# Community onset sepsis in Sweden

## A population based study

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Cover illustration: “Sepsis has many faces” by Lars Duvander

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Dear Sir or Madame,

will you read my book?

It took me years to write

will you take a look?

*Lennon/McCartney1966*

*To*

*Britt-Marie*

*My beloved companion*

*in the ups and downs of life*

# Community onset sepsis in Sweden

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# ABSTRACT

Sepsis is estimated to annually cause 30 million cases and 6 million deaths worldwide. Since 2016, sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection". Previously, and when this study was conducted, the term "severe sepsis" was used to denote organ dysfunction caused by acute infection.

The aims of Study I were to explore the characteristics, epidemiology and outcome of community onset severe sepsis in the adult population in Skaraborg, western Sweden. During a 9-month period, Sept. 2011 – June 2012, 2,462 consecutive episodes in 2,196 patients admitted to Skaraborg Hospital and treated with intravenous antibiotics, were evaluated per protocol. Studies II, III and IV were done on parts of this study population.

The incidence of severe sepsis was estimated to 276/100,000 and of sepsis according to the new 2016 criteria to 856/100,000 (Study I). Risk factors for acquiring severe sepsis were age ≥85 years, cardiovascular disease, and diabetes mellitus. In 429 patients with severe sepsis, the 28-day case fatality rate was 25%, versus 4% in 1,767 with non-severe sepsis or no sepsis. Risk factors for 28-day case fatality were age ≥85 years, renal-, respiratory-, and cerebral dysfunction.

During a six week period, blood samples from 383 consecutive episodes of suspected sepsis in the emergency department were analyzed by multiplex PCR for rapid detection of pathogenic bacteria (Study II). We found that the multiplex PCR added some diagnostic value by detecting clinically relevant bacteria not detected by blood culture.

In Study III, 432 nasopharyngeal samples collected during winter 2012 were examined for respiratory viruses using multiplex PCR. We noted that viral infections or co-infections with bacteria were underestimated in patients with suspected sepsis, especially Influenza A virus, human metapneumovirus and respiratory syncytial virus.

In study IV, we evaluated lactate, C-reactive protein, procalcitonin (PCT) and the neutrophil to lymphocyte count ratio (NLCR) in 1,572 episodes of suspected sepsis. In bacterial sepsis of any severity, either the NLCR or PCT alone exhibited equivalent performance. In the most critically ill patients, combinations of 3 or 4 biomarkers could improve the diagnosis of bacterial sepsis.

Study V, performed in a neighboring hospital in Borås, examined six defined symptoms of sepsis; fever, dyspnea, acute change of mental status, severe pain, vomiting/diarrhea and muscle weakness. Occurrence of ≥3 of these symptoms significantly predicted the presence of severe sepsis or septic shock, especially acute change of mental status and dyspnea.

In conclusion: The Swedish 2011 criteria for severe sepsis appropriately separated those with a high case fatality rate from those with a low. High age was the most significant independent risk factor for both incidence and case fatality. Respiratory viral infections were common and underdiagnosed in patients with suspected sepsis. Multiplex-PCR added diagnostic value to blood culture. Biomarkers were limited in their ability to detect sepsis but improved when combined. Symptoms of sepsis can be defined and can be used for rapidly diagnosing sepsis.

**Keywords**: bacteremia, biomarker, epidemiology, multiplex-PCR outcome, sepsis, severe sepsis, symptoms.

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# SAMMANFATTNING PÅ SVENSKA

Sepsis har uppskattats orsaka 30 miljoner sjukdomsfall och 6 miljoner dödsfall årligen i världen. För att minska sjuklighet och dödlighet är det av största vikt att identifiera sepsis så snabbt som möjligt och att tidigt ge effektiv antibiotikabehandling. Sedan 2016 definieras sepsis som ”livshotande organsvikt orsakad av ett felreglerat immunologiskt svar på en infektion” (Sepsis-3). Tidigare kallades infektion med organsvikt för ”svår sepsis”, vilket var gällande när denna studie genomfördes.

Sepsis förekommer inom alla specialiteter i sjukvården men det finns ingen bra uppskattning av hur vanligt det är i en viss befolkning. Syftet med denna studie var att undersöka förekomsten av sepsis/svår sepsis bland vuxna $\geq$18 år i Skaraborg, riskfaktorer för att insjukna och avlida, samt att undersöka metoder för att snabbt kunna identifiera sepsis. Studieperioden var 9 månader mellan september 2011 - juni 2012.

I den första studien undersöktes alla vuxna patienter som lades in på sjukhuset i Skövde eller Lidköping avseende förekomst eller utveckling av svår sepsis under de första 48 timmarna. Som definition och kriterier användes de svenska från 2011. Då det 2016 kommit en ny internationell definition och nya kriterier (Sepsis-3), utvärderades även dessa. Enligt de svenska kriterierna från 2011 fann vi att 276/100 000 invånare årligen insjuknade i svår sepsis. Dödligheten inom 28-dagar var 25 %. Riskfaktorer för insjuknande var hög ålder och för 28-dagars död hög ålder, nedsatt njur-, lung- respektive hjärnfunktion. Tillämpning av 2016 års kriterier visade att 856/100 000 vuxna årligen insjuknade i sepsis och att dödligheten inom 28-dagar var 12 %.

I den andra studien jämfördes under en 6-veckorsperiod utfallet av 383 blododlingar med PCR, en metod för att snabbt kunna påvisa bakteriers arvsmassa, DNA, i blod. Resultatet visade att metoderna kompletterar, men inte ersätter, varandra.

I den tredje studien utvärderades virusfynd i näsprov hos patienter med misstänkt sepsis. Vi fann att virusinfektioner, främst Influensa A, var vanligare förekommande bland patienter med misstänkt sepsis än vad behandlande läkare trodde. Dubbelinfektioner med både virus och bakterier var inte heller ovanliga, framför allt hos personer med lunginflammation.

I den fjärde studien utvärderades förmågan hos ofta använda markörer såsom C-reaktivt protein (CRP), procalcitonin, neutrofil/lymfocytkvot (NL-kvot) samt laktat att identifiera svåra infektioner. Resultaten visade att inget enskilt prov är riktigt bra, men att kombinationen av de 3 markörerna, laktat, CRP och NL-kvot, resulterade i ökat förmåga att kunna upptäcka patienter med svår sepsis och som därför var i behov av snabbt insatt effektiv antibiotikabehandling.

I den femte studien utförd i Borås, utvärderades förmågan hos sex specifika symtom (feber, andnöd, förvirring, smärta, muskelsvaghet och kräkning/diarré) avseende att tidigt kunna identifiera patienter med svår sepsis. Förekomst av mer än tre av dessa symtom var starkt kopplade till svår sepsis, särskilt andnöd och förvirring.

# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Ljungström L, Andersson R, Jacobsson G. (2017). The epidemiology and outcome of severe sepsis and septic shock in Skaraborg, Sweden. A population based study. Manuscript.

II. Ljungström L, Enroth H, Claesson BE, Ovemyr I, Karlsson J, Fröberg B, Brodin AK, Pernestig AK, Jacobsson G, Andersson R, Karlsson D. (2015). Clinical evaluation of commercial nucleic acid amplification tests in patients with suspected sepsis. *BMC Infectious Diseases*, *15*(1), 199.

III. Ljungström LR, Jacobsson G, Claesson BE, Andersson R, & Enroth H. (2017). Respiratory viral infections are underdiagnosed in patients with suspected sepsis. *European Journal of Clinical Microbiology & Infectious Diseases*, 36(10), 1767-1776. DOI 10.1007/s10096-017-2990-z

IV. Ljungström L, Pernestig AK, Jacobsson G, Andersson R, Usener B, & Tilevik D. (2017). Diagnostic accuracy of procalcitonin, neutrophil-lymphocyte count ratio, C-reactive protein, and lactate in patients with suspected bacterial sepsis. *PLoS One*, *12*(7), e0181704.

V. Edman-Wallér J, Ljungström L, Jacobsson G, Andersson R, & Werner M. (2016). Systemic symptoms predict presence or development of severe sepsis and septic shock. *Infectious Diseases*, *48*(3), 209-214.

# CONTENT

# ABBREVIATIONS

| | |
|---|---|
| AUC | Area under the curve |
| BAS | Blodtryck Andningsfrekvens Saturation |
| CFR | Case fatality rate |
| CI | Confidence interval |
| CRP | C-reactive protein |
| CT | Computed Tomography |
| DIC | Disseminated intravascular coagulation |
| ED | Emergency department |
| EHR | Electronic healthcare record |
| EMS | Emergency medical services |
| $FiO_2$ | Fraction of oxygen in inhaled air (%) |
| GCS | Glasgow Coma Scale |
| ICD | International Statistical Classification of Diseases and Related Health Problems (by the WHO) |
| ICU | Intensive Care Unit |
| LODS | Logistic Organ Dysfunction Score |
| MEWS | Modified Early Warning Signs |
| MEDS | Multiple Emergency Department Score |
| MODS | Mortality Organ Dysfunction Score |
| NAAT | Nucleic Acid Amplification Test |
| NEJM | New England Journal of Medicine |

| | |
|---|---|
| NEWS | National Early Warning Signs |
| NLCR | Neutrophil to Lymphocyte Count Ratio |
| OR | Odds ratio |
| PCR | Polymerase Chain Reaction |
| PCT | Procalcitonin |
| PMN | Polymorphonuclear neutrophil |
| PRESEP | Prehospital Early Sepsis Detection (score) |
| qSOFA | Quick SOFA |
| RETTS | Rapid Emergency Treatment and Triage System |
| ROC | Receiver operating characteristic |
| RLS | Reaction Level Scale |
| SAI | Sjukhusets Antibiotika- och Infektionsuppföljningssystem |
| $SaO_2$ | Oxygen saturation in arterial blood |
| SIR | Swedish Intensive Care Register |
| SIRS | Systemic Inflammatory Response Syndrome |
| SpO2 | Oxygen saturation (in blood) as measured by pulsoximetry |
| SOFA | Sequential (sepsis induced) Organ Failure Assessment |

# DEFINITIONS IN SHORT

| | |
|---|---|
| Sepsis-1 | Sepsis-1 is often used to denote the sepsis definition and criteria according to the 1991 American consensus conference on sepsis [1].<br><br>Sepsis was defined as the systemic inflammatory response syndrome (SIRS) to a confirmed infectious process. Criteria for SIRS were presence of 2/4 of: heart rate >90/min, respiratory rate >20/min, temperature >38 or <36°C or leukocyte count >12 or <4 x $10^9$/ml.<br><br>Severe sepsis was defined as sepsis + organ dysfunction, but criteria for organ dysfunction were not specified. Thus, different criteria for organ dysfunction have been used in clinical studies. |
| Sepsis-2 | Is often used for the definition according to the second sepsis consensus conference in 2001 [2]. The basic definition of sepsis was retained, but the list of sepsis criteria was expanded.<br><br>Criteria for early organ dysfunction were offered. These were not meant to be criteria for severe sepsis, but have often been used as such in clinical studies. |
| The 2011 Swedish sepsis definition | The Swedish definition of sepsis was the same as in Sepsis-1 and -2.<br><br>Severe sepsis, however, was defined as sepsis *or* verified infection + organ dysfunction. More strict criteria for organ dysfunction than Sepsis-2 were presented [3]. *Table 1*, p. 3. |
| Sepsis-3 | Sepsis-3 represents a new definition of sepsis launched in 2016 by a third international consensus sepsis conference [4]. This new definition of sepsis now includes organ dysfunction. Sepsis is now defined as “life |

| | |
|---|---|
| | threatening organ dysfunction caused by a dysregulated immune response to an infection [4].<br><br>Thus, the term “severe sepsis” is no longer needed.<br><br>Criteria for sepsis-3 is an increase of 2 points or more from baseline in the SOFA-score [5]. *Table 2*, p. 4. |
| Septic shock | Septic shock was defined in Sepsis-1 and -2 as sepsis-induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities [1].<br><br>According to Sepsis-3, septic shock is defined as “a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone. Adult patients with septic shock can be identified using the clinical criteria of hypotension requiring vasopressor therapy to maintain mean blood pressure 65 mm Hg or greater and having a serum lactate level greater than 2 mmol/L after adequate fluid resuscitation”[6]. |
| Biomarker | A biomarker, or biological marker, generally refers to a measurable indicator of some biological state or condition, for example a certain disease [7]. In clinical medicine, and in this text, the term “biomarker” refers to laboratory biomarkers. |
| Case Fatality Rate (CFR) | The percentage of patients having a disease that die from that disease. |
| Mortality rate | The percentage of persons in a population that die from a certain disease. |

