This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes
Nebulized Hypertonic Saline in Emergency Department for Acute Bronchiolitis

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1.1 ORIGINAL (INITIAL) TRIAL PROTOCOL

GUÉRANDE

3% HYPERTONIC SALINE TO REDUCE HOSPITALIZATION RATE IN ACUTE VIRAL BRONCHIOLITIS:

A MULTICENTRE RANDOMIZED CONTROLLED TRIAL

BIOMEDICAL RESEARCH PROTOCOL

PHRC National 2012
V1 06/04/2012

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75010 PARIS
### LIST OF INVESTIGATION CENTRES

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1 STATE OF THE ART

1.1 Acute viral bronchiolitis

Bronchiolitis is a disorder most commonly caused in infants by viral lower respiratory tract infection. It is the most common lower respiratory infection in infants less than two years of age. It is characterized by acute inflammation, edema, and necrosis of epithelial cells lining small airways, increased mucus production, and bronchospasm (1).

Most clinicians recognize bronchiolitis as a constellation of clinical symptoms and signs including viral upper respiratory prodrome followed by increased respiratory effort and wheezing in children less than 2 years of age. Clinical signs and symptoms of bronchiolitis consist of rhinorrhea, cough, wheezing, tachypnea, and increased respiratory effort manifested as grunting, nasal flaring, and intercostal and/or subcostal retractions.

The most frequent microorganism involved is Respiratory Syncytial Virus (RSV); it is a virus belonging to the paramyxoviridae family and to the pneumovirus genus, of which there are two types (A and B). This virus is found in 50-80% of infants with acute bronchiolitis (2, 3) and occurs in epidemic (in France, typically between October and February).

Bronchiolitis is a common disease that has a significant impact in terms of public health. In France it is estimated that it affects each year 450,000 to 500,000 infants. According to data from the Institut de Veille Sanitaire (InVS), hospitalization rate of infants admitted in pediatric emergency for acute bronchiolitis is about 35%. In the United States, RSV is found in 18% of children under 5 years of age presenting with respiratory infection and more specifically, in 20% of hospitalized infants, 18% of infants admitted to emergencies and 15% of infants visiting their doctor for acute respiratory infection between November and April (4). This study estimates the annual rate of pediatric emergency room visits to 28 per 1000 children under 5 years and 55 per 1000 children aged less than 6 months. RSV bronchiolitis is the first infectious cause of children hospitalizations (23.5%) and the second in all-cause hospitalizations (10.4%) (5). Worldwide, in 2005, the number of cases of RSV infections among children aged under 5 years has been estimated about 33.8 million, of whom at least 3.4 million required hospitalization (6).

Hence, although acute bronchiolitis is a common disease, its management remains critical.
regarding public health as well as use and cost of care in general practice or hospital pediatric units.

1.2 Treatment of acute viral bronchiolitis

As stated by the American Academy of Pediatrics in 2014, the treatment is mostly supportive, such as oxygen supplementation and hydration, most of drugs and curative therapy having failed to prove their efficacy.(7, 8)

However, since 2001, nebulized hypertonic saline (HS) have been tested in 25 randomized control trial including 3436 infants.(9, 10) Results remains controversial since a first meta-analysis conclude to the efficacy of HS to decrease length of stay,(10) but a reanalysis of the data set and a more recent trial report no benefit to HS in improving length of stay.(9, 11)

Concerning infant admitted in pediatric Emergency Department (ED), evidences are scarce with 951 infants included in 7 randomized control trials.(10) Only one of them showed a statistically significant reduction in admission rates.(12) Following this last study a meta-analysis has concluded to the efficacy of nebulized HS to reduce the risk of hospitalization by 20% (pooled RR was 0.80 (95% CI 0.67–0.96, P = .01) compared with Normal Saline (NS) among infants with bronchiolitis in pediatric ED.(10) Considering the risk of potential adverse event with nebulized HS, the few patients included and the low significance of the results for such a common condition, we conducted a multicenter, randomized, double-blind trial to evaluate the efficacy on admission rate of nebulized HS compared to NS in previously healthy infants, visiting a pediatric ED for a first episode of acute bronchiolitis.

2 HYPOTHESIS

Treatment with nebulized 4 ml of 3% hypertonic saline (HS) every 20 minutes for a total of 2 doses. allows reducing the rate of hospitalization of moderate-to-severe acute viral bronchiolitis seen in the emergency department.
3 **OBJECTIVES**

3.1 Main objective

To determine whether treatment with nebulized HS results in a 10% decrease in hospital admissions among infants with moderate-to-severe bronchiolitis seen in emergency departments.

3.2 Secondary objectives

- Estimate and compare hospital admissions rate by day 28 in each group.
- Estimate and compare the percentages of infants in each group requiring Pediatric Intensive Care Unit (PICU admission).
- Assess whether experimental treatment reduces healing time and duration of hospitalization of infants included in the study and who have been admitted.
- Compare clinical scores of respiratory distress before/after treatment.
- Evaluate the tolerance of experimental treatment.

4 **EVALUATION CRITERIA**

4.1 Primary endpoint

Hospitalization admission rate at the waning of the visit in the emergency department. We chose to evaluate hospital admission by H24 to avoid transient improvement which could delay hospitalization by few hours without clinical pertinence.(13)

4.2 Secondary endpoint

- Hospital admission rate by day 28 following examination in the emergency ward.
- PICU admission rate following examination in the emergency ward.
- Length of hospitalization for infants admitted for bronchiolitis in the following 28 days after inclusion.
- Variation of Retraction Distress Assessment Instrument (RDAI score) before and after nebulization (Table 1).
- Adverse events.
- Emergency visits, hospital and ICU duration of stay, use of physical therapy, parental time.
Table 1: Retraction Distress Assessment Instrument (RDAI score) (14)

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<tr>
<th>Symptom</th>
<th>Points</th>
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<th>2</th>
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<td>End</td>
<td>Fist half</td>
<td>First three quarters</td>
<td>Throughout</td>
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<td>Part</td>
<td>Throughout</td>
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<td>N° of involved lung fields</td>
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<td>3 or 4</td>
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</table>

5 **SELECTION OF THE POPULATION**

5.1 Inclusion criteria

- Infants aged 6 weeks to 12 months
- visiting a pediatric emergency department
- between October 2012 and January 2014
- with bronchiolitis (history of viral upper respiratory tract infection plus wheezing and/or crackles on chest auscultation)
- with a respiratory distress defined if at least two of the following conditions were met:
  (i) altered general condition and/or reduce alimentary intake,
  (ii) respiratory rate > 50 per minutes,
  (iii) oxygen saturation < 95% while awake,
  (iii) at least one severe retraction sign or two moderate according to the Respiratory Distress Assessment Instrument (RDAI) score.(14)
- Agreement of at least one of the parents for his child to participate in biomedical research.
**Justification for the waiver of the need for the collection of the consent of both parents:**

Our study goes through acts of urgent care and has only negligible risks and stress. Article L.1122 - 2 of the public health act thus applies to our study. Accordingly, if only one of the parents is present and allows the participation of the child in the study, it will be proceeded to the inclusion, and permission from the second holder of parental authority will be sought in a second time. If the second holder of parental authority does not permit the continued participation of his child, it will put an end to it and, unless parents do not agree, only the data acquired up to the expression of the denial of parental involvement will be analyzed.

