# University of Thessaly

## School of Medicine


# Postgraduate dissertation

*«Research Methodology in Biomedicine, Biostatistics and Clinical Bioinformatics at University of Thessaly»*

***"The relationship between Procalcitonin and mortality in septic ICU patients"***

**"Η σχέση προκαλσιτονίνης και θνησιμότητας σε ασθενείς της ΜΕΘ με σήψη"**

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# 1. Abstract

## 1.1.English abstract

This diploma thesis is part of the Postgraduate Department "Biomedical Research Methodology, Biostatistics and Clinical Bioinformatics" and aims to highlight the relationship of procalcitonin and mortality in ICU patients with sepsis.

Its subject is the design of a prospective observational study protocol. Patients participating in the study will be in intensive care center units having already experienced a septic stage. The hospitals that will be used are: General Hospital of Volos, General Hospital of Larissa and General University Hospital of Larissa

Along with the writing of the thesis, an Access database will be created where the PCT measurements will be recorded according to the study timetable, thus making the statistical analysis and the management - publication of the results easier. In this way, it is possible to draw conclusions about the relationship of procalcitonin and mortality.

*Key words: sepsis, procalcitonin, mortality, ICU*

## 1.2. Greek abstract

Η παρούσα εργασία αποτελεί κομμάτι του Μεταπτυχιακού Τμήματος «Μεθοδολογία Βιοϊατρικής Έρευνας, Βιοστατιστική και Κλινική Βιοπληροφορική» και έχει ως στόχο την ανάδειξη της σχέσης προκαλσιτονίνης και θνησιμότητας σε ασθενείς της ΜΕΘ με σήψη.

Αντικείμενο είναι ο σχεδιασμός ενός πρωτοκόλλου μελέτης παρατήρησης για μία προοπτική μελέτη. Οι ασθενείς που θα συμμετάσχουν στην μελέτη θα βρίσκονται σε μονάδες εντατικής θεραπείας κέντρων υγείας και θα παρουσιάζουν ήδη ένα στάδιο σήψης. Τα νοσοκομεία που θα εξεταστούν είναι τα: Γενικό Νοσοκομείο Βόλου, Γενικό Νοσοκομείο Λάρισας και τέλος το Πανεπιστημιακό Νοσοκομείο Λάρισας.

Παράλληλα με την συγγραφή της εργασίας, δημιουργήθηκε και μία βάση δεδομένων Access όπου θα γίνεται η καταχώρηση των μετρήσεων της PCT σύμφωνα με το χρονοδιάγραμμα της μελέτης με αποτέλεσμα να είναι πιο έυκολη η στατιστική ανάλυση και η διαχείριση – έκδοση των αποτελεσμάτων. Με αυτό τον τρόπο θα μπορέσει να γίνει και η εξαγωγή συμπερασμάτων για την σχέση της προκαλσιτονινης και του θανάτου.

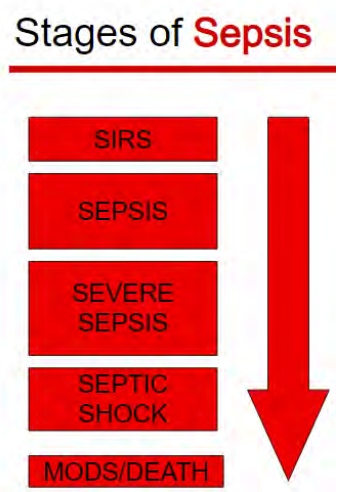
# 2. Introduction

In this chapter, basic information will be presented on the theoretical background of the postgraduate dissertation. More specifically, the definitions of sepsis, PCT will be analyzed.

## 2.1.Sepsis

Sepsis is a dramatic change of an infection which can lead to death. It has four steps: SIRS, Sepsis, Severe sepsis, septic shock and mortality rate in sepsis remains extremely high. Early diagnosis is critical in order to reduce mortality associated with sepsis. Diagnostic uncertainty sometimes still remains high despite the available clinical information which leads to laboratory measurements where serum biomarkers like procalcitonin may aid in the early diagnosis of sepsis and therapeutic intervention.

If sepsis progresses to septic shock, which is the last step, then blood pressure drops dramatically and may lead to death. Sepsis is more common and most dangerous in elder people or those who already suffer a weakened immune system. In the end, if sepsis is treated early, most common with antibiotics and intravenous fluids then the risk of mortality is drastically decreased.




