANTEBRAQUIAL VENOPUNCTION AND THE IMPACT ON THE INTENSITY OF HEMOLYSIS.

CODE: ECYPVEN-H / 17

SPONSOR AND PRINCIPAL INVESTIGATOR: LETICIA CARMEN SIMÓN LÓPEZ (Nurse in the Clinical Trials Unit of the University Hospital of the Princess, Clinical Pharmacology Service).

SETTING: Clinical Trials Unit of the University Hospital of La Princesa.

INTRODUCTION

You have been invited to participate in this study by volunteering for a clinical trial with medications that will be carried out in the same center (indicated above). Before proceeding to explain in detail what it is, I would like to thank you for the time and interest you devote to this study. If you have any questions during the explanation, please let us know.

BASIS

It is an academic study, linked to the Doctorate program in Health Care of the Complutense University of Madrid. The objective of the study is to identify if the use of heat and / or pressure facilitate the obtaining of "one way" at the first attempt, analyze if it influences the quality of the blood sample, that is, detect the intensity of the color of the plasma (hemolysis). Finally, to know the degree of perceived pain, as well as the possible discomfort and its relationship with skin types.

STUDY DEVELOPMENT

The study consists of applying two different methods of implantation of venous catheters followed by a blood extraction, in two different non-consecutive days, which correspond to the entries in the Clinical Trials Unit of the Hospital of La Princesa.

Day (A). The implantation of the catheter will be carried out in a habitual way, with a normal pressure in an arm (COMPARATOR). It consists in the application of rubber band (blue or green) as it is usually done.

Day (B). Catheter implantation may be carried out through one of the following three interventions:

- LOCAL HEAT INTERVENTION. It consists of the application of two thermal bags deposited in the forearm where it is expected to click, for 7 minutes. Subsequently they are removed, and normal pressure is applied to the arm (with an elastic band).

- HIGH PRESSURE INTERVENTION. It consists of applying pressure with the tensiometer (device to measure the tension), instead of the rubber band.

- INTERVENTION LOCAL HEAT AND HIGH PRESSURE. Both interventions will be applied together. Heat will first be applied, as described above; and then the pressure will be applied with the tensiometer instead of the rubber band.

The maximum duration of the whole interventions will be approximately 15 minutes.

Each participant has the same probability of having a blood sample taken with any of the three interventions. Each participant has the same probability of sequence with respect to the extraction method, being able to submit the day (A) in a habitual way (comparator) and the day (B) with some of the three interventions; or vice versa, first day (B) and then day (A).

After the installation of the catheter, the extracted blood sample is evaluated, in particular, the degree of plasma staining; component of the blood that is stained by the destruction of red blood cells (hemolysis). The analysis of the degree of hemolysis will be compared with two blood samples taken at other times of the study (there is no additional blood samples withdrawn from registered in the trial with drugs), and the extraction method in these two samples will be the standard and normal way. The remaining plasma of your blood samples will be converted into a biological container and destroyed according to the usual procedure and marked in the clinical trial with drugs which you are volunteer. We will only determine the intensity of the staining, at no time will be determined the blood parameters.

WHAT DOES YOUR PARTICIPATION CONSIST OF?

After the implementation of "the vein line", you will be asked to answer some questions of a scale to assess the following, in both entries.

- Pain assessment (VAS scale).
- Evaluation of skin type (Fitzpatrick Scale).

In addition, you will be asked to, to the extent possible:
- Do not exceed the intake of two and a half glasses of water or 500 ml from 00h on the night of admission.
- Communicate any discomfort to the nurses and, if possible, to the principal investigator.

RISKS AND BENEFITS OF THE STUDY

It has been described the possibility of local redness in the forearm, appearing only in "sensitive skin" that abates in a few minutes without the need for treatment.

No case of temporary numbness sensation in the arm has been described when high pressure is applied with the blood pressure monitor, but it is considered that it could be produced; equivalent to the usual blood pressure procedure.

Likewise, beneficial effects have been registered in the increase of the visualization and palpation of the veins of the forearm, previous step to the placement of the venous line, with the combined intervention of heat and pressure.