5.2 Non-inclusion criteria

- Previous episode of wheezing or diagnosis of asthma
- Previous nebulized HS use
- Chronic cardiopulmonary disease or immunodeficiency
- Bone malformation of the chest
- Critically ill infants requiring PICU admission
- Prematurity (less than 37 weeks of gestation)
- Inability to communicate with the family (a language barrier or lack of telephone on the part of the parent or guardian).

5.3 Justification for the inclusion of specially protected persons

This study concerns the inclusion of infants who are considered specially protected persons. Under the terms of Art L1121-7 of the code of public health, the inclusion of infants is justified since the research of a comparable effectiveness cannot be carried out on major individuals and since the importance of the expected benefit for these people may justify the foreseeable risk which is here negligible. In addition the constraints inherent in the participation in this research can be considered as negligible.
5.4 Procedures for recruitment of patients

Antoine Béclère (Clamart), Jeanne de Flandre (Lille), Necker - Enfants Malades (Paris), Pediatric Hospital of Caen, Robert Debré (Paris), Children hospital of Nantes, Centre Hospitalier Sud-Francilien (Corbeil Essonne), Jean Verdier (Bondy), hospital mother and child (Limoges), CHU de Bicêtre (Kremlin-Bicetre), children's Hospital (Toulouse), André Mignot Hospital (Le Chesnay), Hôpital Nord (Marseille), Ambroise Paré (Boulogne), Centre Hospitalier Laennec (Quimper), Children Hospital (Nancy), Mother-Children Hospital (Lyon), CHI Créteil (Créteil), Hôpital Sud (Rennes), CHRU Morvan (Brest), CHRU Clocheville (Tours), CHU Léval (Nice), CHU de Rouen, Centre hospitalier de Fontainebleau.

Patients’ recruitments will be carried out consecutively. In each center, an eligibility list will be held, on which every infant who meets inclusion criteria will be registered (first letter of both name and first name) as well as whether he was included or not in the study. If the child was not included, the reason for non-inclusion will be specified (medical, refusal of parents, technical reason, non-availability of the investigator), as well as the outcome (hospitalization or not, successful diagnosis).
6 EXPERIMENTAL DESIGN

6.1 Research methodology: study type

National double blind randomized clinical trial with comparison of an experimental treatment (nebulized HS) to control (nebulized NS). The rationale for the choice of a methodology with randomization and blindness is the need to ensure the initial comparability of both groups and its maintenance during the trial and to decrease the risk of skewing the evaluation of judgment criteria, of which all or part, is not a completely objective characteristic.

6.2 Inclusion of patients

The investigator physician in charge of the child will check criteria for inclusion and non-inclusion of eligible children, propose to parents the participation of their child and, should they accept, collect the informed consent.

The investigator will collect family and personal previous medical events and the history of the disease and will conduct a clinical review.

6.3 Randomization

Randomization list will be established beforehand by the Paris South research clinic unit from the specification written by the unit Inserm U 1018 - team No. 02. The two panels (Experimental, Control) will be randomized by center with a block size of four (software nQuery). Randomization will be stratified on the center and the size of the blocks will be unknown to investigators.

At each new inclusion, the investigator will connect to a web site (https://cleanweb.aphp.fr), that will indicate to which group (experimental or control) the child must be assigned: nebulized HS (experimental group) or nebulized NS (control group).

6.4 Intervention

6.4.1 Intervention for patients

Prior to nebulization, nasal aspiration will be performed and the product of this aspiration will be sent for virological investigations (multiplex PCR).
According to their randomization group, the patient received two nebulizations, 4mL of 3% Hypertonic Saline (MUCOCLEAR 3%, PARI Pharma GmbH, Stanberg, Germany) or 4 mL of 0.9% Normal Saline (NaCl 0.9%, UNITHER, FRANCE), lasting 20 minutes and 20 minutes apart. The study medication was delivered using a jet nebulizer (PARI LC® SPRINT SP BABY, PARI Pharma GmbH, Stanberg, Germany) through a firmly applied face mask (ref “« coccinelle ») with an oxygen flowrate of 6 Liters per minute. The preparations were packaged in identical clear plastic vials labeled only with the randomization numbers. Both Hypertonic Saline and Normal Saline were clear and odorless, and thus were indistinguishable in the syringe and nebulization chamber. Additional therapies were ordered in accordance with routine care at the discretion of the treating physician.

6.4.2 Observations in emergencies

Before each nebulization and 20 minutes after the last one, a standardized respiratory clinical examination will be performed (heart rate and breathing, percutaneous measure of oxygen saturation, respiratory retraction signs, auscultation, RDAI score). Possible adverse events will be collected and reported.

Following the last evaluation the child will be taken care by the investigator as is usually an infant who consults for bronchiolitis in pediatric emergency department: the investigator may decide hospital admission or discharge. The case report form will be completed indicating the data of the final clinical examination, all treatments received during stay in the emergency, additional treatment prescribed and the final decision (hospitalization or not).

6.5 Collection of primary endpoint (phone call at the 48th hour)

If the child was admitted after the pediatric emergencies visit, the information on the primary endpoint (type of hospitalization, date and time of hospitalization) will be completed in real time by the investigator and his team.

If the infant is discharge to home after the pediatric emergencies visit, the infant is considered "not admitted", a telephone appointment will be made with parents by H24 (or in the morning after) to collect information about the primary endpoint (hospitalization: yes - no, if any, type of admission, date and time of hospitalization).
6.6 Monitoring beyond the 48th hour (until day 28)

For children who are not admitted, the child’s parents will be contacted at different times to administer a telephone questionnaire to collect infant health status, health and consumer information for a hospitalization.