**Picture 1 Stages of sepsis**

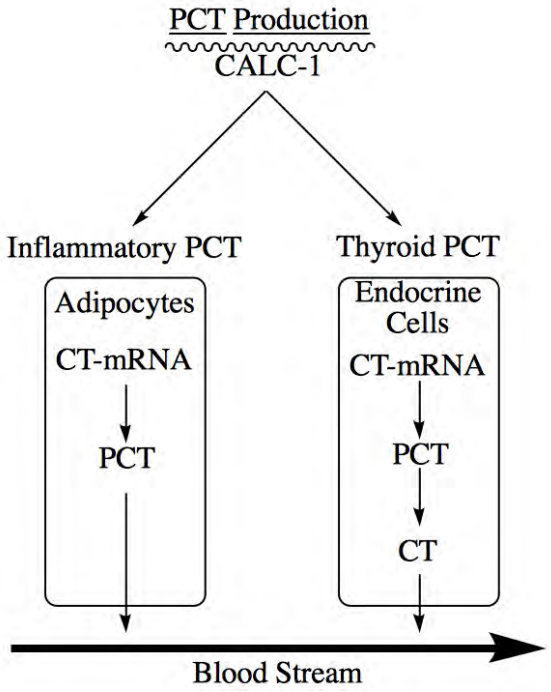
### 2.1.1. Sepsis risk factors

The most common risk factors of sepsis are based on:

- the age of the patient (either very young or very old),
- the state of the patient's immune system,
- the state of the patient's sickness level ( Intensive care unit),
- patient's injuries (burns, severe cuts etc),
- patient's invasive devices(catheters or breathing tubes)

## 2.2.Procalcitonin

Procalcitonin (PCT) is a protein that consists of 116 amino acids and forms the protein precursor form of calcitonin. It is produced and secreted by the thyroid gland and physiologically is undetectable in the blood of healthy individuals. In response to infection and systemic inflammation, the secretion of Procalcitonin in the bloodstream increases and in combination with proteolytic enzymes break down into the active hormone, calcitonin. After infection, procalcitonin is increased in a short time.




**Picture 2 : Production pathway of PCT and CT in healthy and infected individuals**

When inflammation is caused by a bacterial infection, the presence of procalcitonin is particularly important because it is also released from the liver,

kidneys, lungs, muscles and fatty tissue, elevating serum levels of procalcitonin significantly above normal. Thus, measuring procalcitonin is useful in the differentiation of bacterial infections from other pathological conditions and has proven to be more sensitive and specific marker for this purpose, relative to C-reactive protein (CRP).

The level of procalcitonin rises in a response to a proinflammatory state. It is often classed as an acute phase reactant. Blood levels of procalcitonin may rise up to 100 μg/L. As a matter of fact, monitoring of serum procalcitonin levels may be particularly useful in monitoring the response to antibiotic therapy.

### 2.2.1. Limitations of procalcitonin

There is a number of limitations where using procalcitonin as a marker of infection and sepsis. High measurements in PCT levels where there is absence of a bacterial infection can take place in situations like stress, after surgery, or in patients with cardiac shock.

That is the reason why the power of procalcitonin to discriminate between sepsis and sterile inflammation is better for medical than for surgical patients.

## 2.3.Procalcitonin and sepsis

Procalcitonin has been used in Europe and US (FDA) for many years as a diagnostic aid for sepsis and has recently become of interest as a possible marker of the systemic inflammatory response to infection. Procalcitonin gained an FDA indication in 2016 for serial use to assess sepsis progression and 28-day mortality risk.

## 2.4.Possible Interpretation of Pathological values procalcitionin levels

- <0.50 ng / mL do not rule out the infection, as localized inflammations can be combined with such values.

- > 2.0 ng / mL are indicative of systemic bacterial infection or severe localized inflammation. Levels> 2.0 ng / mL may be related also with trauma, burn, multi-organ failure.