# 1 INTRODUCTION

## 1.1 Already the ancient Greeks…

Sepsis was known already to the Greeks 4000 years ago. The word "sepsis" is Greek and means "to rot". It was mainly used for skin and soft tissue infections causing high fever, foul smelling tissue destruction, weakening of the individual and eventually death in just a few days. In modern medicine, "a septic patient" refers to someone with high fever, rapid breathing, high pulse, low blood pressure, vomiting or diarrhea, general weakness, and sometimes an altered mental status.

## 1.2 Sepsis definitions

For many decades and for many Swedish doctors, "sepsis" still equals an infection where bacteria or their toxins, have spread to the circulation, (Cronberg 1986) [8] and can be detected by blood cultures.

In 1991, an American consensus meeting (Sepsis-1) defined sepsis as the systemic inflammatory response syndrome (SIRS) to a confirmed infection process [1]. There were four SIRS criteria: heart rate >90/min, respiratory rate >20/min, temperature >38 or <36°C and leukocytes >12 or <4 x $10^9$/ml. For sepsis diagnosis, a suspicion of infection plus $\geq$2/4 of those criteria were needed. Detection of bacteria in blood culture was not obligatory. The definition focused on inflammation. An overwhelming inflammatory response could cause progressive organ dysfunction, and organ dysfunction was associated with high case fatality rates (CFR). Sepsis-induced organ dysfunction was termed "severe sepsis" and severe sepsis accompanied by circulatory failure was as previously termed "septic shock". Criteria for hypotension were defined, but for "hypoperfusion abnormalities" only suggested: "Lactic acidosis, acute alteration of mental status, and oliguria." Organ dysfunction was recognized as a progressive event, not dichotomous, and studies were called for that could define the progressive organ dysfunction observed in many patients with sepsis.

Soon there were many suggestions for organ dysfunction definitions, among those the Multiple Organ Dysfunction Score (MODS) in 1995 [9], the Sequential Organ Failure Assessment score (SOFA) in 1996 [10], and the Logistic Organ Dysfunction Score (LODS) in 1996 [11]. However, none of

these have come into general use, especially not outside the intensive care units, outside clinical studies.

Critique against the SIRS-criteria for being too insensitive and too unspecific, led to a second international consensus conference in 2001 (Sepsis-2) [2]. The basic definition of sepsis was retained, but the list of sepsis criteria was expanded. Still there were no criteria formulated for organ dysfunction in severe sepsis. The criteria for hypotension remained from Sepsis-1, and some criteria for "early organ dysfunction" were suggested in the expanded list of criteria for sepsis. As criteria for severe sepsis, the suggestion was to use the MODS or the SOFA-score [2]. In practice, however, the criteria for "early organ dysfunction" in the Sepsis-2 document were used in many clinical studies to define organ dysfunction in severe sepsis.

Some clinical studies though, used more strict criteria for organ dysfunction than those for "early organ dysfunction" suggested in Sepsis-2. One such study was the PROWESS study in 1998-2000 on the efficacy of drotrecogin alfa, recombinant human activated protein-C, in treating patients with severe sepsis. In the PROWESS study, the criteria for respiratory dysfunction were stricter. Instead of $PaO_2/FiO_2$ <300 (mm Hg) for respiratory dysfunction, the level chosen was $PaO_2/FiO_2$ <250 and if the lung was the focus of the infection the level was $PaO_2/FiO_2$ <200 [12]. In 2012 the Surviving Sepsis Campaign (SSC) Guidelines presented dichotomous criteria for organ dysfunction in severe sepsis [13], adopting these respiratory criteria.

It hardly needs saying, that because of this lack of clear-cut criteria for organ dysfunction in severe sepsis, different criteria have been used in almost every study since 1991, making studies on the epidemiology of severe sepsis difficult to compare.

In 2011 a work group within the Swedish Infectious Disease Society published a consensus document together with representatives for the Swedish Intensive Care Society, with a definition and criteria of severe sepsis and septic shock [3]. This was based on the Sepsis-1 and Sepsis-2 definitions and criteria with some exceptions. The main difference was that the Swedish definition did not demand $\geq$2/4 SIRS criteria for the diagnosis of severe sepsis. A verified infection + organ dysfunction was sufficient. ***Table 1.*** The second difference was that "acute alteration of mental status" was added as an independent criterion for severe sepsis, as suggested in Sepsis-1. The third difference was that the stricter criteria for respiratory dysfunction, as used for example in the PROWESS study, were adopted. Fourthly, only urine output was used for renal dysfunction, not creatinine level.

***Table 1.*** *The 2011 Swedish consensus definition and criteria of severe sepsis and septic shock. Adapted from Ljungström [3].*

| | |
|---|---|
| **Sepsis** | Suspected infection + ≥2 SIRS[1]-criteria |
| **Severe sepsis** | Sepsis *or* documented infection + either hypotension, hypoperfusion *or* organ dysfunction |
| *Hypotension* | Systolic blood pressure ≤90 mm Hg *or* mean arterial pressure ≤70 mm Hg |
| *Hypoperfusion* | Blood lactate >3 mmol/l or + >1 mmol/l above the upper reference limit, *or*, base excess ≤-5 mmol/l |
| *Organ dysfunction:* | |
| *Respiratory* | PaO2/FiO2 <33 kPa (corresponding to 86% saturation on air breathing) *or* PaO2/FiO2 <27 kPa (corresponding to 78% saturation) if the lung is the focus of infection. |
| *Renal* | <0.5 ml urine/kg/2 hours despite adequate volume resuscitation |
| *Hematologic* | Thrombocytes <100 x $10^6$/ml, *or* INR[2] >1.5, or APTT[3] >60 seconds |
| *Cerebral* | Acute change of mental status |
| *Hepatic* | Serum bilirubin >45 µmol/l |
| **Septic shock** | Persisting hypotension despite adequate volume resuscitation (500-1000 ml of crystalloid given within 30 minutes) *plus* either hypoperfusion or organ dysfunction |

[1]SIRS criteria consist of a. Heart rate >90/min. b. Respiratory rate >20/min. c. Temperature >38.0$^0$ C or <36.0$^0$ C. d. Leukocyte count >12.0 x $10^9$/ml or <4.0 x $10^9$/ml or >10% bands. INR, International Normalized Ratio; APTT, activated partial thromboplastin time.

Since 2011, This Swedish definition and criteria have been used in the coding of severe sepsis according to ICD-10. It has also been used in the Swedish quality register for severe sepsis since 2012. The register is for patients who within 24 hours of arrival are referred to the ICU. The Swedish criteria, however, have never been evaluated in an epidemiological study.

In 2016, based on new research findings, a new definition of sepsis was suggested by the Third International Sepsis Definitions Task force. The concept of sepsis as being caused by hyper-inflammation was abandoned. Instead, sepsis was defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection" (Sepsis-3) [4]. Organ dysfunction was incorporated into the very definition of sepsis, and thus the term "severe sepsis" is no longer needed. The criterion for sepsis is now +≥2 points from base-line in the Sequential Organ Dysfunction Score, SOFA-score [10]. ***Table 2.*** This is said to correspond to a case fatality rate of ≥10% and <2 points to a case fatality rate of <5% [4].

***Table 2.*** *The SOFA score. Modified from Vincent 1999 [5], Singer 2016 [4] and Edman-Waller 2017 [14].*

| | SOFA-score | | | | |
|---|---|---|---|---|---|
| **Variabel** | 0 | 1 | 2 | 3 | 4 |
| Respiration: $PaO_2/FiO_2$, kPa | >53 | ≤53 | ≤40 | ≤27 | ≤13 |
| Corresponding saturation $SaO_2$ % | ≥96 | <96 | <92 | <79 | <49 |
| Coagulation: Thrombocytes, x $10^9$/l | >150 | ≤150 | ≤100 | ≤50 | ≤20 |
| Liver: bilirubin, µmol/l | <20 | 20-32 | 33-101 | 102-204 | >204 |
| Hypotension: mean arterial pressure, MAP | ≥70 mm Hg | <70 mm Hg | Dopamin ≤5[1] Dobutamin[2] | Dopamin >5[1] Adrenalin ≤0.1[1] Noradrenalin ≤0.1[1] | Dopamin >15[1] Adrenalin >0.1[1] Noradrenalin >0.1[1] Levosimendan[2] Vasopressin[2] |
| Cerebral: GCS-points | 15 | 13-14 | 10-12 | 6-9 | 3-5 |
| RLS-points | 1 | 2 | 3 | 4-5 | 6-8 |
| Renal: Creatinine µmol/l | <110 | 110-170 | 171-299 | 300-440 | >440 |
| Diures, ml/day | ≥500 | ≥500 | ≥500 | <500 | <200 |

[1)] Catecholamine doses are given as µg/kg/min.
[2)] Regardless of dose.
$FiO_2$, Fraction of inspired oxygen; $PaO_2$, partial pressure of oxygen; $SaO_2$, arterial oxygen saturation; GCS, Glasgow Coma Scale; RLS, Reaction Level Scale.

The definition of septic shock was also changed, so that septic shock is now defined as "a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone". The clinical criteria for septic shock were changed to "hypotension requiring vasopressor therapy to maintain mean arterial blood pressure 65 mm Hg or greater and having a serum lactate level greater than 2 mmol/L after adequate fluid resuscitation" [6].

## 1.3 Sepsis epidemiology

A recent review has estimated that there are 27-30 million annual cases of sepsis and 6-9 million deaths from sepsis worldwide [15]. Though approximations, these figures give an apprehension of the magnitude of the problem that sepsis poses.

There are many pitfalls in describing the epidemiology of severe sepsis in a population. Definitions and criteria have changed over time, have not been consistently used, and have been applied to different study populations [16].

### 1.3.1 Sepsis incidence

The incidence of severe sepsis in high-income countries has been explored by two main methods, chart-based or code-based. These methods have yielded incidences ranging between 3-1,074/100,000 [16]. Lately a third method, extracting clinical criteria from electronic health care records, EHRs, has been developed for this purpose [17]. The incidence in low-income countries is difficult to estimate because of lack of reliable data.