- 48 hours after the inclusion
- 7, 14, 21 and 28 days after enrollment

Phone calls will be made by the clinical research nurse.

For hospitalized children, a clinical questionnaire will be filled after hospitalization and telephone follow-up will be repeated in the same ways mentioned above.

**The primary outcome** (hospital admission up to H24 after enrollment) will be determined through telephone follow-up and confirmed by chart review. Secondary outcomes of length and severity of symptoms will be determined by standardized telephone follow-up. Time to discharge, determined by chart review, will be defined as the time between the triage time at the enrollment and the time of discharge from the last emergency department visit or from the last hospitalization for each patient.
6.7 Summary diagram of the timeline of the research

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7 STATISTICAL CONSIDERATIONS

7.1 Required number of patients

The calculation of the required number of patients was based on published data (15).

In order to show a 10% decrease in hospitalization (25% vs 35%) 349 infants per group have to be included. Statistical error risks will be set at $\alpha=5\%$ and $\beta=20\%$. The tests will be two-sided test, without interim analysis. As participation withdrawals or deviations of protocol cannot be completely avoided, it is planned to include 15% additional patients. Finally, 800 children have to be included.

7.2 Strategy management and data analysis

Statistical analysis will be performed by team n° 2 of the centre for research in epidemiology and health of populations (Inserm U1018).

After monitoring, the data processing will be carried out in accordance with the guide of good clinical practices of the European Community. Specifically, the data will be validated prior to statistical analysis (following a protocol provided to the clinical study technicians and
physicians involved in the study). Once the data frozen, they will be analyzed with statistical software Stata®.

The statistics analysis plan may be revised during the study, to take into account any change made to the protocol or any other modification of the study progress having an impact on the originally planned statistical analyses. A final statistical analysis plan will be written and validated data-blind. All versions of the plan will be retained in the file of the study.

Statistical analysis will be carried out according to the principle of 'intent to treat': the intervention considered in the analysis will be the one coming from the randomization, even if this intervention could not be implemented, regardless of the reason.

7.3 Description of the planned statistical methods

7.3.1 Descriptive analysis

A flow chart will describe the number of eligible patients, the number of included patients and among them, the number of patients lost to follow-up or withdrawn from the study (and reasons).

The demographic characteristics of the sample will be described, overall, for each center and according to the arm of randomization. The decision of hospitalization as well as various medical procedures performed will also be described.

The qualitative criteria will be described by sample size and percentage and quantitative criteria by sample size, mean and standard deviation. In the case of quantitative criteria with non-normal distribution, median and interquartile range (25th percentile - 75th percentile) will be displayed.

7.3.2 Primary outcome: hospital admission up by H24 after enrollment

The occurrence of the event (hospitalization by H24 after enrollment) will be determined through H48 phone follow-up (phone appointments will have been made with the family at the time of inclusion). A multileveled mixed effects logistic regression analyses examined the effect of adjustment for potential clinically relevant covariates and random effect for center.(16) The strength of the association between the intervention and the rate of hospitalization at H24 will be quantified by the odds ratio and its 95% confidence interval.
7.3.3 Secondary analyses

7.3.3.1 PICU Admission rate following examination in the emergency ward
The rate of PICU hospitalization will be compared between both arms of the study with random effects logistic models. Adjustment will be performed for known prognostic variables.

7.3.3.2 Length of hospitalization for infants admitted for bronchiolitis following inclusion
Comparison of hospitalization duration for infants admitted for bronchiolitis in the 28 days following inclusion will be carried out by Cox model. This model allows taking into account censorship (ie, infants lost to follow-up before the end of the monitoring planned in the protocol). If there is no information regarding the event “discharge”, causes of censorship will be clarified. The association between intervention and length of hospitalization will be quantified by the hazard ratio and its 95% confidence interval.

7.3.3.3 Variation of RDAI scores before and after nebulization
The Respiratory Distress Assessment Instrument (RDAI) score will assigned by the study investigator before and 30 minutes after each treatment). This score will be converted into the Respiratory Assessment Change Score (RACS), calculated by adding together changes in RDAI score from before to after treatment, plus a point for each 10% change in respiratory rate above 5% (eg, –1 for a decrease of 6%-15% and –2 for a decrease of 16%-25%; negative values signify improvement) (14).
Multiple linear regression analysis will be used to compare treatment effects on RACS, controlling for demographic variables, potentially related clinical factors and pretreatment RDAI score.

7.3.3.4 Adverse events
Adverse events will be described in each arm. There frequency will be compared between groups using the Fischer test.
8 ETHICAL ASPECTS AND LEGAL

8.1 Commitments of the investigator - good practices

Audits may be decided by the promoter, local authorities or authorities to whom informations concerning this study have been submitted. All documents in relation to this study should be available for such an inspection after prior notice.

8.1.1 Conservation of the documentation and reports

The investigator will archive and retain, for at least 15 years after the end of the study, the following documents related to the study:
- updated version of protocol and annexes; any amendments
- CRFs
- informed consent of included patients
- correspondence relating to the test
- Sources of the patients should also remain available during this time.

8.1.2 The study premature end

At any time, the sponsor and/or investigator may interrupt the test prematurely for medical or administrative reason. In all cases, the end will only happen after mutual consultation and proper documentation of the reasons (ex: letter of abandonment to the investigator). The investigator will then return CRFs and all documentation pertaining to the study to the sponsor.

The promotor will inform the Ethic Committee.

8.1.3 Monitoring

The level of risk of research has been estimated at B according to the rules laid down by the direction of clinical research department (DCRD). 10-20% of notebooks will be integrally monitored.

For other notebooks, monitoring will check:
- The existence of the patient
- The conformity of signed consents
- The existence of serious adverse events
The study will be monitored by clinical research associate on-site visits and regular phone calls. Sufficient time should be devoted by the investigator to these on-site visits. The patient data will be made available for a possible inspection by the competent authorities or by the representatives of the sponsor. However, patients are not identified by their name and a strict confidentiality will be preserved at any time.

Data verification is also required and will be made by direct source documents consultation if the patient gave his agreement, in respect for confidentiality. The main parameters of assessment must be found in the source documents. Parental informed consent of each included patient will be checked.

8.2 Quality assurance and quality control

8.2.1 Sponsor

The sponsor and the promoter of the study is the Assistance Publique - Hôpitaux de Paris. It is represented by the Regional Delegation in clinical research (DRCD), saint Louis hospital, 1, rue Claude Vellefaux, 75010 Paris.