- > 10.0 ng / mL are indicative of septicemia.

| Guidelines for initiating antibiotics according to PCT value | | | |
|---|---|---|---|
| < 0.25 ng/ml | 0.25 - 0.5 ng/ml | 0.5 - < 1.0 ng/ml | ≥ 1.0 ng/ml |
| Antibiotics strongly discouraged | Antibiotics discouraged | Antibiotics encouraged | Antibiotics strongly encouraged |
| **Guidelines for stopping, continuing or changing antibiotics according to daily measured PCT value** | | | |
| < 0.25 ng/ml | Decline more than 80% or 80% of peak (maximum) value or ≥ 0.25 to < 0.5 ng/ml | Decline of PCT less than 80% of peak value and PCT ≥ 0.5 ng/ml | Increase of PCT above previous and PCT ≥ 0.5 ng/ml |

**Table 1 PCT and antibiotics guidelines [17]**

## 2.5.Prospective or retrospective?

A prospective cohort study is a study that follows over time a group of similar individuals (cohorts) who differ with respect to certain factors under study, to determine how these factors affect rates of a certain outcome.

A retrospective cohort study, is a study where a cohort of individuals that share a common exposure factor is compared to another group of equivalent individuals not exposed, in order to determine the factor's influence on the incidence of a condition such as disease or death.

The reason that the study is prospective is that those type of studies provide future proof for biomarkers (which is the key point of our study) and has also strong validity where a retrospective study could provide fast data but there could be potential bias (missing data etc.)

## 2.6. Cohort study

In these studies, all people in the surveyed population are ranked in one exposure category at the start of the follow-up period. The exposure classification may be bitsome, ie exposed / un-exposed or may include many categories (un-exposed, little exposed, very exposed). These individuals are monitored for a specified period and all new cases of the disease under study are identified.

# 3. Methods

This chapter will demonstrate all the information about the patients and the study and will be divided into seven subchapters. As mentioned in the Abstract (Chapter 1) an MS Access Database will be created to accompany the patient's CRF's and lead the way for further statistical analysis.

## 3.1.Interventions

Procalcitonin was measured daily over the first 5 days. Primary blood measurement should occur in the first 12 hours (Baseline PCT) after the severe sepsis or septic shock is detected. This point is critical as antibiotics shouldn't be delayed.

## 3.2.Study design

The study design is prospective for six months, multicenter observational clinical trial. Three hospitals will take place in the study:

I. General Hospital of Volos
II. General Hospital of Larissa
III. General University Hospital of Larissa

Each hospital will have a defined sample size of patients which will be computed in the next subchapter (3.4 Sample Size).


**Picture 3 Study design**

## 3.3.Study discontinuation

Basic reasons for the termination of the study are:

- Ethical concerns (will also be mentioned in the sub-chapter 3.4.1),
- alterations in accepted clinical practice,
- reaching a positive or negative statistical end point earlier than expected
- lack of funding.

Last but not least, the discontinuation of a clinical trial can be decided by *either the investigator, the study sponsor, or by mutual agreement*.

## 3.4.Sample size

Based on Demirdal et al 2018, the mean PCT value for septic ICU patients that survived was [5.7±13.7 ng/dL] and [10.1 ± 18.0] for the non survivors. Thus, we need an 100 % increase in the study group mean value, leading us to a sample of n=61 patients.

$$N = \frac{\sigma^2(z1 - \beta + z1 - \alpha/2)^2}{(\mu 0 - \mu 1)^2}$$

$$N = \frac{13{,}7^2(1{,}28 + 1{,}96)^2}{(5{,}7 - 11{,}4)^2}$$

$$N = 61$$

Having three different hospitals where the sample can be gathered, it will be divided proportionally based on demographic criteria. More specifically, two hospitals are based in Larissa (Greece) and one in Volos (Greece). The proportions will be set to 66,7% and 33,3%, $n_1$=21, $n_2$= 20 and $n_3$= 20.

The power of the study is set to 90% and p-value at 0.05

## 3.5.Patients

Patients meeting criteria for severe sepsis or septic shock who were admitted to the ICU from the emergency department, other hospital clinics, or directly from out of hospital were included. Minimum three hospitals will be included in the study. Consecutive sampling will be used for all patients until we reach the sample size which was calculated in the previous subchapter.

### 3.5.1. Inclusion criteria

Inclusion criteria for this study were adult patients who were diagnosed with severe sepsis or septic shock. Those patients were either treated in the ICU or were transferred there from the emergency department or even out of the hospital.

Blood sampling performed within the first 12 hours after diagnosis of severe sepsis or septic shock and at last, their or their escort's willingness to provide written informed consent.

### 3.5.2. Exclusion criteria

Patients without initial blood draw, unwilling to provide written informed consent were excluded.