The chart-based method is considered the "gold standard" for studying sepsis incidence [17], since patients are individually evaluated. The drawback is that it is very labor intense, useful only for smaller cohorts, and thus rarely used for population-based studies. One such study was performed in a twelve month period in 2011-2012 by Henriksen, [18] evaluating all patients admitted to the medical ED at the University Hospital in Odense, Denmark. Using modified Sepsis-2 criteria, they found an incidence of severe sepsis of 457/100,000 person-years at risk.

A variant of this method is to perform chart-based population studies based on point prevalence data and extrapolating the results. This method was used in a study by Mellhammar [19], evaluating all hospitalized patients in southern Sweden who had received intravenous antibiotic treatment at four evenly distributed dates during the year 2015. Using criteria similar to those of the 2012 Surviving Sepsis Campaign, they found an annual incidence of severe sepsis of 687/100,000 and of Sepsis-3 of 780/100,000. The incidence did not differ significantly between the dates studied.

The most recent study on the incidence of Sepsis-3 in the United states 2009-2014, compared clinical criteria from EHRs, to code-based abstraction [17] in almost 174,000 patients treated with antibiotics. The results in turn were evaluated against "gold standard", which in this study was chart-based point prevalence data from 510 randomly chosen EHRs. The incidence using EHR data showed 70% concordance with the results of the chart based evaluation, but only 32% concordance with the results of code-based abstraction. The reported incidence of Sepsis-3 was 6% of all admissions. Using clinical criteria from EHR data, no increase in the incidence of sepsis could be demonstrated during those 6 years. Using code-based abstraction, the less sensitive method, there was an increase in incidence of 13% per year.

Code-based abstraction is suitable for large patient populations and is the method used in most previous American studies. However, the reliability depends on the accuracy in coding. One problem is that coding has changed

over the years. In the United States, ICD-9 codes were used until 2008, and in 2003 classification codes for severe sepsis and septic shock were added to ICD-9. Sweden started using ICD-10 for coding in 1997, but not until 2011 were there additional ICD-10 codes for severe sepsis and septic shock.

Three American studies have used code abstraction on large population groups. In a patient cohort from 1995, Angus estimated the overall annual incidence of severe sepsis to be 300/100,000 [20]. In a population study from 2000, Martin found an incidence of 81/100,000 [21]. In a cohort from 2003, Dombrowsky reported an incidence of severe sepsis of 134/100,000 [22]. The abstraction methods used in these three studies plus the method used in a study by Wang [23] was applied by Gaieski to a population of nearly 40 million hospitalizations during a six year period 2004-2009 [24]. This resulted in a 3.5-fold variation in incidence, between 300-1,031/100,000 depending on the method used. However, the annual increase in incidence was 13%, regardless of the method used.

A study exploring the incidence of severe sepsis in Sweden between 1987-2005 by Wilhems [25] applied the abstraction methods by Angus, [20] Martin, [21] and Flaatens [26] to Swedish data. This resulted in an incidence in 2005 of only 13-47/100,000. In addition, the sepsis populations identified using these three methods were almost entirely different. Most likely, this reflects poor quality in coding in Sweden rather than a ten-fold lower incidence compared to the United States.

### 1.3.2 Risk factors for acquiring sepsis

Age, co-morbidities, male sex, ethnicity, genetic factors, and geographical location are known risk factors for acquiring sepsis [27]. The increasing incidence with age has been a consistent finding in many studies [17-21, 27, 28]. Co-morbidities associated with increased risk of sepsis are diabetes mellitus, congestive heart failure, chronic pulmonary disease, immunosuppression, chronic renal failure, cancer, liver disease [29] and chronic alcohol abuse [18, 30]. Male sex is also a risk factor [20, 21, 30], as is African-American [29-31], nonwhite [30], and Aboriginal ethnicity [32]. In the United States there is more sepsis in the winter season which coincides with the Influenza epidemic [29], but the study from Sweden in 2015 by Mellhammar found no seasonal variation [19].

### 1.3.3 Case fatality in severe sepsis

As the incidence of severe sepsis varies with definitions used and populations studied, so do case fatality rates. In the study by Gaieski [24], the case fatality

rate varied two fold, between 15-30%, depending on the method used for code-abstraction. In a study by Rhee based on clinically defined EHR data, the in-hospital mortality of Sepsis-3 between the years 2009-2014 was 15.0%, and decreased by 3.3% annually [17]. If including those referred to hospice care after being treated for severe sepsis, on the average 6.2%, there was no change of the annual in-hospital mortality rate.

A large comprehensive Australian study on patients with severe sepsis treated in the ICU, found a decrease of in-hospital mortality from 35-18.4% between the years 2000-2012. The decrease was mainly attributed to overall improved ICU treatment during this time period [33], since the same decrease could be observed in patients with other diagnoses treated in the ICU as well.

Increasing age was shown to correlate to case fatality in severe sepsis already in the study by Angus in 2001 [20], later also by others [21, 27, 28]. The study by Martin in 2007 [28] showed age $\geq$65 years to be a statistically significant independent risk factor for in-hospital mortality.

The number of dysfunctioning organ systems has in many studies been identified as a risk factor for case fatality [34, 35].

The type of organism causing severe sepsis also relates to outcome [36]. In patients with bacteremia, *Staphylococcus aureus* is associated with higher case fatality rates than bacteremia caused by *Escherichia coli* [37]. In a large international multicenter study on patients treated in ICUs, infections with *Enterococcus* spp., *Pseudomonas* spp., and *Acinetobacter* spp., were independent risk factors for case fatality [38].

In the study by Angus [20], male sex was associated with higher case fatality, as shown also in later studies.

Time to appropriate antibiotic therapy is another factor affecting case fatality rates in severe sepsis, which may become more and more important, as multi-drug resistance is increasing worldwide. An American study on bacteremia in ICU-patients found that >24 hours delay to start of effective antibiotic therapy was an independent risk factor for case fatality, and was mainly due to bacteria with multiple antibiotic resistances [39].

### 1.3.4 Long term effects of severe sepsis

Acute infections may worsen pre-existing chronic diseases, leading to poorer long term outcome [27]. Severe sepsis may lead to decreased cognitive and autonomous functions [40] as well as to decreased quality of life [41]. Severe

sepsis is a long-term risk factor for death. Shapiro [34] found the one-year case fatality in patients with severe sepsis to be significantly higher than in those who had an infection but not organ dysfunction. This increase in 1-year case fatality was even more pronounced in patients with septic shock. A Danish study by Storgaard [42] reported that 1 year and even 4 year case fatality rates were significantly increased in patients with severe sepsis compared to a control group. Similar results were found by the Finnsepsis study group [43].

### 1.3.5 Bacteremia

The incidence and outcome of patients having bacteremia, or "blood stream infection" (BSI) is often treated as an entity of its own among patients with sepsis, and is therefore evaluated even in this thesis. Bacteremia ranks among the seven most common causes of death in North America and several European countries [44]. Ever since the dawn of bacteriology in the 19$^{th}$ century, detection of pathogenic bacteria in blood has been considered a sign of severity, associated with increased case fatality rates. This has repeatedly been verified in clinical studies and reviews [44-48]. As severe sepsis, bacteremia has been shown to influences long term case fatality rates up to twelve years after an episode [48].

### 1.3.6 Respiratory tract infections

In sepsis studies, the respiratory tract is frequently found to be the most common focus of infection, and has therefore received special attention in this study. The bacteria most commonly found in community acquired pneumonia and associated with the highest case fatality rates is *Streptococcus pneumoniae*, followed by *Haemophilus influenzae* and *Mycoplasma pneumoniae*, which occurs in epidemics with 3-5 year intervals [49].

Respiratory viruses may also cause severe disease, the most obvious being influenza A and B viruses. Further, by several mechanisms, viral respiratory infections enhance colonization and secondary infection by respiratory bacteria [50]. One example is pneumonia caused by *S. pneumoniae*, *H. influenza* or *S. aureus* following infection with the influenza virus. In daily practice, it is often difficult to distinguish whether a patient with pneumonia has a bacterial or a viral infection only, or a combination. In clinical cases of "clear cut" pneumonia, many clinicians do not even consider the possibility of co-infections with virus.

One reason why it is important to know if a patient has a viral infection is that some viral infections, like influenza, may be treated with anti-viral drugs in the early phase. Another reason is the need of infection control. Viral infections

are highly contagious. These patients are often cared for in wards together with other old and frail patients for whom it might be detrimental to have a severe viral infection on top of the condition they already have. Another reason is that mixed viral-bacterial infections are related to disease severity, as described by Voiriot [51].

## 1.4 Sepsis is an emergency

An infection leading to organ dysfunction is an emergency; early identification and adequate antibiotic treatment is imperative for reducing case fatality. This can be shown for large groups of patients with severe sepsis (Sepsis-2) [52-54] and for patients with septic shock [55]. There are conflicting study results, where the influence of the time factor on case fatality cannot always be verified. Mostly, this is because patient populations are heterogeneous and groups studied not large enough to demonstrate a statistically significant difference. Apart from time to antibiotic treatment, the outcome in sepsis depends on age, co-morbidities, focus of infection, number of dysfunctioning organ systems, and on the causing agent, as described in the previous section.

## 1.5 Early identification of sepsis patients

There is strong consensus on the need to recognize sepsis early, so that effective antibiotic treatment can be instituted without unnecessary delay. Tools used are the medical history, ongoing medication, symptoms, clinical signs, vital signs, clinical examination, laboratory parameters, cultures and rapid tests for detection of pathogenic microorganisms, and imaging techniques.

One main challenge in diagnosing patients with sepsis is that neither symptoms nor changes in vital signs or laboratory parameters are specific for sepsis. Another challenge is that sepsis is both a syndrome and a dynamic process where not all characteristics of the syndrome appear at the same time in the same individual. This means that changes indicating severe sepsis may not be present at the time of investigation or sampling. Therefore, repeated examinations, most easily done by recognizing symptoms and measurements of vital signs, are of great importance.

## 1.6 Clinical markers of sepsis

Most clinical markers of sepsis, symptoms, vital signs and laboratory biomarkers (hereafter referred to only as "biomarkers"), carry both diagnostic and prognostic information. To the clinician in the ED, the prime interest is the diagnostic information. Is this an infection? Is this an infection that needs antibiotic treatment? Is urgent treatment needed? It is also of interest to determine whether this is a severe infection or not. Can the patient be discharged home or is inpatient care at a ward or ICU indicated?

A patient arriving with high temperature, high respiratory rate, and lowered level of consciousness, displays variables with both diagnostic and prognostic information. They tell us that an infection is most likely at hand (high temperature), and that antibiotic treatment is urgently called for (high respiratory rate and lowered level of consciousness). More specifically, they may draw our attention to bacterial meningitis as a possible cause of the infection (high fever, lowered level of consciousness). Further, they tell us that if this is an infection, the patient has sepsis, since lowered level of consciousness in infection is a criterion for Sepsis-3 as well as for severe sepsis (as defined in this study). Finally, the lowered level of consciousness and the high respiratory rate tell us that this patient has in increased risk of in-hospital case fatality, and should probably be treated in a special unit.

Today, many new biomarkers are launched as important diagnostic or prognostic biomarkers. However, in most cases there is already much such information available in commonly used vital signs and biomarkers. "What does this add to the information we already have?" is an important question to ask before introducing new biomarkers on the arena.