8.2.2 Informed consent of patients

The parents of the patients will be informed by the physician who supports emergency of the objective, nature, constraints and foreseeable risks of the test. A leaflet will be given to them and an informed consent form that he must date and sign before beginning the test.

To ensure medical confidentiality and privacy data, written consent forms will be retained by the investigator for a period of fifteen years after the end of the test. The investigator will certify in the CRF that the patient's consent was obtained by dating and signing.

The investigator will start no investigation specifically required by the test before obtaining the written consent of the parents of the patient. The parents of the patients will be informed that all data from the trial will be computerized and stored in a confidential manner. The names of patients being kept secret, documentation and evaluation of the data will be identified only by a code defined with the center number, the individual patient number and the first letter of both first and last names of the child.
Our study goes through acts of urgent care and has only negligible risks and stress. Article L.1122 - 2 of the public health Act thus applies to our study. Accordingly, if one of the parents is present and allows the participation of the child in the study, it will be proceeded to the inclusion, and permission from the second holder of parental authority will be sought in a second time. If the second holder of parental authority does not permit the continued participation of the child, it will put an end to it and unless parents do not agree, only the data acquired up to the expression of the denial of parental involvement will be analyzed.

8.2.3 Committee for the Protection of persons (CPP).

The technical Protocol and related documents will be submitted by the proponent to the CPP – île de France XI of Saint Germain - en - Laye for advice.

The sponsor will declare the research to the ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé).

8.2.4 Letter of intent - formalization of the beginning of the study.

Curriculum vitae of the coordinating investigator and other investigators participating in the study will be asked, as well as the agreement signed by each responsible investigator of a centre, for his participation in the study.

8.2.5 Insurance

The proponent shall cover for the duration of the study, insurance guaranteeing civil liability as well as the responsibility of all investigators involved in the study. It will also ensure full compensation for the harmful consequences to searching for the person who lends himself, unless there is evidence to charge that the damage is due to his fault, without however that may be opposed due to a third party or voluntary withdrawal from the person who had originally consented to lend research (Law No. 88-1138, s. L. 209-7).
8.3 Final research report

A final report will be prepared. It will include tables of raw data and statistical report of the data. This report will be submitted for approval and signature to the coordinating investigator.

The analysis of the results will also be presented in congresses and published in medical journals.

8.4 Publications and data properties

The data are the property of the APHP and no use or transmission to a third party can be carried out without its prior agreement. The text of the publications and communications will be discussed with all investigators participating in the trial. The co-authors will be drawn according to consensual rules holding between another account of the number of inclusions in each centre and the involvement of different investigators.

9 BIBLIOGRAPHY.

1.2 FINAL TRIAL PROTOCOL

GUÉRANDE

3% HYPERTONIC SALINE TO REDUCE HOSPITALIZATION RATE IN ACUTE VIRAL BRONCHIOLITIS:

A MULTICENTRE RANDOMIZED CONTROLLED TRIAL

BIOMEDICAL RESEARCH PROTOCOL

PHRC National 2012
v7 20/01/2014

Supported by a grant from the French Health Ministry (P110143 / IDRCB2012-A00228-35).

Coordinating investigator: Pr. GAJDOS Vincent
General Pediatrics
Paris Sud Hospital Group - Antoine Béclère

Statisticians: M. BOUYER Jean (CESP, U1018, Equipe n°2)

Sponsor: APHP, Department of clinical research and Development
Saint Louis Hospital
1, avenue Claude Vellefaux
75010 PARIS
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<td>V. GAJDOS</td>
</tr>
<tr>
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<td>Jeanne de Flandre (Lille)</td>
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1 STATE OF THE ART

1.1 Acute viral bronchiolitis

Bronchiolitis is a disorder most commonly caused in infants by viral lower respiratory tract infection. It is the most common lower respiratory infection in infants less than two years of age. It is characterized by acute inflammation, edema, and necrosis of epithelial cells lining small airways, increased mucus production, and bronchospasm (1).

Most clinicians recognize bronchiolitis as a constellation of clinical symptoms and signs including viral upper respiratory prodrome followed by increased respiratory effort and wheezing in children less than 2 years of age. Clinical signs and symptoms of bronchiolitis consist of rhinorrhea, cough, wheezing, tachypnea, and increased respiratory effort manifested as grunting, nasal flaring, and intercostal and/or subcostal retractions.

The most frequent microorganism involved is Respiratory Syncytial Virus (RSV); it is a virus belonging to the paramyxoviridae family and to the pneumovirus genus, of which there are two types (A and B). This virus is found in 50-80% of infants with acute bronchiolitis (2, 3) and occurs in epidemic (in France, typically between October and February).

Bronchiolitis is a common disease that has a significant impact in terms of public health. In France it is estimated that it affects each year 450,000 to 500,000 infants. According to data from the Institut de Veille Sanitaire (InVS), hospitalization rate of infants admitted in pediatric emergency for acute bronchiolitis is about 35%. In the United States, RSV is found in 18% of children under 5 years of age presenting with respiratory infection and more specifically, in 20% of hospitalized infants, 18% of infants admitted to emergencies and 15% of infants visiting their doctor for acute respiratory infection between November and April (4). This study estimates the annual rate of pediatric emergency room visits to 28 per 1000 children under 5 years and 55 per 1000 children aged less than 6 months. RSV bronchiolitis is the first infectious cause of children hospitalizations (23.5%) and the second in all-cause hospitalizations (10.4%) (5). Worldwide, in 2005, the number of cases of RSV infections among children aged under 5 years has been estimated about 33.8 million, of whom at least 3.4 million required hospitalization (6).

Hence, although acute bronchiolitis is a common disease, its management remains critical.
regarding public health as well as use and cost of care in general practice or hospital pediatric units.

1.2 Treatment of acute viral bronchiolitis

As stated by the American Academy of Pediatrics in 2014, the treatment is mostly supportive, such as oxygen supplementation and hydration, most of drugs and curative therapy having failed to prove their efficacy.\(^{(7, 8)}\)

However, since 2001, nebulized hypertonic saline (HS) have been tested in 25 randomized control trial including 3436 infants.\(^{(9, 10)}\) Results remains controversial since a first meta-analysis conclude to the efficacy of HS to decrease length of stay,\(^{(10)}\) but a reanalysis of the data set and a more recent trial report no benefit to HS in improving length of stay.\(^{(9, 11)}\)

Concerning infant admitted in pediatric Emergency Department (ED), evidences are scarce with 951 infants included in 7 randomized control trials.\(^{(10)}\) Only one of them showed a statistically significant reduction in admission rates.\(^{(12)}\) Following this last study a meta-analysis has concluded to the efficacy of nebulized HS to reduce the risk of hospitalization by 20\% (pooled RR was 0.80 (95\% CI 0.67–0.96, P = .01) compared with Normal Saline (NS) among infants with bronchiolitis in pediatric ED.\(^{(10)}\) Considering the risk of potential adverse event with nebulized HS, the few patients included and the low significance of the results for such a common condition, we conducted a multicenter, randomized, double-blind trial to evaluate the efficacy on admission rate of nebulized HS compared to NS in previously healthy infants, visiting a pediatric ED for a first episode of acute bronchiolitis.