### 3.5.3. Subject's withdrawal

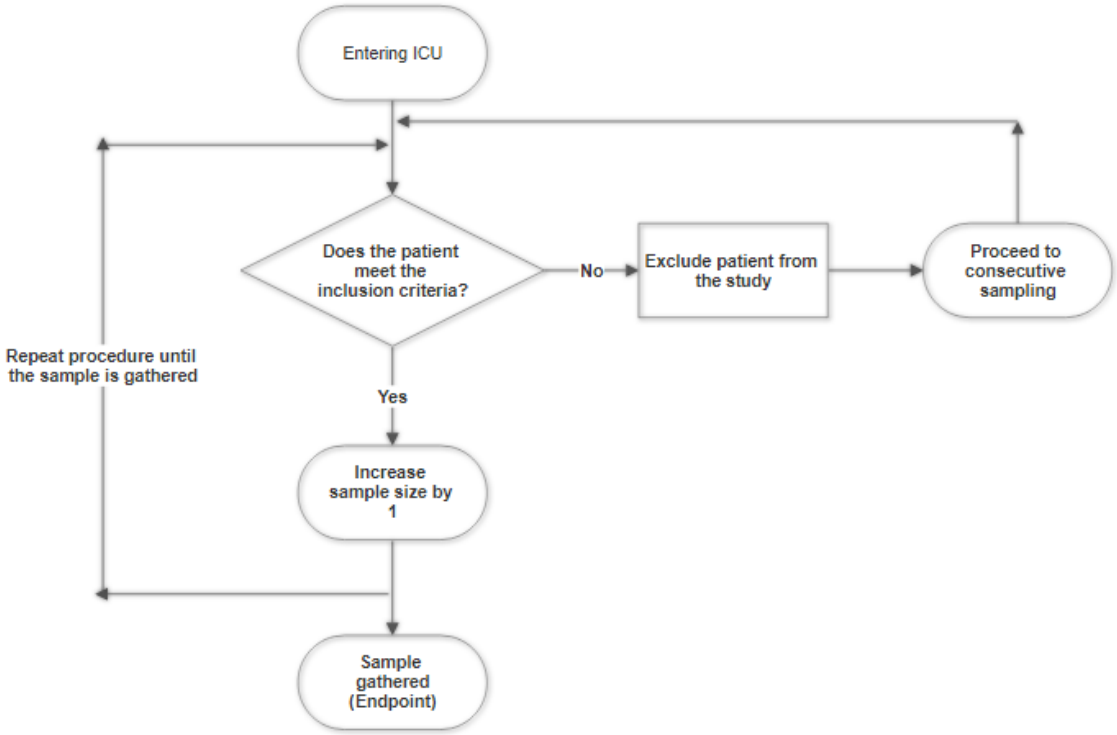
Every subject has the right to withdraw from the study anytime. By the time this occurs, all of the following research activities involving the subject's participation such as:

- Interaction with the subject in order to obtain data
- additional identifiable private information

will be stopped. In addition, patients might be withdrawn by the investigator if the investigator feels that the patient is not gaining any clinical benefit or because of unacceptable toxicity. Patients who are withdrawn or are removed from the study will be required to have an off-study clinic visit at the time of discontinuation, a 30-day follow-up safety visit.

## 3.6.Flow chart

Each patient that enters the Intensive Care Unit in each of the three medical centers is controlled whether meets the inclusion criteria or not. If they meet all the criteria, then they can enter the study. If they do not, then we proceed to the consecutive sampling until all the three sub samples are gathered. Below there is a flow chart that demonstrates the "*route*" of its patient.




**Picture 4 Flow chart**

## 3.7.Access Database

As mentioned in the introduction, a database will also escort the monitoring of the study and the recording data on each CRF. The primal reason for this is to assure that the *single data entry with second look* is registered properly so that a clear outcome could be drawn in a very short time.

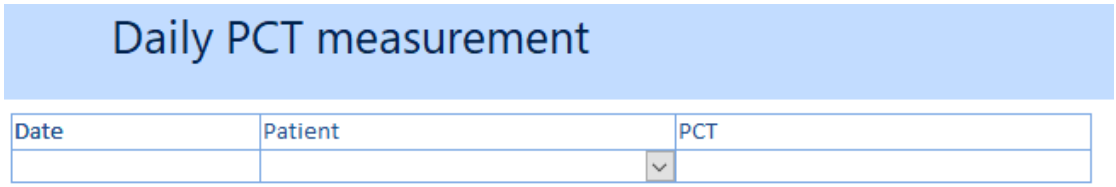
Due to the fact that the sample size is not very large, not many specialized staff is required thus the implementation of this database could provide us instant data for queries that the operator could set such as:

- mean PCT value,
- mortality (survivors – nonsurvivors),
- average days in the ICU
- age
- gender
- antibiotics used etc.