## 1.7 Symptoms of sepsis

In our study, a 64-year-old woman with Crohn's disease, hypertension, and overweight, arrived at the ED because of sudden onset of severe upper abdominal pain, pronounced respiratory distress and high fever. An experienced surgeon suspected bowel perforation. He ordered cultures, broad-spectrum intravenous antibiotics and a computed tomography (CT) scan of the abdomen. The patient was then referred to the surgical ward. The CT-scan revealed nothing abnormal. Upon arrival to the ward, a nurse discovered that the patient had redness of the left lower limb, and she was diagnosed with erysipelas. Recovery was rapid and uncomplicated. Blood cultures were negative. Apart from respiratory dysfunction, she fulfilled no other criteria for either severe sepsis or Sepsis-3. The leg was the focus of infection, not the

abdomen or the lungs. Then why did she have such severe pain in the upper part of the abdomen? Why such respiratory distress?

Many speak about the importance of symptoms in early recognition of sepsis. An editorial in the New England Journal of Medicine (NEJM) commenting on the PROcess study on septic shock treatment, wrote[56]:

"The critical role of the clinician in the early recognition of sepsis continues to this day to be fundamental to our efforts to improve the rate of survival. Identification of *the combination of signs and symptoms* that make up the systemic inflammatory response syndrome (SIRS) in the context of an infection allows the astute clinician to recognize the malady" [57].

However, which are these "symptoms of sepsis"? If sepsis is a collective term for many different acute serious infections, are there any "symptoms of sepsis" to look for? Should we not instead examine for symptoms of the focal infection causing sepsis? Yet, the immune response in sepsis to an invading organism is the same, regardless of the organism or the focus of the infection. Thus, there are good reasons to believe that there really are "symptoms of sepsis", "fever" being the most obvious. Until very recently there have been no studies on this topic.

Why are symptoms important? Because symptoms cause patients with acute medical conditions to seek medical care. *"Listen to your patient, he is telling you the diagnosis",* is maybe the most well-known saying by Sir William Osler, "the founding father of modern medicine" [58]. When doctors had less technological support, they had to rely more on the patient history and their clinical investigation than we often do today. This is still true in poor-resource settings. Maybe we are focusing too heavily on changes in vital signs and laboratory parameters in making a diagnosis?

There is a detailed description with many interesting observations from 1852 by James Hudson Taylor [59] of what we today would call "sepsis" (with organ dysfunction). Hudson Taylor, then 20 years old, wanted to become a missionary to inland China, until then a country closed to foreigners. The way to being accepted in China was by becoming a doctor. Thus, he started studying medicine at the Royal College of Surgeons in London, where he was staying with an uncle. One night at home while sewing, he accidentally pricked the first finger of his right hand, *"but in a few moments forgot all about it".* The next morning the students started dissecting as before. *"The body was that of a person who had died of fever, and was more than usually disagreeable and dangerous. I need scarcely say that those of us who were at work upon it*


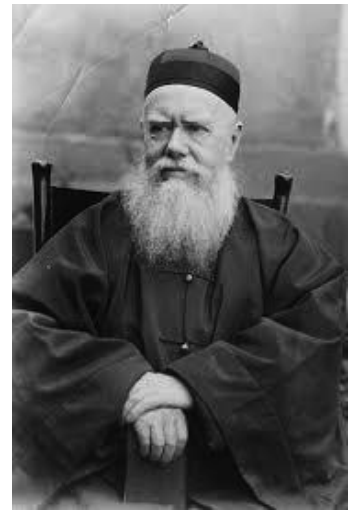

*Figure 1. James Hudson Taylor in England at the age of 20 and at the age of around 70 when he resided in China. Published with permission of the OMF, Overseas Missionary Fellowship www.omf.org.*

*dissected with special care, knowing that the slightest scratch might cost our lives. Before the morning was far advanced I began to feel weary, and while going through the surgical wards at noon was obliged to run out, being suddenly very sick – a most unusual circumstance with med, as I took but little food and nothing that could disagree with me".*

After lunch, the pain in his whole arm and right side became more and more intense. He got weaker and weaker until he could no longer hold his pencil, realized he could not continue class and went back to the dissecting room to clean up before going home. There he met "the demonstrator, himself a skilled surgeon", and asked him about the illness. *When describing the symptoms*, the surgeon replied *"Why",* said he, "*what has happened is clear enough. You must have cut yourself in dissecting, and this is a case of malignant fever".* He advised me to take a hansom, drive home as fast as I could and arrange my affairs forthwith: "*For",* said he, *"you are a dead man".* By that time the young student was unable to walk all the way home and finally had to take a ride with an Omnibus. At home, *"the pain was very severe. I fainted away, and was so long unconscious that when I came to myself I found I had been carried to bed".* "Days and weeks passed by" before he was able to leave bed. However, he did survive, and made it to China where he died at the age of 73 years. The full recount is found in the Appendix.

This is a vivid description of what most probably was a streptococcal infection, a common cause of death in those days. Apart from fever, the symptoms he mentions are “severe pain” (in his whole right side), muscle weakness (unable to walk home), gastrointestinal upset (vomiting), and unconsciousness. It is interesting that when “describing the symptoms”, the demonstrator could both diagnose the sickness (“malignant fever”) and give a prognosis (“you are a dead man”).

Which is the pathophysiological basis for these symptoms? All symptoms in acute infections are caused by the systemic inflammatory response, orchestrated by cytokines and other mediators, many of which, at high concentrations, also have other biological effects. Disease severity is related to cytokine levels, so it is reasonable to believe that the intensity of symptoms is as well.

Fever is caused by pro-inflammatory cytokines or bacterial components increasing the temperature level in the thermostat in the hypothalamus. The body tries to adjust to the new temperature setting, often by initiating muscle contractions known as “rigors” or “shivering”. The end result is increased body temperature, fever [60, 61].

Acute cerebral dysfunction is a severe symptom in patients with sepsis, associated with increased CFRs. The pathophysiology is multifactorial, including excessive microglial activation, impaired cerebral perfusion, blood–brain-barrier dysfunction, and altered neurotransmission [62].

Dyspnea is a result of increased vascular leakage of fluid into the lung tissue, as part of the systemic inflammatory reaction to an infection. This leads to increased compliance and a need to breathe harder to satisfy the requirement of oxygen. When fluid starts filling the alveoli, saturation decreases. Taken together, this causes heavier breathing and decreased saturation which the patient experiences as dyspnea [63].

The acute muscular weakness observed in septic patients is caused by excessive production of pro-inflammatory cytokines, hampering muscle contraction at several sub-cellular levels [64]. This affects both skeletal muscles, but also the muscle of the heart and the intestinal smooth muscles. In younger persons, the effect is a decreased ability to perform more heavy exercise, whereas elderly patients may be unable to either stand or walk. The effect on the heart can be visualized by ultracardiography as dilatation of the myocardium and decreased ejection fraction. Decreased smooth-muscle contractions in the bowels may result in paralytic ileus.

Vomiting and/or diarrhea are symptoms mainly seen in abdominal infections, but may occur also in infections unrelated to an abdominal focus. The same is true for severe localized pain, which is mostly a sign of the focal infection but may be seemingly unrelated to the focus of the acute infection. Though mechanisms are manifold, these symptoms are common in patients with sepsis. One illustration of this is the well-known case of Rory Staunton, a 12-year old boy in New York, who fell ill with fever, vomiting and severe pain in his right leg. The pediatrician diagnosed him with gastroenteritis and dehydration, when actually he had a streptococcal bacteremia, from which he succumbed. Afterwards, his mother pointed out that it was the severe pain in his leg that was the main problem, not the fever or the vomiting [65]. The tragic death of Rory Staunton led the governor of New York in 2013 to introduce Rory's Regulations, demanding that all hospitals in New York implement routines in order to recognize and treat sepsis early.

# 1.8 Vital signs in sepsis

## 1.8.1 Vital signs in general

Vital signs are rapidly and easily measured by all health care personnel, and, compared to laboratory sampling, can be performed repeatedly. Vital signs also contain information about organ dysfunction, though not specific for sepsis. Studies show that abnormal vital signs can be used to predict in-hospital mortality, and thus give a clue to understanding which vital signs and at which cut-offs should be a warning sign. In a large consecutive study on vital signs in the ED, Buist [66]found unconsciousness to be the strongest predictor of case fatality. The second strongest predictor was respiratory rate of $\geq$30/minute or lowered level of consciousness.

Vital sign data can thus be used for clinical decision making in emergency care and for surveillance of hospitalized patients that may be deteriorating. In Sweden, the Rapid Emergency Triage and Treatment System, RETTS, is commonly used in the prehospital setting and in many EDs [67]. For surveillance purposes in the wards, NEWS, National Early Warning Score, is frequently used [68]. The general idea is that the more deviating the vital signs, the more serious the condition. This information can be linked to alarm systems to call for extra help if a patient is deteriorating. There are several other systems used, but few have been evaluated specifically in their ability to identify patients with sepsis. One study by Bayer [69] found the Modified Early Warnings Signs, MEWS, score $\geq$4 to have a positive predictive value of 0.74 for sepsis. (MEWS is very similar to NEWS).

### 1.8.2 Vital signs in early identification of patients with sepsis

Fever, an elevated temperature, is the hallmark of infection, and often the key to suspecting that symptoms and changes in a patient's vital signs are caused by an infection. Fever is believed to be part of our adaptive response to an infection. With increasing temperature, the multiplication rate of many bacteria, virus, and fungi decreases and many functions of the immune system are activated, such as migration of neutrophils, T-cell proliferation and the production of interferon and other cytokines [70]. Yet, far from all patients with sepsis or severe sepsis have fever in the ED. In a Swedish study on patients with suspected sepsis by Gille-Johnson, only 65% had a temperature of >38°C or <36°C on arrival [44].

In a pre-hospital setting, Bayer [69] validated vital signs in their ability to differentiate patients with sepsis of any severity from patients with non-sepsis. They found that each of temperature >38°C or <36°C, heart rate >90/min, respiratory rate >22/min, saturation <90%, and systolic blood pressure >90 mm Hg, significantly discriminated those with sepsis from those with non-septic conditions. Though the study design is commendable, it suffers from a large number of dropouts due to missing data, and does not stratify patients according to age group.

To the clinical doctor, it is of prime interest to identify patients with infections in need of antibiotic treatment. One study aiming at this by Gille-Johnson [71] found the maximum respiratory rate within the first 4 hours after arrival in hospital to be the only independent vital sign for this purpose. The median respiratory rate for patients with severe sepsis, bacteremia, or infection in need of antibiotic treatment, was $\geq$24/min. For neither heart rate nor temperature was there such a correlation.

Apart from using single vital signs, there are composite algorithms for sepsis identification, based on several vital signs and often some laboratory parameter. The SIRS criteria, is one such algorithm, based on the presence of $\geq$2 of temperature >38 or <36°C, respiratory rate >20/minute, heart rate >90/min or leukocyte count >12 or <4 x $10^9$/L. SIRS has been criticized for being too insensitive and too unspecific depending on population studied, especially patients in the ICU [72]. Yet, in the prehospital setting or in the EDs, SIRS may still be a useful tool for identifying patients with an infection that might be at risk for developing organ dysfunction [4].

Another composite algorithm for early sepsis identification is the modified Robson screening tool. This is based on a medical history indicating infection, plus ≥2/5 criteria similar to the SIRS criteria, adding acute alteration of mental state and substituting leukocyte count for blood glucose >6.6 mmol/L in the absence of diabetes [73]. Evaluations in pre-hospital use have found a high sensitivity for sepsis but a low specificity [69]. It is also considered somewhat complicated to use [74].