2 HYPOTHESIS

Treatment with nebulized 4 ml of 3\% hypertonic saline (HS) every 20 minutes for a total of 2 doses. allows reducing the rate of hospitalization of moderate-to-severe acute viral bronchiolitis seen in the emergency department.
3 **OBJECTIVES**

3.1 Main objective

To determine whether treatment with nebulized HS results in a 10% decrease in hospital admissions among infants with moderate-to-severe bronchiolitis seen in emergency departments.

3.2 Secondary objectives

- Estimate and compare hospital admissions rate by day 28 in each group.
- Estimate and compare the percentages of infants in each group requiring Pediatric Intensive Care Unit (PICU admission).
- Assess whether experimental treatment reduces healing time and duration of hospitalization of infants included in the study and who have been admitted
- Compare clinical scores of respiratory distress before/after treatment
- Evaluate the tolerance of experimental treatment

4 **EVALUATION CRITERIA**

4.1 Primary endpoint

Hospital admission rate up to H24 after enrollment, which occurred during the visit in the emergency department. We chose to evaluate hospital admission by H24 to avoid transient improvement which could delay hospitalization by few hours without clinical pertinence.(13)

4.2 Secondary endpoint

- Hospital admission rate by day 28 following examination in the emergency ward
- PICU admission rate following examination in the emergency ward
- Length of hospitalization for infants admitted for bronchiolitis in the following 28 days after inclusion
- Variation of Retraction Distress Assessment Instrument (RDAI score) before and after nebulization (Table 1)
- Adverse events
- Emergency visits, hospital and ICU duration of stay, use of physical therapy, parental time
Table 1: Retraction Distress Assessment Instrument (RDAI score) (14)

<table>
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<tr>
<th>Symptom</th>
<th>0</th>
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<th>2</th>
<th>3</th>
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<td></td>
<td></td>
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<td>During expiration</td>
<td>None</td>
<td>End</td>
<td>Fist half</td>
<td>First three quarters</td>
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<td>4</td>
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<td>During Inspiration</td>
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<td>Part</td>
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<td>---</td>
<td>---</td>
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</table>

5 **SELECTION OF THE POPULATION**

5.1 **Inclusion criteria**

- Infants aged 6 weeks to 12 months
- visiting a pediatric emergency department
- between October 2012 and April 2014
- with bronchiolitis (history of viral upper respiratory tract infection plus wheezing and/or crackles on chest auscultation)
- with a respiratory distress defined if at least two of the following conditions were met:
  (i) altered general condition and/or reduce alimentary intake,
  (ii) respiratory rate > 50 per minutes,
  (iii) oxygen saturation < 95% while awake,
  (iii) at least one severe retraction sign or two moderate according to the Respiratory Distress Assessment Instrument (RDAI) score.(14)
- Agreement of at least one of the parents for his child to participate in biomedical research.
Justification for the waiver of the need for the collection of the consent of both parents:
our study goes through acts of urgent care and has only negligible risks and stress. Article L.1122 - 2 of the public health act thus applies to our study. Accordingly, if only one of the parents is present and allows the participation of the child in the study, it will be proceeded to the inclusion, and permission from the second holder of parental authority will be sought in a second time. If the second holder of parental authority does not permit the continued participation of his child, it will put an end to it and, unless parents do not agree, only the data acquired up to the expression of the denial of parental involvement will be analyzed.

5.2 Non-inclusion criteria

- Previous episode of wheezing or diagnosis of asthma
- Previous nebulized HS use
- Chronic cardiopulmonary disease or immunodeficiency
- Bone malformation of the chest
- Critically ill infants requiring PICU admission
- Prematurity (less than 37 weeks of gestation)
- Inability to communicate with the family (a language barrier or lack of telephone on the part of the parent or guardian).

5.3 Justification for the inclusion of specially protected persons

This study concerns the inclusion of infants who are considered specially protected persons. Under the terms of Art L1121-7 of the code of public health, the inclusion of infants is justified since the research of a comparable effectiveness cannot be carried out on major individuals and since the importance of the expected benefit for these people may justify the foreseeable risk which is here negligible. In addition the constraints inherent in the participation in this research can be considered as negligible.
5.4 Procedures for recruitment of patients

Antoine Béclère (Clamart), Jeanne de Flandre (Lille), Necker - Enfants Malades (Paris), Pediatric Hospital of Caen, Robert Debré (Paris), Children hospital of Nantes, Centre Hospitalier Sud-Francilien (Corbeil Essonne), Jean Verdier (Bondy), hospital mother and child (Limoges), CHU de Bicêtre (Kremlin-Bicetre), children's Hospital (Toulouse), André Mignot Hospital (Le Chesnay), Hôpital Nord (Marseille), Ambroise Paré (Boulogne), Centre Hospitalier Laennec (Quimper), Children Hospital (Nancy), Mother-Children Hospital (Lyon), CHI Créteil (Créteil), Hôpital Sud (Rennes), CHRU Morvan (Brest), CHRU Clocheville (Tours), CHU Lenval (Nice), CHU de Rouen, Centre hospitalier de Fontainebleau.

Patients’ recruitments will be carried out consecutively. In each center, an eligibility list will be held, on which every infant who meets inclusion criteria will be registered (first letter of both name and first name) as well as whether he was included or not in the study. If the child was not included, the reason for non-inclusion will be specified (medical, refusal of parents, technical reason, non-availability of the investigator), as well as the outcome (hospitalization or not, successful diagnosis).
6 EXPERIMENTAL DESIGN

6.1 Research methodology: study type

National double blind randomized clinical trial with comparison of an experimental treatment (nebulized HS) to control (nebulized NS). The rationale for the choice of a methodology with randomization and blindness is the need to ensure the initial comparability of both groups and its maintenance during the trial and to decrease the risk of skewing the evaluation of judgment criteria, of which all or part, is not a completely objective characteristic.