Below is an example of a query where instant patient outcome is provided. The database searches all the patients that are included in the study and their outcome which is also fulfilled from the personnel.

This query can be adjusted by date and even measure monthly, annually etc. outcomes. Such queries can be generated by the data exported from the CRF's.

```
SELECT Patient.Fullname, Patient.Outcome, Patient.[ExportDate]
FROM Patient
WHERE (((Patient.[ExportDate]) Between #  /  /2018# And # /  /2018#));
```




**Picture 5 Form in MS Access for daily PCT measurement**

In picture 5, a simple form of daily PCT measurement is demonstrated. By keeping records of those forms, the operator can extract results for the PCT value such as:

- minimum PCT value
- maximum PCT value
- Average PCT value etc.

that can be combined with factors such as age, gender or even Medical History for further statistical analysis ( Chapter 4 –Results).

# 4. Results

## 4.1.Statistical analysis

The entire patient's data will be recorded for this study:

- Age

- Gender
- Sepsis stage
- PCT measurement (5 days)
- Antibiotics
- Days in ICU
- Outcome

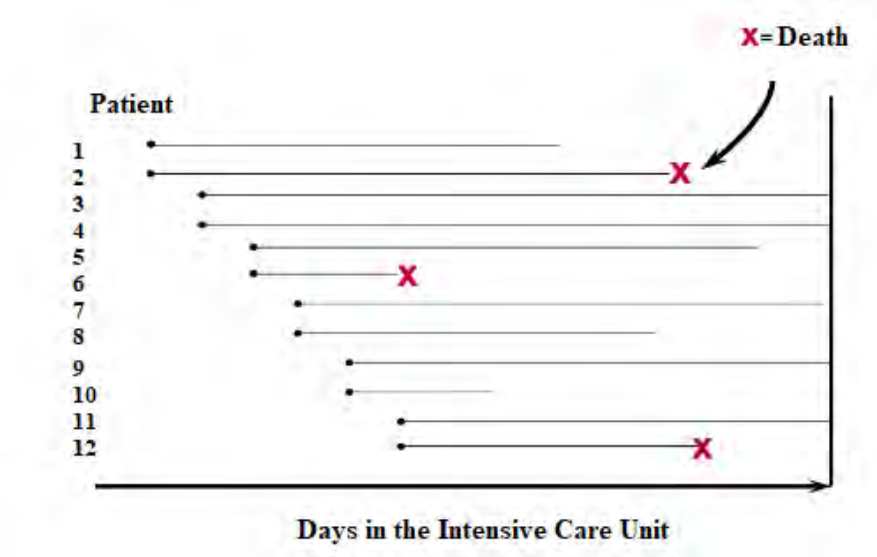
For the quantitative variables, descriptive statistics will produce mean value, median, quartiles, standard deviation, 95% CI and extreme values. As for the categorical values, proportions will be measured.

| Patient | Age | Gender | Sepsis stage | PCT day1 | PCT day2 | PCT day3 | PCT day4 | PCT day5 | Antibiotics | Days in ICU | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - | - | - | - | - | - | - | - | - | - | - | - |