A third composite algorithm for early detection of patients with sepsis is the PRESEP score suggested by Bayer [69]. This is a score where each parameter has been validated and assigned certain weight: temperature >38ºC = 4 points, <36ºC = 1 point, heart rate >90/min = 2 points, respiratory rate >22/min = 1 point, saturation <90% = 2 points and systolic blood pressure <90 mm Hg = 2 points. If a patient has ≥4 points in the PRESEP score, the sensitivity and specificity for sepsis is 0.85 and 0.86 respectively [69] and thus performed better than both MEWS and BAS 90-30-90.

BAS 90-30-90 is a local algorithm used in our own hospital, aiming at identifying patients with organ dysfunction. Each parameter in BAS 90-30-90 targets organ dysfunction, not early changes in vital signs due an infection. The idea is that if a patient has a systolic blood pressure of ≤90 mm Hg, *or* a respiratory rate of ≥30/minute *or* ≤90% saturation by pulse oximeter, the patient should be evaluated also for severe sepsis, regardless of temperature or whatever other diagnosis is suspected. The rationale is that hypotension is a serious sign, an elevated respiratory rate is an early sign and respiratory dysfunction is common in severe sepsis. In one study evaluating methods for sepsis identification by the emergency medical services, BAS 90-30-90 was found to identify 70.4% of the patients with severe sepsis compared to 16% clinically suspected by the ambulance nurse [74]. Another study found BAS 90-30-90 to identify 62% of patients with sepsis of any severity [69].

### 1.8.3 Vital signs in identification of patients with sepsis at risk of poor outcome

Many vital signs are part of the criteria for organ dysfunction and can be used for identifying patients in the ED at risk of poor outcome, mostly in-hospital mortality. Not surprisingly, patients with an infection and persisting hypotension despite adequate fluid resuscitation, septic shock, have the highest risk of dying in hospital [34]. Even single organ dysfunction increases the risk of in-hospital case fatality, a risk that increases with the number of dysfunctioning organ systems [20, 34].

Temperature is a vital sign that is not a criterion for organ dysfunction. A recent Swedish study on temperature at baseline in patients with severe sepsis treated in the ICU, showed a linear inverse relation of temperature to in-hospital case fatality rate (CFR) [75]. Patients with temperature <35° C had an in-hospital CFR of 50%, whereas those with temperature >40° C had an CFR of only 14%. A similar relationship to temperature has also been shown for patients with bacteremia [36].

Vital signs can also be used in algorithms for predicting in-hospital mortality or poor outcome. One such algorithm including vital signs is the mortality in emergency department sepsis, MEDS, score [76]. Of vital signs, respiratory rate >20/min, saturation <90 %, persisting hypotension (septic shock), and altered mental status were independent predictors contributing to the score.

The most recent predictive scoring system is the qSOFA, quick Sequential (sepsis-related) Organ Failure Assessment, score. qSOFA is based on systolic blood pressure ≤100 mm Hg, respiratory rate ≥22/min or altered mentation. The score is positive if two out of the three criteria are fulfilled[77]. A positive qSOFA was found in a large derivation and validation study to predict 81% of patients with "poor outcome", defined as in-hospital mortality or ≥3 days in the ICU. qSOFA has been suggested to be used outside the ICU for identifying patients at risk that should be assessed for possible sepsis [77]. The usefulness of qSOFA has been debated, since not all later studies have been able to show the same good predictive ability. A Swedish study by Mellhammar [19] found a sensitivity of 55% for predicting severe sepsis and 42% for predicting Sepsis-3. An Australian study by Williams [35] found a sensitivity of only 29%.

## 1.9 Biomarkers in sepsis and severe sepsis

### 1.9.1 General comment

The perfect biomarker for identifying patients with sepsis does not exist. For commonly used biomarkers in the clinic, sensitivity and specificity at optimal cut-offs rarely exceed 70% [78]. Taking into account the multitude of infectious agents with varying pathogenicity that can cause sepsis in patients with different age, sex, ethnicity, genetic set-up, co-morbidities, medication and patient delay, this is not surprising [79]. More than 180 distinct molecules have been assessed for potential use in sepsis [80, 81], but few are in clinical use. The majority of these biomarkers have been evaluated as prognostic markers; and only ten as diagnostic markers [81]. However, combinations of biomarkers may improve sensitivity and specificity for sepsis identification [82-84].

Whichever biomarkers are available in the clinic, it is important for the clinician to know the strengths and weaknesses of the biomarkers used, not least the kinetics. Timing of sampling is an important factor. Like a football match, sepsis is a highly dynamic process where changes can occur rapidly, but, unlike vital signs, biomarkers are not always tested for when the patient is the most sick.

### 1.9.2 Blood cells in sepsis and severe sepsis

Sepsis and severe sepsis leads to changes in peripheral blood cell counts and distribution, which is often used by clinicians for infection- and sepsis diagnosis, mainly white blood cells, but also platelets. In severe sepsis, hemoglobin levels go down due to red cell destruction [85], leukocytes increase, mainly because neutrophils are released from the bone marrow, and platelets decrease because of consumption. There are many studies reporting significant changes in size [86, 87], distribution [88] and ratios [89] of different cells in sepsis patients. The most commonly used in the clinic are discussed below.

More important than changes in numbers or size of various blood cells, is that sepsis or severe sepsis has profound negative effects on the function of almost all white blood cells [90], not measured in the lab, but affecting the course and outcome in sepsis patients.

### 1.9.3 Leukocytes, neutrophils and lymphocytes

Leukocytes are white blood cells engaged in the immune system. In peripheral blood more than 100 different populations can be distinguished by molecular markers [91]. The most commonly found leukocyte is the polymorph nuclear neutrophil, PMN, or simply “neutrophil”. The neutrophil count in peripheral blood can increase as the result of any acute inflammatory process, so it is not specific for bacterial infections. Yet, leukocytosis or neutrophilia is often used for diagnosing bacterial infections. Neutrophils adhering to the vascular cell walls are rapidly mobilized on stimulation by an infectious agent and more neutrophils are then recruited from the bone marrow. In experimental inflammation, this is seen within 1-2 hours after injecting lipopolysaccharides subcutaneously in healthy volunteers [92], and maximum levels are seen within 4-6 hours. However, in most studies, not more than half the patients with bacterial severe sepsis have leukocytosis or neutrophilia. Leukopenia or neutropenia occurs in a few percent of those patients and is generally regarded as a less favorable prognostic sign.

As neutrophil counts often increase in inflammatory events, lymphocyte counts decrease equally rapid [92], and this decrease is generally more pronounced in severe sepsis. In sepsis patients this occurs among lymphocytes in all tissues, but in non-infectious inflammation only in peripheral blood [93]. The decrease in peripheral blood is due to both re-distribution of lymphocytes back to the tissues, but even more to induced apoptosis, which eventually leads to immune suppression and increased risk of secondary infections with less pathogenic bacteria [93, 94]. In the individual septic patient, the degree of lymphocytopenia is directly related to the intensity of the infection and to outcome, especially if not normalized in 4-6 days [95]. The lymphocytopenia in septic shock may even be a main component of sepsis induced immune dysfunction [95].

### 1.9.4 The neutrophil to lymphocyte count ratio

The fact that neutrophils rapidly increase in bacterial infections and that lymphocytes rapidly decrease [92], can be used in a ratio, the neutrophil to lymphocyte count ratio (NLCR) as a measure of acute systemic inflammation. This was first described by Zahorec in 2001 [96]. In 2010, deJaeger found the NLCR to be useful for identifying patients with bacteremia [89] and later also Lowsby [97]. The NLCR was shown by deJaeger to identify patients with severe disease and risk of poor outcome in community acquired pneumonia, CAP [98]. Recently, Naess found the NLCR to be a diagnostic tool, not only for patients having bacteremia, but also having bacterial infection [99].

Though useful for detecting patients with bacteremia and severe pneumonia, the NLCR has limitations. Sensitivity for bacteremia is at best 70% at a cut-off of >10 [89]. In my own experience, the NLCR is not specific for bacterial infections. Values >10 can be seen also in viral infections, such as influenza or norovirus infections. Probably, the NLCR reaches the highest levels when the inflammatory response is the most intense, mainly in the early phase of an infection, and then gradually returns to normal as the inflammatory reaction subsides. This was described by Naess [99], who found lower levels of the NLCR in patients with fever for >1 week compared to <1 week. This is in accordance with our clinical experience.

What about patients without infection? In a master thesis in medicine at the University of Gothenburg, Landgren, investigated the NLCR in almost 900 patients in the medical ED, and found a median value of 4. Very few patients with chest pain, angina pectoris, acute myocardial infarction, pulmonary embolism or congestive heart failure had a NLCR >10 (unpublished data).

In clinical studies, lymphocytopenia performs even slightly better than the NLCR in identifying patients with bacteremia [89, 100], but maybe a high value (NLCR) is more didactic than a low value for lymphocyte counts. However, using both parameter can be of even greater help than only evaluating only one of these.

### 1.9.5 Platelets (thrombocytes)

"Platelets are small circulating anucleate cells that are of crucial importance in haemostasis. Over the last decade, it has become increasingly clear that platelets play an important role in inflammation and can influence both innate and adaptive immunity. Dysbalanced immune response and activation of the coagulation system during sepsis are fundamental events leading to sepsis complications and organ failure. Platelets, being major effector cells in both haemostasis and inflammation, are involved in sepsis pathogenesis and contribute to sepsis complications. Platelets catalyse the development of hyperinflammation, disseminated intravascular coagulation and microthrombosis, and subsequently contribute to multiple organ failure. Inappropriate accumulation and activity of platelets are key events in the development of sepsis-related complications such as acute lung injury and acute kidney injury. Platelet activation readouts could serve as biomarkers for early sepsis recognition; inhibition of platelets in septic patients seems like an important target for immune-modulating therapy and appears promising based on animal models and retrospective human studies"[101].

In clinical practice, thrombocytopenia occurs as an effect of an activated coagulation in sepsis, and is usually seen a few days after onset of a severe infection. However, in certain infections, as meningococcemia, thrombocytopenia may be present within few hours after start of symptoms. A thrombocyte level of $<100 \times 10^6$/L caused by an infection is one of the criteria for organ dysfunction in both severe sepsis and Sepsis-3.

### 1.9.6 Coagulation

Coagulation is an intrinsic part of our local defense mechanisms against infections that interacts closely with platelets. In sepsis, there is almost invariably a systemic activation of the coagulation system. Normally this is not harmful, but may in severe cases lead to intravascular coagulation, causing microthrombosis in all major organs. This contributes to organ dysfunction and circulatory disturbances often seen in severe sepsis. If pronounced, these changes may lead to disseminated intravascular coagulation, DIC [102], which can cause both thrombosis and bleedings and is associated with increased

CFRs. Currently, the best treatment for DIC in patients with sepsis is treating the infection, but in a near future there may be specific treatment options [103].

### 1.9.7 C-reactive protein

The C-reactive protein, CRP, is an acute phase reactant originating from the liver. The CRP binds to the surface of bacteria and injured cells, facilitating phagocytosis by macrophages. The production of CRP is stimulated by cytokines, mainly interleukin-6, secreted by macrophages and T-cells, which can be activated by a wide range of inflammatory conditions such as infections, inflammatory diseases, malignancies and traumatic tissue injury. Thus, it is not specific for infection, but studies have shown CRP production to be more pronounced in bacterial infections than in other inflammatory conditions [104]. CRP is a rather slow biomarker. After activation by a bacterial infection, it takes 6-8 hours before CRP can be measured in plasma and 24-48 hours before maximum concentrations are reached [92]. Therefore, in patients arriving in hospital few hours after onset of suspected sepsis, CRP is of little use. The CRP-level has not been shown to have any prognostic value.