6.2 Inclusion of patients

The investigator physician in charge of the child will check criteria for inclusion and non-inclusion of eligible children, propose to parents the participation of their child and, should they accept, collect the informed consent.

The investigator will collect family and personal previous medical events and the history of the disease and will conduct a clinical review.

6.3 Randomization

Randomization list will be established beforehand by the Paris South research clinic unit from the specification written by the unit Inserm U 1018 - team No. 02. The two panels (Experimental, Control) will be randomized by center with a block size of four (software nQuery). Randomization will be stratified on the center and the size of the blocks will be unknown to investigators.

At each new inclusion, the investigator will connect to a web site (https://cleanweb.aphp.fr) that will indicate to which group (experimental or control) the child must be assigned: nebulized HS (experimental group) or nebulized NS (control group).

6.4 Intervention

6.4.1 Intervention for patients

Prior to nebulization, nasal aspiration will be performed and the product of this aspiration will be sent for virological investigations (multiplex PCR).
According to their randomization group, the patient received two nebulizations, 4mL of 3% Hypertonic Saline (MUCOCLEAR 3%, PARI Pharma GmbH, Stanberg, Germany) or 4 mL of 0.9% Normal Saline (NaCl 0.9%, UNITHER, FRANCE), lasting 20 minutes and 20 minutes apart. The study medication was delivered using a jet nebulizer (PARI LC® SPRINT SP BABY, PARI Pharma GmbH, Stanberg, Germany) through a firmly applied face mask (ref: “Baby n°1” and “Baby n°2”) with an oxygen flowrate of 6 Liters per minute. The preparations were packaged in identical clear plastic vials labeled only with the randomization numbers. Both Hypertonic Saline and Normal Saline were clear and odorless, and thus were indistinguishable in the syringe and nebulization chamber. Additional therapies were ordered in accordance with routine care at the discretion of the treating physician.

6.4.2 Observations in emergencies
Before each nebulization and 20 minutes after the last one, a standardized respiratory clinical examination will be performed (heart rate and breathing, percutaneous measure of oxygen saturation, respiratory retraction signs, auscultation, RDAI score). Possible adverse events will be collected and reported. Following the last evaluation the child will be taken care by the investigator as is usually an infant who consults for bronchiolitis in pediatric emergency department: the investigator may decide hospital admission or discharge. The case report form will be completed indicating the data of the final clinical examination, all treatments received during stay in the emergency, additional treatment prescribed and the final decision (hospitalization or not).

6.5 Collection of primary endpoint (phone call at the 48th hour)
If the child was admitted after the pediatric emergencies visit, the information on the primary endpoint (type of hospitalization, date and time of hospitalization) will be completed in real time by the investigator and his team.
If the infant is discharge to home after the pediatric emergencies visit, the infant is considered "not admitted", a telephone appointment will be made with parents by H24 (or in the morning after) to collect information about the primary endpoint (hospitalization: yes - no, if any, type of admission, date and time of hospitalization).
6.6 Monitoring beyond the 48th hour (until day 28)

For children who are not admitted, the child's parents will be contacted at different times to administer a telephone questionnaire to collect infant health status, health and consumer information for a hospitalization.

- 48 hours after the inclusion
- Between day 8 and day 11, between day 15 and day 18, and between day 28 and day 31 after enrollment

Phone calls will be made by the clinical research nurse.
A logbook will be given to parents. They indicate the questions put to them at the time of the phone call and allowing them to take notes to optimally respond to questions during each call. This book will include, besides the medical information, the information needed to estimate any consumer outpatient care.
For hospitalized children, a clinical questionnaire will be filled after hospitalization and telephone follow-up will be repeated in the same ways mentioned above.

The primary outcome (hospital admission up to H24 after enrollment) will be determined through telephone follow-up and confirmed by chart review. Secondary outcomes of length and severity of symptoms will be determined by standardized telephone follow-up. Time to discharge, determined by chart review, will be defined as the time between the triage time at the enrollment and the time of discharge from the last emergency department visit or from the last hospitalization for each patient.
6.7 Summary diagram of the timeline of the research

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7 Statistical Considerations

7.1 Required number of patients

The calculation of the required number of patients was based on published data (15).

In order to show a 10% decrease in hospitalization (25% vs 35%) 349 infants per group have to be included. Statistical error risks will be set at $\alpha = 5\%$ and $\beta = 20\%$. The tests will be two-sided test, without interim analysis. As participation withdrawals or deviations of protocol cannot be completely avoided, it is planned to include 15% additional patients. Finally, 800 children have to be included.

7.2 Strategy management and data analysis

Statistical analysis will be performed by team n° 2 of the centre for research in epidemiology and health of populations (Inserm U1018).

After monitoring, the data processing will be carried out in accordance with the guide of good clinical practices of the European Community. Specifically, the data will be validated prior to statistical analysis (following a protocol provided to the clinical study technicians and
physicians involved in the study). Once the data frozen, they will be analyzed with statistical software Stata ®.

The statistics analysis plan may be revised during the study, to take into account any change made to the protocol or any other modification of the study progress having an impact on the originally planned statistical analyses. A final statistical analysis plan will be written and validated data-blind. All versions of the plan will be retained in the file of the study. Statistical analysis will be carried out according to the principle of 'intent to treat': the intervention considered in the analysis will be the one coming from the randomization, even if this intervention could not be implemented, regardless of the reason.

7.3 Description of the planned statistical methods

7.3.1 Descriptive analysis

A flow chart will describe the number of eligible patients, the number of included patients and among them, the number of patients lost to follow-up or withdrawn from the study (and reasons).

The demographic characteristics of the sample will be described, overall, for each center and according to the arm of randomization. The decision of hospitalization as well as various medical procedures performed will also be described.

The qualitative criteria will be described by sample size and percentage and quantitative criteria by sample size, mean and standard deviation. In the case of quantitative criteria with non-normal distribution, median and interquartile range (25th percentile - 75th percentile) will be displayed.

7.3.2 Primary outcome : hospital admission up by H24 after enrollment

The occurrence of the event (hospitalization by H24 after enrollment) will be determined through H48 phone follow-up (phone appointments will have been made with the family at the time of inclusion). A multileveled mixed effects logistic regression analyses examined the effect of adjustment for potential clinically relevant covariates and random effect for center.(16) The strength of the association between the intervention and the rate of hospitalization at H24 will be quantified by the odds ratio and its 95% confidence interval.
7.3.3 Secondary analyses

7.3.3.1 PICU Admission rate following examination in the emergency ward

The rate of PICU hospitalization will be compared between both arms of the study with random effects logistic models. Adjustment will be performed for known prognostic variables.