Potentially, it is considered that redness of local skin that remits in minutes would reflect greater benefit than the appearance of a hematoma of potential duration prolonged to days produced by failed punctures. The temporary numbness sensation, with easy solution, could reduce the perceived pain, whose impact would be beneficial.

No risk has been recorded from observing the intensity of plasma staining in blood samples. It could be considered that the indirect benefit would be directly related to the transcendence of the clinical findings. Therefore, a reduction of failed punctures, or a successful puncture on the first attempt, increases the comfort of the subject and decreases the pain.

There is **no serious risk and potential benefits** from participating in this study. Likewise, all the material used is commercialized.

OTHER IMPORTANT CONSIDERATIONS

Data related to the health of the participants will be recorded, always non-profit body and in the nature of the development of scientific knowledge.

The confidentiality of the data collected will be maintained through a reversible coding and anonymization once the entry and / or inconvenience, if any, has ended. Any data is subject to professional secrecy during and after the study, likewise, the transfer of data can only be carried out when they are anonymized according to Organic Law 15/1999, of December 13, on the Protection of Personal Data.

If there is any adverse event, it will be followed by the principal investigator and the collaborating researcher Dr. Dolores Ochoa Mazarro of this study until the end of the study, either in person or by telephone (see contact of the principal investigator). Likewise, these would be covered by the insurance of the clinical trial of which, in the first instance, you are a participant.

You will be allowed to ask all the questions that you consider pertinent and will be answered. No procedure described above will be carried out if informed consent form has not been duly completed by each subject, as established in Royal Decree 1090/2015, of December 4, which regulates clinical trials with drugs, the Research Ethics Committees with medicines and the Spanish Registry of Clinical Studies and Royal Decree 1616/2009, of October 26, which regulates active implantable medical devices.

In the same way, no procedure for the evaluation of blood samples will be carried out if their express agreement and informed consent form has not been previously collected and duly completed after having received all the necessary information; as established in Law 14/2007, of July 3, on Biomedical Research.

Participation is totally **voluntary**, you can revoke your agreement at any time without negative consequences for you. You can be excluded from the study if the research team of the study considers it appropriate, either for your safety or because you are not following the instructions provided.

CONTACT OF THE PRINCIPAL INVESTIGATOR. Clinical Trials Unit. University Hospital of La Princesa. C / Diego de León 62. Postal code 28006. Contact telephone number: 91 520 24 25/91 520 22 47. Email address: leticia.simon@salud.madrid.org

Own elaboration.

INFORMED CONSENT FORM

I, _____,
have understood everything that is reflected in writing in the information sheet, the information provided has been sufficient and has been able to ask questions about the study whose code is ECYPVEN-H / 17.
Likewise, my doubts have been resolved by _____.
I declare that it has been delivered to an original copy of this document.

I understand that it is voluntary and that I can withdraw my participation without negative consequences, at any time and without having to provide justifications.

Therefore, I freely agree to participate in this study and consent to the use of my data, always for non-profit purposes and for the promotion of scientific development.

Also, I agree to assess the intensity of plasma staining / hemolysis in some blood samples that are extracted during my admission.

Signature of the participant Signature of the researcher

Name: Name:

Date: Date:

ECYPVEN-H/17

Version 1.0 (Original)

Own elaboration.

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ANNEXES.

ANEXE I. DECLARATION OF HELSINKI

World Medical Association Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan, October 1975

35th WMA General Assembly, Venice, Italy, October 1983

41st WMA General Assembly, Hong Kong, September 1989

48th WMA General Assembly, Somerset West, South Africa, October 1996

52nd WMA General Assembly, Edinburgh, Scotland, October 2000

53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification on paragraph 29 added)

55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification on Paragraph 30 added)

59th WMA General Assembly, Seoul, Korea, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the

consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances: The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Source: World Medical Association. Declaration of Helsinki (2008). Ethics Codes Collections. 2011. Available From: https://ethics.iit.edu/ecodes/node/4618