### 1.9.8 Procalcitonin

Procalcitonin (PCT), is a marker of infection and sepsis described in 1993 [105]. PCT is a peptide and a precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid and involved in calcium homeostasis. In acute inflammatory disorders, PCT production increases rapidly. Elevated plasma levels can be detected within 2-4 hours after an insult, reaching peak values within 12-24 hours [106]. It thus reacts much faster than the CRP but slower than the NLCR.

In septic patients, PCT is “regarded as a helpful biomarker for early diagnosis in critically ill patients, though the results need to be interpreted in the context of medical history, physical examination, and microbiological assessment” [107, 108]. PCT has performed well in detecting bacteremia [109-111], and has also been used to guide time for antibiotic treatment in the ICU [112, 113] and for prognosis [114].

### 1.9.9 Lactate

Lactate may be highly elevated within few hours after disease onset, but the elevation may in many other patients occur at a later stage of the disease. Until a few years ago, increased lactate in sepsis was thought to be an effect of hypoperfusion of tissues, leading to diminished oxygen delivery, anaerobic glycolysis and hyperlactatemia. Today we know that hyperlactatemia is also

an effect of metabolic dysregulation in sepsis and septic shock. Regardless of its origin, hyperlactatemia in sepsis is significantly associated with increased CFRs. Conversely, a rapid reduction in lactate level is significantly associated with improved survival rates in sepsis and septic shock [115-117]. Increased lactate level is one of the severe sepsis criteria, but not one of the criteria for sepsis according to the new Sepsis-3 definition. Despite this, lactate can still be, and should be, used as a marker of disease severity in sepsis [4]. And, lactate is part of the new Sepsis-3 definition of septic shock, which is now defined as a plasma lactate >2 mmol/L plus need of vasopressor to maintain a mean arterial blood pressure >65 mm Hg [6].

## 1.10 Etiology of infection

In order to give sepsis patients appropriate antimicrobial treatment, it is of utmost importance to identify the microorganism(s) causing the infection. This may be done in various ways, most commonly by obtaining cultures from the suspected focus of the infection, preferably before start of antibiotic treatment. In this study, cultures were performed in the laboratory according to accredited methods. Today, molecular techniques for more rapid identification of microorganisms are being developed, especially techniques based on detecting DNA or RNA of microorganisms. Only methods of special relevance for this study are commented on in this section.

### 1.10.1 Blood culture

Blood culture is still "gold standard" for detecting bacteria in the blood, though molecular methods are under rapid development and might in the near future change this view. A significant finding in blood culture carries much useful information for the clinician. It not only reveals the pathogen responsible for the disease. The pathogen detected gives a clue to what might be the focus of the infection, which in turn directs further investigations and procedures. It affects the mode and length of antibiotic treatment, and provides an antibiogram so that an appropriate antibiotic treatment can be selected. During the past ten years, it has been more and more common in Swedish hospitals to draw blood cultures before initiating intravenous antibiotic treatment. In our hospital, this has been compulsory since 2011.

Detection of bacteria in blood culture is dependent on the volume of blood used. Today, 40 ml from two puncture sites, divided into 4 different bottles, is the recommended volume. Lesser volumes significantly decrease the rate of positive cultures. Theoretical calculations favor the use of 80 ml blood for optimal yield [118].

### 1.10.2 Nasopharyngeal culture

Though there are more sophisticated diagnostic methods on respiratory specimen, culture from the nasopharynx is a method easy to perform that can be used in clinical routine. However, nasopharyngeal culture is generally not regarded as proof of etiology in community acquired pneumonia in adults [49]. Many accept only detection of pathogens from other compartments, such as blood, sputum, trachea (trans-tracheal aspirate), the bronchial tree (bronchoalveolar lavage), thorax (pleural effusions, lung biopsies), urine or serology. In Swedish tradition, identification of *S. pneumoniae* in nasopharyngeal culture in adults, is regarded as being a rather specific, though not very sensitive method for possible etiologic diagnosis of pneumococcal pneumonia.

### 1.10.3 Nucleic acid-based testing

Nucleic acid-based amplification techniques, NAATs, for detecting bacteria and viruses in patient samples is an evolving diagnostic field with many benefits, but also pitfalls. Among advantages are high analytic sensitivity and high specificity for the organism aimed at. Other advantages are short turnaround time, being faster than culture, and that the analyses can be automated and performed in a closed system with no need for a microbiology laboratory. Thus, they can be used, and are used, in low-resource settings, in some cases with revolutionary results. Yet another advantage is the ability to detect microorganisms that are not easily cultured or slow growing. One good example is the GeneXpert, used for detection of *Mycobacterium tuberculosis* and rifampicin resistance in sputum samples [119]. A complete analysis takes a few hours compared to many weeks for culture. NAATs may also be combined with other techniques, increasing the detection rate even more. Among disadvantages are the question of clinical significance, lack of antibiograms, costs, and that the technique only detects the organisms it is designed for.

One difficulty in using NAATs to investigate blood lies in detecting the minute amounts of bacterial DNA among the vast amounts of human DNA. Therefore, many NAATs, like PCR or microarray, have been developed for diagnosing bacteria in blood cultures that have already turned positive. Other techniques are being developed for this purpose, for example mass spectrometry of bottles that have signaled positive, Maldi-TOF®. If automated, these methods may save some time, but compared to today's routine in the labs, the gain is minimal. Better if methods could identify bacteria directly in whole blood, without the need of culture.

The first such commercial test was Septifast® by Roche in 2006, a multiplex-PCR method which detects the eight most commonly found gram-negative bacteria, the six most common gram-positive bacteria, the five most common Candida species plus Aspergillus. In a review article by Pasqualini [120], the Septifast® was compared to blood culture results as gold standard, and was found to have 68% sensitivity and 85% specificity. The conclusion was that it was difficult to make firm recommendations about the clinical utility of the method. Since then, similar tests have appeared on the market, but have not yet received broad acceptance among clinical doctors.

In several studies the findings of bacterial DNA in blood have exceeded the findings by blood culture. This has led to the invention of the term "DNAemia" [121], suggested to be a significant finding, since these patients have more severe disease and more pronounced inflammatory markers than those who are DNA-negative. Another explanation may be that in patients with an acute inflammatory reaction, bacterial DNA may more easily reach the blood stream through leakage from mucosal membranes. At any rate, the concept of "DNAemia" has not gained recognition and is not used in clinical practice.

For respiratory tract infections, multiplex PCR on respiratory specimens have greatly improved detection rates, not least of viral infections and difficult-to-culture bacteria such as *Mycoplasma pneumoniae, Bordetella pertussis* and *Chlamydophila pneumophila*. Nasopharyngeal specimens are in daily practice easily obtained using a swab and universal transport medium, though throat swabs, nasopharyngeal aspirates, sputum, trans-tracheal aspirates or bronchoalveolar lavage may result in higher yields and higher specificity [122, 123]. Methodological obstacles lie in the questions of sensitivity and specificity – are all relevant viruses detected by this method? Which is the analytical sensitivity of the method in detecting the viruses included in the test? Are the viruses detected proof of an acute or ongoing infection? For improving clinical sensitivity and specificity, serology has been shown to add diagnostic value to NAATs in diagnosing several different respiratory viral infections [124].

# 2 AIMS

The epidemiology of sepsis is important for health care planning and resource allocation in order to provide the best possible medical care for the population. Since code based estimations are inferior, we wanted to perform a prospective study using "gold standard" methodology, evaluating patients individually per protocol. The manageable size of the population of Skaraborg and the health care infrastructure in Sweden provided excellent prerequisites for performing such a study.

The overall aims were

- To investigate the incidence and epidemiology of community onset severe sepsis among adults in the former county of Skaraborg in western Sweden. (Study I).
- To investigate factors affecting outcome in community onset severe sepsis in adults in this study population (Study I).
- To evaluate the performance of the 2011 Swedish consensus criteria for severe sepsis (Study 1).
- To evaluate the new Sepsis-3 definition and criteria launched in February 2016 (Study I).
- To investigate the clinical value of a nucleic acid amplification test in early detection of bacteria in whole blood on part of the study population (Study II).
- To investigate the clinical value of nucleic acid amplification test in detection of respiratory viruses in nasopharyngeal samples on part of the study population (Study III).
- To evaluate the clinical performance of commonly used biomarkers for sepsis identification (Study IV).
- To evaluate the use of six defined systemic symptoms in early identification of severe sepsis in the emergency department. (Study V).

# 3 PATIENTS AND METHODS

## 3.1 PATIENTS

### 3.1.1 Studies I-IV.

The epidemiological study was conducted in the former county of Skaraborg, since 1998 part of Region Västra Götaland in western Sweden. The region has a population of about 1.6 million, the main city being Gothenburg. Skaraborg is a rural area, with a population of 256,700 by Jan 1 2012, 206,900 being adults. Skaraborg has one public secondary care hospital in two locations, Lidköping and Skövde. In Sweden, there is open access to all hospitals. For admitted patients, medical care is free of charge, apart from a small daily administrative fee.

Skaraborg hospital has approximately 640 beds, 60,000 annual visits to the emergency department (ED), and 24,000 admissions. During the study period, one electronic patient record, Melior (Siemens), was used throughout the hospital. Unilabs, an accredited private laboratory, served the hospital with laboratory diagnostics in both clinical chemistry and clinical microbiology. Unilabs performed all routine laboratory analyses on patient samples included in this study. The Emergency Medical Services, EMS, is public and the same throughout Skaraborg. The EMS used a separate electronic patient record, AmbuLink, for medical history and vital signs, available through the hospital electronic patient record. The EMS and the ED used the same triage system, Rapid Emergency Triage and Treatment System, (RETTS) [67], which included documentation of vital signs. Both electronic patient records were used for retrieving information on patient medical history, vital signs, results of biochemistry, cultures, and imaging, in order to assess the presence or development of severe sepsis or septic shock.