7.3.3.2 Length of hospitalization for infants admitted for bronchiolitis following inclusion

Comparison of hospitalization duration for infants admitted for bronchiolitis in the 28 days following inclusion will be carried out by Cox model. This model allows taking into account censorship (i.e., infants lost to follow-up before the end of the monitoring planned in the protocol). If there is no information regarding the event “discharge”, causes of censorship will be clarified. The association between intervention and length of hospitalization will be quantified by the hazard ratio and its 95% confidence interval.

7.3.3.3 Variation of RDAI scores before and after nebulization

The Respiratory Distress Assessment Instrument (RDAI) score will assigned by the study investigator before and 30 minutes after each treatment). This score will be converted into the Respiratory Assessment Change Score (RACS), calculated by adding together changes in RDAI score from before to after treatment, plus a point for each 10% change in respiratory rate above 5% (e.g., −1 for a decrease of 6%-15% and −2 for a decrease of 16%-25%; negative values signify improvement) (14).

Multiple linear regression analysis will be used to compare treatment effects on RACS, controlling for demographic variables, potentially related clinical factors and pretreatment RDAI score.

7.3.3.4 Adverse events

Adverse events will be described in each arm. Their frequency will be compared between groups using the Fischer test.
8 ETHICAL ASPECTS AND LEGAL

8.1 Commitments of the investigator - good practices

Audits may be decided by the promoter, local authorities or authorities to whom informations concerning this study have been submitted. All documents in relation to this study should be available for such an inspection after prior notice.

8.1.1 Conservation of the documentation and reports

The investigator will archive and retain, for at least 15 years after the end of the study, the following documents related to the study:
- updated version of protocol and annexes; any amendments
- CRFs
- informed consent of included patients
- correspondence relating to the test
- Sources of the patients should also remain available during this time.

8.1.2 The study premature end

At any time, the sponsor and/or investigator may interrupt the test prematurely for medical or administrative reason. In all cases, the end will only happen after mutual consultation and proper documentation of the reasons (ex: letter of abandonment to the investigator). The investigator will then return CRFs and all documentation pertaining to the study to the sponsor.

The promotor will inform the Ethic Committee.

8.1.3 Monitoring

The level of risk of research has been estimated at B according to the rules laid down by the direction of clinical research department (DCRD). 10-20% of notebooks will be integrally monitored.

For other notebooks, monitoring will check:
- The existence of the patient
- The conformity of signed consents
- The existence of serious adverse events
The study will be monitored by clinical research associate on-site visits and regular phone calls. Sufficient time should be devoted by the investigator to these on-site visits. The patient data will be made available for a possible inspection by the competent authorities or by the representatives of the sponsor. However, patients are not identified by their name and a strict confidentiality will be preserved at any time.

Data verification is also required and will be made by direct source documents consultation if the patient gave his agreement, in respect for confidentiality. The main parameters of assessment must be found in the source documents. Parental informed consent of each included patient will be checked.

8.2 Quality assurance and quality control

8.2.1 Sponsor

The sponsor and the promoter of the study is the Assistance Publique - Hôpitaux de Paris. It is represented by the Regional Delegation in clinical research (DRCD), saint Louis hospital, 1, rue Claude Vellefaux, 75010 Paris.

8.2.2 Informed consent of patients

The parents of the patients will be informed by the physician who supports emergency of the objective, nature, constraints and foreseeable risks of the test. A leaflet will be given to them and an informed consent form that he must date and sign before beginning the test.

To ensure medical confidentiality and privacy data, written consent forms will be retained by the investigator for a period of fifteen years after the end of the test. The investigator will certify in the CRF that the patient's consent was obtained by dating and signing.

The investigator will start no investigation specifically required by the test before obtaining the written consent of the parents of the patient. The parents of the patients will be informed that all data from the trial will be computerized and stored in a confidential manner. The names of patients being kept secret, documentation and evaluation of the data will be identified only by a code defined with the center number, the individual patient number and the first letter of both first and last names of the child.
Our study goes through acts of urgent care and has only negligible risks and stress. Article L.1122 - 2 of the public health Act thus applies to our study. Accordingly, if one of the parents is present and allows the participation of the child in the study, it will be proceeded to the inclusion, and permission from the second holder of parental authority will be sought in a second time. If the second holder of parental authority does not permit the continued participation of the child, it will put an end to it and unless parents do not agree, only the data acquired up to the expression of the denial of parental involvement will be analyzed.

8.2.3 Committee for the Protection of persons (CPP).
The technical Protocol and related documents will be submitted by the proponent to the CPP – île de France XI of Saint Germain - en - Laye for advice. The sponsor will declare the research to the ANSM (Agence Nationale de Sécurité du Médicament et des Produits de Santé).

8.2.4 Letter of intent - formalization of the beginning of the study.
Curriculum vitae of the coordinating investigator and other investigators participating in the study will be asked, as well as the agreement signed by each responsible investigator of a centre, for his participation in the study.

8.2.5 Insurance
The proponent shall cover for the duration of the study, insurance guaranteeing civil liability as well as the responsibility of all investigators involved in the study. It will also ensure full compensation for the harmful consequences to searching for the person who lends himself, unless there is evidence to charge that the damage is due to his fault, without however that may be opposed due to a third party or voluntary withdrawal from the person who had originally consented to lend research (Law No. 88-1138, s. L. 209-7).
8.3 Final research report

A final report will be prepared. It will include tables of raw data and statistical report of the data. This report will be submitted for approval and signature to the coordinating investigator.

The analysis of the results will also be presented in congresses and published in medical journals.

8.4 Publications and data properties

The data are the property of the APHP and no use or transmission to a third party can be carried out without its prior agreement. The text of the publications and communications will be discussed with all investigators participating in the trial. The co-authors will be drawn according to consensual rules holding between another account of the number of inclusions in each centre and the involvement of different investigators.

9 BIBLIOGRAPHY.

1.3 SUMMARY OF TRIAL PROTOCOL CHANGES

1. Clarification of the primary endpoint (Paragraph 4.1).
2. A modification of the reference of the mask used for the production of aerosols (same kind of material, with a CE marking and used in this indication) (paragraph 6.4.1).
3. Change in the number and frequency of phone calls to families during the follow-up period (paragraph 6.6 and 6.7).
4. Suppression of the H8 evaluation (paragraph 6.7)
5. Adding a logbook for parents. They indicate the questions put to them at the time of the phone call and allowing them to take notes to optimally respond to questions during each call. This book will include, besides the medical information, the information needed to estimate any consumer outpatient care. (Paragraph 6.6)
6. Extension of the inclusion period from 16 months (October 2012 to January 2014), to 19 months (October 2012 to April 2014). (paragraph 5.1)
7. Addition of further lead site investigators as further centers joined the trial (5 centers).