**Table 2 Patients primary measurements**

## 4.2.Evaluation

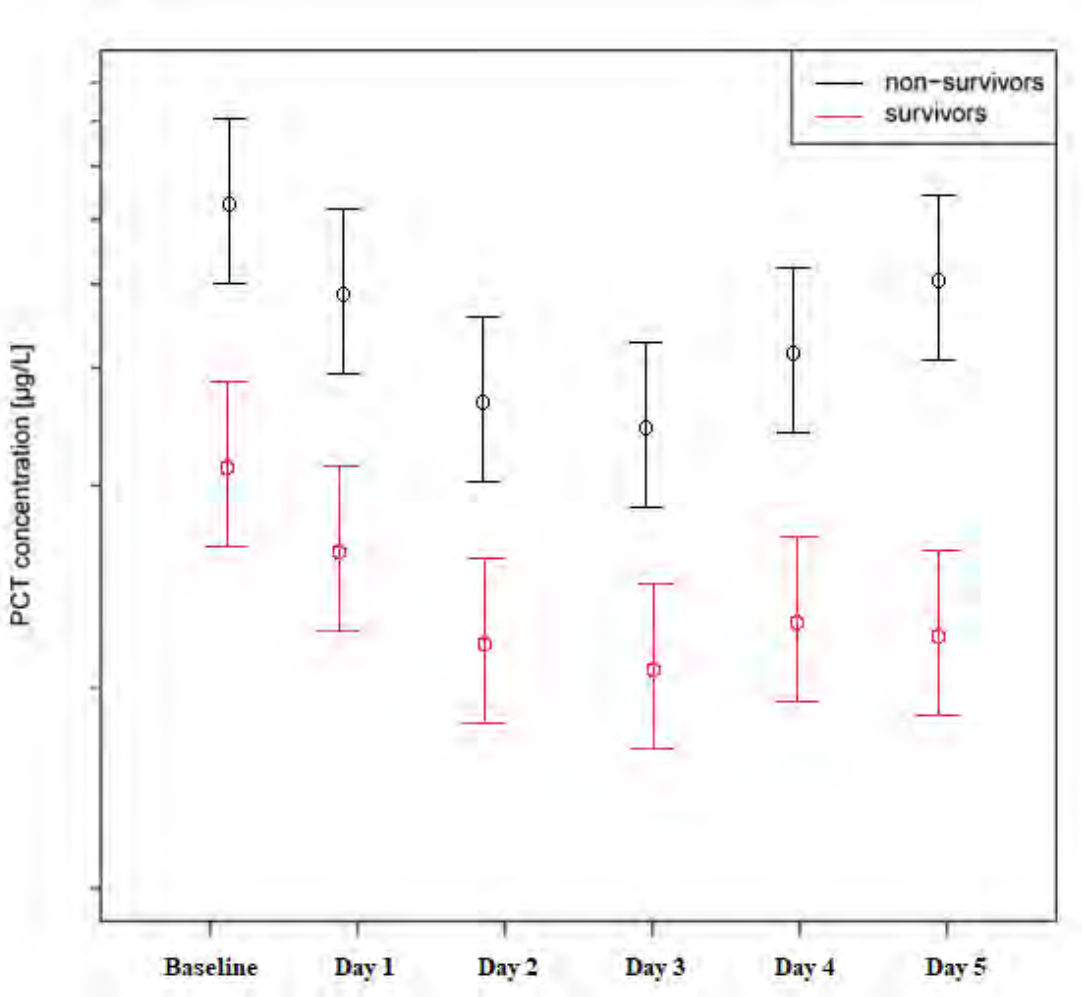
At the end of the study, survival analysis will be performed as the event of study is mortality. Patients will be followed over the period of the study. Focus will be on the time that the event occurs, where the closest PCT value that will be recorded, will indicate us a relationship between PCT and mortality. Linear regression cannot be used as it cannot effectively handle the censored observations.




**Picture 6 Incident Rate**

A plot about PCT concentration will be created for survivors and non-suvivors demonstrating the 95% confidence interval:

- LL (Lower Limit)
- UL (Upper Limit)
- Mean (Mean value)




**Picture 7 Daily PCT concentration**

### 4.3.Study limitations

As the study is observational, non-randomized, different biases may occur that can potentially introduce channeling bias and confuse the relationship between procalcitonin and the risk of the safety outcome (mortality).

## 5. Discussion

This diploma dissertation presented a draft protocol with the aim to examine the relationship between procalcitonin and mortality in septic ICU patients. Basic

information about sepsis, procalcitonin was presented in the "*Introduction*" chapter as well as the definitions of prospective and retrospective type of studies with their pros and cons.

Also, in "*Methods*" chapter a flow chart was designed based on the inclusion-exclusion criteria of the study. Study's sample size was calculated based on a recent study about procalcitonin and bacteremia prediction in Intensive care units. Last but not least, a Microsoft Access Database was created in order to escort the data fulfilled in the Case Report/Record Forms and strengthen the integrity of the results. With such combination, further research could use the database's data for other studies subjects.

To sum up, it is known that procalcitonin is a prognostic marker for sepsis, what we need to study is not only its relationship with mortality but also a multivariate analysis with gender – age – and mortality.

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