During a 9 month period, September 8, 2011 – June 7, 2012, all adult permanent residents of Skaraborg, admitted to the hospital and within 48 hours started on intravenous antibiotic treatment on clinical suspicion of a bacterial infection, were evaluated for presence or development of severe sepsis or septic shock during the first 48 hours. (Study I). All admissions were evaluated, but incidence and 28-day case fatality rate (CFR), were calculated using the first episode for each patient only. Exclusion criteria were non-residents of Skaraborg, re-admissions within 30 days for the same infection, patients with post-operative infections within 30 days of surgery and patients who never received intravenous antibiotics even though first intended. *Figure 2.*

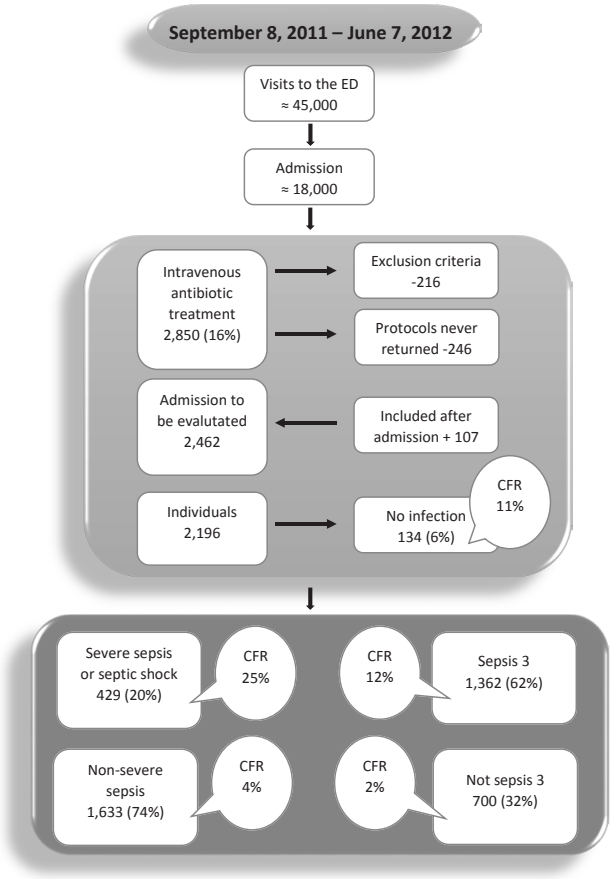


*Figure 2. Patient selection to the study on severe sepsis as well as distribution of patients having severe sepsis or Sepsis-3. 28-day CFRs are included. CFR, Case fatality rate.*

Study II–V were performed during parts of Study I. ***Table 3***.

***Table 3.*** *Time periods and locations of the five studies in this thesis.*

| September 8, 2011 – June 7, 2012 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sept 8, 2011** | **Oct 2011** | **Nov 2011** | **Dec 2011** | **Jan 2012** | **Feb 2012** | **Mar 2012** | **Apr 2012** | **May 2012** | **June 7, 2012** |
| *Skaraborg Hospital, Lidköping and Skövde* | | | | | | | | | |
| Study I – Epidemiology and outcome n=2,196 individuals, 2,462 episodes | | | | | | | | | |
| | | | | | | Study II – Multiplex PCR on whole blood | n=383 | | |
| | | | | | Study III – Respiratory viral infections | | n=432 | | |
| Study IV – Biomarkers n=1,572 episodes | | | | | | | | | |
| *Södra Älvsborg Hospital Borås* | | | | | | | | | |
| | | | | | | Study V – Symptoms of sepsis | n=289 | | |

### 3.1.2 Study V.

The study on symptoms was performed in another population at Södra Älvsborg Hospital in Borås, with a catchment area of 190,000 inhabitants. The hospital in Borås is also a public secondary care hospital, similar in size to the hospital in Skövde, and part of the same region, Region Västra Götaland. The study was conducted during the month of March in 2012 on all patients having received intravenous antibiotic treatment for a community onset infection.

## 3.2 Methods

When infection needing intravenous antibiotic treatment was suspected on admission or within the first 48 hours, the patient and/or a close relative received oral and written information about the study by the attending nurse. Routine biochemistry taken on arrival consisted of a full hemogram, blood neutrophils and lymphocytes, the neutrophil to lymphocyte count ratio (NLCR), electrolytes, creatinine, liver enzymes, prothrombin complex, and a venous blood gas with lactate. The initial sampling included 1.5 ml plasma and 3 ml whole blood. If patients or a close relative within 3 days after admission gave a written consent to participate in the study, the plasma and whole blood was stored at -80$^{o}$ C for later analyses of sepsis biomarkers. Apart from blood cultures, all other cultures were performed at the discretion of the attending physician. Urine culture was performed in most patients. In patients with respiratory symptoms or sepsis with unknown focus, culture from the nasopharynx was desired, as was culture from wounds if present.

For diagnosis of hypoperfusion in severe sepsis, venous lactate was used on arrival for routine screening of all patients with suspected sepsis. In many severely ill patients, further saturation measurements were preferrably assessed in arterial blood. A lactate value >1 mmol/L above the upper reference limit in venous blood, in our lab 0.9-2.5 mmol/l, was used as a criterion for hypoperfusion. For respiratory dysfunction, saturation measurements of oxygen saturation ($SpO_2$) were routinely assessed using pulse oximetry. The correlation to saturation assessed in arterial blood is debated, different studies showing different results, but in patients with severe sepsis there seems to be a tendency for pulse oximetry to overestimate the saturation in arterial blood ($SaO_2$) [125]. In patients receiving supplementary oxygen, saturation values by pulse oximetry were corrected for using the $FiO_2$ values for supplementary oxygen found in the Swedish Intensive Care Register (SIR). For cerebral dysfunction, the Reaction Level Scale (RLS) was used instead of the Glasgow Coma Scale (GCS), since RLS is the method used by the EMS and in the triage in the ED in our hospital.

During the study period, the laboratory delivered two lists on a daily basis; one list of all patients who had study samples taken during the past 24 hours and one list of all blood cultures drawn within the past 24 hours. On weekdays, a study nurse would take the lists and visit the patients in the wards to repeat information about the study, answer questions about the study if any, and to collect written consent. After 3 days in the ward or more, the study protocols were returned by the internal mail to the Infectious Disease Clinic for evaluation by either of the study doctors LL or GJ. Patients who had not consented to participate in the study were evaluated anonymously for epidemiological data only. Severe sepsis or septic shock was diagnosed using the 2011 Swedish consensus definition and criteria for severe sepsis and septic shock. The protocols were entered into an IBM SPSS database version 22.0 (Inc, Chicago, IL) by a secretary at the Skaraborg Hospital Research and Development Center. Later, after the launch in February 2016 of the new Sepsis-3 definition and criteria of sepsis, the study cohort was evaluated also according to Sepsis-3[4].

We thus chose to include patients admitted and within 48 hours started on intravenous antibiotic treatment, according to the definition of community onset infection. Since inclusion depended on the attending nurse, some patients may have been missed to be included. To analyze the rate of patients missed, we analyzed a list of patients during the study period who had a significant finding in blood culture. That way we found 30 patients who had been missed to be included. For the month of March 2012 we obtained a list from the EHR of patients who had received intravenous antibiotic treatment within 48 hours of admission. Comparison with study patients revealed that 23/343 (7%) of admissions had been missed to be included in the study.

This study method, chart-based or protocol-based, is considered “gold standard” but is very labor intense and suited only for a smaller hospital where almost all patients can be surveilled. Through the Swedish unique identification number, every study patient could easily be retrieved in the EMS- and hospital EHR. That way all vital signs and laboratory values could be accessed, and no patients were lost to follow-up.

Today, there is an EHR-linked, online-based, hospital developed, program, “the infection tool” where all antibiotic prescriptions in hospitals are compulsory registered. This allows for rapid access to all patients who have received any antibiotic treatment and for whatever reason. The “infection tool” was used in the study by Mellhammar [19], though in a point prevalence fashion. This method is probably well suited for future studies on sepsis epidemiology, and can also include hospital acquired infections.

Did we miss other patients? Obviously we could have missed patients with community onset sepsis who were diagnosed more than 48 hours after admission, but we believe those were few. If missed altogether, they would probably not survive a severe infection. One way of identifying such patients could be to review the EHRs of all patients who died during the stay in hospital. Another group of patients probably missed were those with acute infections who never sought medical care or patients in nursing homes who were never referred to hospital because of some concomitant terminal illness. There were most certainly such patients, but the extent was difficult to determine, since they were not subject to diagnostic efforts. Thus, our results should be regarded as a minimum.

Did we diagnose correctly? In the Swedish definition, "acute alteration of mental status" is one criterion of severe sepsis. We used RLS $\geq$2, since this is the scale used in our triage system RETTS. That way we probably missed some patients, since not all who had an acutely altered mental status were considered to be RLS 2. This is a question of training, since most personnel in the EDs are not trained to either ask for or to document "acute change of mental status". How accurately does oxygen saturation by pulse oximetry reflect the oxygen concentration in blood? In a Canadian study on patients with severe sepsis, pulse oximetry was found to overestimate the arterial oxygen saturation by 2.75%, even more in the presence of hypoxemia [125]. How accurate is the estimation of the inhaled fraction of oxygen in patients receiving supplementary oxygen treatment? How much can venous lactate measurements be trusted compared to arterial measurements? Does medication, like metformin, affect the lactate levels in patients with diabetes mellitus? Methodological problems like those mentioned may well have affected the outcome of this study, but are difficult to answer. Nevertheless, the results obtained with the method used did separate patients with a high CFR from those with a low and therefore seems useful in a Swedish setting.

Did we miss any risk factor? Chronic alcohol abuse is a known but probably underestimated risk factor for acquiring infections such as pneumonia and bacteremia [30]. Since information on a patient's alcohol consumption is often lacking in our EHRs and since biomarkers for alcohol consumption is not routinely measured, we could not evaluate alcohol as a risk factor in our study. This, however, was done in a Danish study by Henriksen [18], who found that 10% of cases of severe sepsis and 20% of cases of septic shock were alcohol related.

The study on symptoms, Paper V, was performed in Borås (see 3.1.2). Patients were identified using Sjukhusets Antibiotika- och Infektionsuppföljnings-system (SAI), Neotide, Vasa, Finland. SAI is a Windows-based on-line tool

for hospital antibiotic use and infection control that is linked to the electronic healthcare record and automatically registers all antibiotic prescriptions. Using chart-based method, patients identified were retrospectively evaluated for severe sepsis according to the Swedish 2011 definition and criteria.

## 3.3 Statistical analysis

Statistics used in the different studies are described in detail in the different papers. In study III on respiratory tract viral infections, only descriptive statistics were used.

## 3.4 Ethics

The study in Skaraborg on sepsis epidemiology, characteristics and outcome (papers I-IV), was approved by the regional ethical review board in Gothenburg, reference number 376/11.

The study on symptoms of sepsis conducted in Borås (study V), was approved by the regional ethical review board in Gothenburg, reference number 617-14.

# 4 RESULTS AND DISCUSSION

## 4.1 Paper I. Epidemiology and outcome of severe sepsis

Using the 2011 Swedish consensus criteria, we found an incidence of community onset severe sepsis and septic shock of 276/100,000. This is lower than in contemporary chart-based studies from Denmark and Sweden. The Danish study by Henriksen [18] used saturation <92% as criterion for severe sepsis, which probably accounts for the higher incidence in their study, 457/100,000. The Swedish study by Mellhammar [19] investigated both community onset and hospital acquired severe sepsis, and found an incidence of 687/100,000. They used a 4-day point prevalence evaluation and extrapolated the results for the year of 2015, which may account for some of the difference.

The incidence of Sepsis-3 in our study was high, 878/100,000, in the same range as reported by Mellhammar [19]. This incidence was three times as high as that of severe sepsis. The reason was mainly the high proportion of patients having ≥2 points in SOFA score for respiratory dysfunction. Still, this may have been an underestimation of the incidence, since using pulse oximetry for measuring oxygen saturation has a tendency to yield higher values than the actual saturation measured in arterial blood [125].