No changes other than those indicated above were made to the trial design or clinical protocol during the trial.
2.1 ORIGINAL STATISTICAL ANALYSIS PLAN

3% HYPERTONIC SALINE TO REDUCE HOSPITALIZATION RATE IN ACUTE VIRAL BRONCHIOBITIS: A MULTICENTRE RANDOMIZED CONTROLLED TRIAL - GUÉRANDE

Statistical Analysis Plan
Supported by a grant from the French Health Ministry: P110143 / IDRCB2012-A00228-35.
Clinicaltrial.gov number: NCT01777347

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Sponsor: APHP, Department of clinical research and Development
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A. TAHIR; Mother children Hospital (Limoges); France
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O. CHARARA; Hospital André Mignot (Le Chesnay); France
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A. BORSA-DORION; Children Hospital (Nancy); France
N. CABET; Mother-Children Hospital (Lyon); France
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P. BABE; CHU Lenval (Nice); France
INTRODUCTION
This document outlines the proposed presentation and analyses for the GUERANDE trial. Subsequent analyses of a more exploratory nature will not be bound by this strategy, but are expected to follow the broad principles described. Suggestions for subsequent analyses by journal editors or referees will be considered carefully and carried out, as far as possible, in line with the principles of this analysis plan.

TRIAL SUMMARY
TRIAL DESIGN
GUERANDE is a multi-centre, double bind, randomised, controlled, superiority trial, comparing the efficacy and safety of 3% hypertonic saline (HS) to physiologic saline (NS) as control for treatment of severe to moderate acute viral bronchiolitis in emergency department. Full information on the design and conduct of the trial is included in the trial protocol.

SAMPLE SIZE CALCULATION
In order to show a 10% decrease in hospitalization (25% vs 35%) 349 infants per group have to be included. Statistical error risks will be set at alpha= 5% and beta= 20%. The tests will be two-sided test, without interim analysis.

ANALYSIS
ANALYSIS OVERVIEW
Statistical analysis will be performed by the trial statistician (JB & JPT) with assistance from the principal investigators (FA & VG). Primary analysis will be by intention to treat (ITT). Statistical analysis will be performed by team n ° 2 of the centre for research in epidemiology and health of populations (Inserm U1018).
Data will be exported from a secure, web-based electronic database (Cleanweb) to an electronic statistical package (Stata I/C software, version 13.1) for analysis. After external monitoring, the data processing will be carried out in accordance with the guide of good clinical practices of the European Community. Specifically, the data will be validated prior to statistical analysis (following a protocol provided to the clinical study technicians and physicians involved in the study). Once the data frozen, they will be analyzed with statistical software Stata ®.
The statistics analysis plan may be revised during the study, to take into account any change made to the protocol or any other modification of the study progress having an impact on the originally planned statistical analyses. A final statistical analysis plan will be written and validated data-blind. All versions of the plan will be retained in the file of the study.
Statistical analysis will be carried out according to the principle of 'intent to treat': the intervention considered in the analysis will be the one coming from the randomization, even if this intervention could not be implemented, regardless of the reason.

The qualitative criteria will be described by sample size and percentage and quantitative criteria by sample size, mean and standard deviation. In the case of quantitative criteria with non-normal distribution, median and interquartile range (25th percentile - 75th percentile) will be displayed.

ANALYSIS POPULATION
The analysis population will consist of 777 infants (387 in the HS group, 390 in the NS group).
A flow chart will describe eligible population, included population and reason for non inclusion and analyzable population (children for whom, primary outcome is available).

DESCRIPTION OF THE POPULATION
The baseline characteristics of patients will be described according to the arm of randomization.

CONSORT DIAGRAM
A CONSORT diagram describing patient flow with total numbers randomised to each treatment. This diagram will include all patients randomized for the intent to treat analysis. It will also described per-protocol population (receiving at least one study medication).

Demographic variables: Age, sex, number of sibling, smoker(s) in home, atopy, daycare. History of disease variables: Previous treatment (bronchodilatators, steroids, antibiotics), duration of symptoms before enrollment, feeding status, Clinical variables: Respiratory Rate, Heart Rate, RDAI score, Oxygen saturation, Temperature, Virological variables: RSV status

PRIMARY OUTCOME ANALYSIS
The occurrence of the event (hospitalization by H24 after enrollement) will be compared between patient groups with a chi2-exact test.

SECONDARY OUTCOME ANALYSIS
1. The proportion of hospitalization by H24 will be compared in two age subgroups : less than 3 months, 3 months or more.

2. To detect a center effect and to take into account known prognosis variables, a multilevel logistic regression using the center as the random effect will be done. Prognosis variables will be: age, duration of symptoms before inclusion, reduced feeding, Heart Rate, Oxygen Saturation, initial RDAI, RSV. Difference between treatment groups was estimated via Odds Ratio (OR) and 95 % confidence interval.

3. Proportion of PICU admission among hospitalized infants will be compared using a chi2 exact test.

4. RDAI score post nebulization, change in RDAI Pre to Post nebulization and Respiratory Assessment Change Score will be compared using a student’s t-test (RACS). The RACS will be calculated by adding together changes in RDAI score Pre/post treatment, plus a point for each 10% change in respiratory rate above 5% (eg, −1 for a decrease of 6%-15% and −2 for a decrease of 16%-25%; negative values signify improvement) according to Lowell DI et al, Pediatrics 1987;79:939-45

5. Length of stay for hospitalized patients in the first 24 hours will be compared using a student’s t-test.
6. The proportion of hospitalization by day28 will be compared between patient groups with a chi$^2$ test. Denominators were calculated by adding patients with complete follow-up and patients with a known hospitalization before being lost to follow-up.

7. 5. Adverse events will be described for each group in a per protocol analysis and will be compared using a chi$^2$ exact test.

**PROCEDURE FOR MISSING AND SPURIOUS DATA**
Missing data will be described, for example, by presenting the number of individuals in the missing category for each treatment group. No data will be considered spurious in the analysis since all data will be checked and cleaned before analysis.
2.2 STATEMENT REGARDING STATISTICAL ANALYSIS

PLAN CHANGES

There were no changes made to the original statistical analysis plan.