When comparing the two sets of criteria for organ dysfunction, we found that the Swedish 2011 criteria identified a smaller cohort with a 28-day case fatality rate (CFR) of 25%, and the Sepsis-3 criteria a more than three times as large cohort with a 12% 28-day CFR. Those who did not have severe sepsis had a 28-day CFR of only 4%. *Figure 3*. Thus the Swedish 2011 criteria were able to distinguish patients with a high 28-day CFR from those with a low, not much higher than that of the general hospital population. We retrospectively applied Sepsis-3 criteria to the study cohort of 2,196 individuals. Of the 429 patients with severe sepsis, 413 (97%) also fulfilled the Sepsis-3 criteria, as did 949 of those not having severe sepsis according to Sepsis-2. The additional 949 patients that also fulfilled the Sepsis-3 criteria had the same average age, 78 years, as those with severe sepsis, but had a 28-day CFR of only 6%, so that the 28-day CFR in the Sepsis-3 group dropped to 12%. This shift led to a drop in average age in the non-Sepsis-3 group from 67 to 60 years and to a 28-day CFR of only 2%. This should serve as a basis for debate among doctors. Do we want 2/3 of all admitted patients who receive intravenous antibiotics to be diagnosed as “sepsis-patients”?

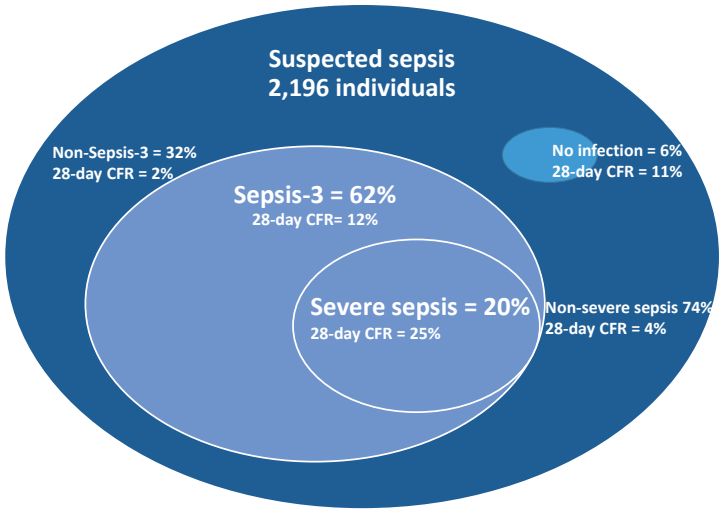


*Figure 3. Distribution of patients with severe sepsis according to the Swedish 2011 criteria and of patients with Sepsis-3 within the study population. Includes 28-day CFRs for the different groups. CFR, case fatality rate.*

In our study, the most common organ dysfunction in severe sepsis was hypoperfusion, due to increased lactate levels, in 52% of the patients. This rate is more than twice as high as in most other studies. One reason may have been the high median age of our study population, 58% being ≥75 years of age. Another may have been the high rate of venous lactate taken on arrival in the ED, 93%. However, in a recent large American study by Rhee of nearly 174,000 patients with Sepsis-3 during the years 2009-2014, the most common organ dysfunction was also an increased lactate, in 52% of the patients [17].

An interesting observation in this study was that the rate of some organ dysfunctions was constant regardless of age group, whereas respiratory dysfunction and to some extent cerebral dysfunction increased with increasing age. *Figure 4.*

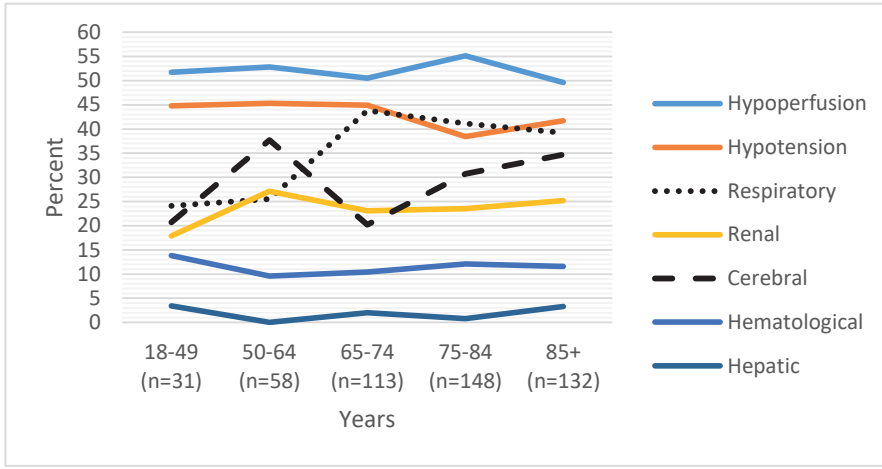


*Figure 4. Rates of organ dysfunction in 482 episodes of severe sepsis relative to age groups.*

Maybe more interesting was the influence of age, both on incidence and case fatality rates. These findings are not new (section 1.3.1 and 1.3.3), but maybe not highlighted as they should. The incidence increased about 40-fold between the youngest and the oldest age groups, for both severe sepsis and Sepsis-3. The 28-day CFR increased about 10-fold between the youngest and the oldest age groups, for both severe sepsis and Sepsis-3. Below the age of 50 years, severe sepsis was a rare event and the 28-day CFR was low. Above the age of 65 years, the incidence was 30-40% higher in men than in women, but the 28-day CFR was not higher in men. *Figure 5*. (see Supplement 1, Table, Paper I).

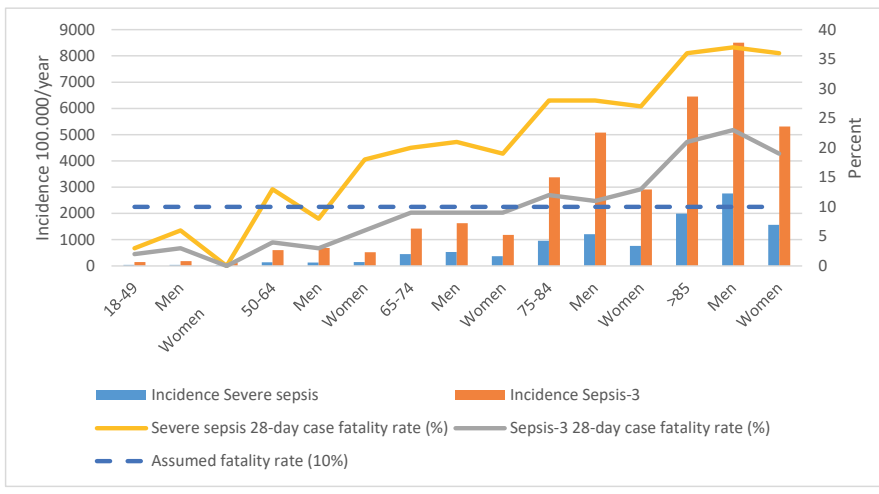


*Figure 5. Incidence and 28-day case fatality rates from severe sepsis and sepsis-3 in men and women in different age groups*

In sepsis studies, incidence and case fatality rates are often presented for whole populations. However, the age distribution should always be accounted for, since it highly affects both parameters.

The average co-morbidities per age group were rather constant in the age groups ≥65 years, even slightly lower in the ≥85 year group. The average of organ dysfunctions in those who died was also rather constant, around 2.5 in all age groups ≥65 years. If co-morbidities do not increase with age, why does the incidence increase? Is the explanation to be found in the impaired function of the immune system that comes with age [126, 127]? Is this "a hidden co-morbidity" present in many elderly patients that we are unaware of, since it is not as easily measured as co-morbidities in other organ systems?

In the multivariate regression analysis, age ≥75 years turned out as an independent risk factor for 28-day case fatality in those having severe sepsis. If the average of organ dysfunctions does not increase with age, why does the 28-day CFR? Is this a reflection of the profound dysfunction of the immune system inflicted upon an immune system compromised by age [90]? Can we liken the ageing immune system to an ageing heart and the bout of severe sepsis to a myocardial infarction of the immune system with concomitant high CFRs, not only within 28 days, but also in many years to come?

Of vital signs, renal dysfunction, respiratory dysfunction, acute change in mental status, and temperature on arrival, were significant risk factors for 28-day case fatality in patients with severe sepsis. In a Swedish study on patients with community onset severe sepsis treated in the ICU, Sundén-Cullberg reported an inverse relationship between temperature on arrival and in-hospital case fatality rates [75]. This was found also in our population-based study. One explanation may be that hypothermia in patients with severe sepsis is an indicator of a compromised immune system that is unable to react properly to the assault of an invading pathogen [70].

The incidence of community onset bacteremia found in this study cohort 203/100,000 was higher than in another Swedish study from the same time-period by Holmberg, 156/100,000 [128]. It was high compared to other contemporary Scandinavian studies that also included hospital acquired bacteremia; Norway 223/100,000 [45], Denmark 199/100,000 (2008) [129], and Finland 167/100,000 (2007) [46]. The incidence is higher than in older studies in Europe and North America [44, 47] yet this was in community onset patients only. The most likely explanation is the high frequency of blood cultures drawn in our study, before initiating intravenous antibiotic therapy.

Bacteremia, or "blood stream infection", is often highlighted as a disease of its own with high CFRs. The overall 28-day CFR among patients with bacteremia in this study was 13%, in the same range as contemporary Scandinavian studies [45, 46]. However, we found a co-variation with severe sepsis, linking the 28-day CFR to organ dysfunction rather than to bacteremia *per se*. In fact, in the severe sepsis group the 28-day CFR was even slightly lower in those who had bacteremia compared to those without. In the group having non-severe sepsis, the 28-day CFR was also slightly lower among those with bacteremia than without. Bacteremia was more common in patients with organ dysfunction than in patients without, 25% versus 5 %, but did not increase the 28-day CFR in either group.

Further, like sepsis, bacteremia is not a disease in itself, but rather a term used for all bacteria found in blood cultures, having different pathogenicity, different concomitant focal infections, and different CFRs. We found that the 28-day CFR from *S. aureus* bacteremia was significantly higher than that from *E. coli* bacteremia, though the rate of severe sepsis among those with *S. aureus* infection was lower than among those with *E. coli* bacteremia.

This study was performed in a setting with very low frequency of resistance to intravenous antibiotics commonly used for treating septic patients. In 93% of the patients with bacteremia, the initial treatment was effective and given early after arrival in the hospital. Therefore, the CFRs can be regarded as reflecting the outcome under rather optimal conditions. The CFRs from bacteremia is probably different in countries with higher rates of antibiotic resistance or multi-drug resistance, where initial treatment may not always be appropriate.

The reason why we looked at community onset severe sepsis was mainly because we wanted a description of a population that could be extrapolated to the whole Swedish population. If we had looked at hospital-acquired sepsis or postoperative sepsis as well, it would have been valid only for the situation in a secondary care hospital.

We chose to evaluate all patients that were prescribed intravenous antibiotic treatment for a suspected bacterial infection. This method has been used in other studies as well, for example by Rhee [17] and Mellhammar [19]. This way we missed some patients, whom we found when we retrospectively compared our study patients with a list form the electronic health record of patients who had received intravenous antibiotic treatment within 48 hours of arrival. This latter method would have been a better way to find the patients we wanted to study, but such lists were not readily available and did not indicate whether the antibiotic given was for treatment or for prophylaxis.

## 4.2 Paper II. Multiplex-PCR on whole blood

In this study, we primarily evaluated the clinical significance of findings by a commercial multiplex PCR test for bacteria on whole blood, Magiplex™ Sepsis Real-Time Test. The results were compared to the results of blood culture, still regarded as "gold standard", despite known incomplete sensitivity. The laboratory work was performed by Unilabs Department of Clinical Molecular Biology. During a six week period of the epidemiological sepsis study, patient blood from 383 consecutive episodes of suspected sepsis was tested. An algorithm was developed for interpretation of the findings by either method, *Figure 6*.

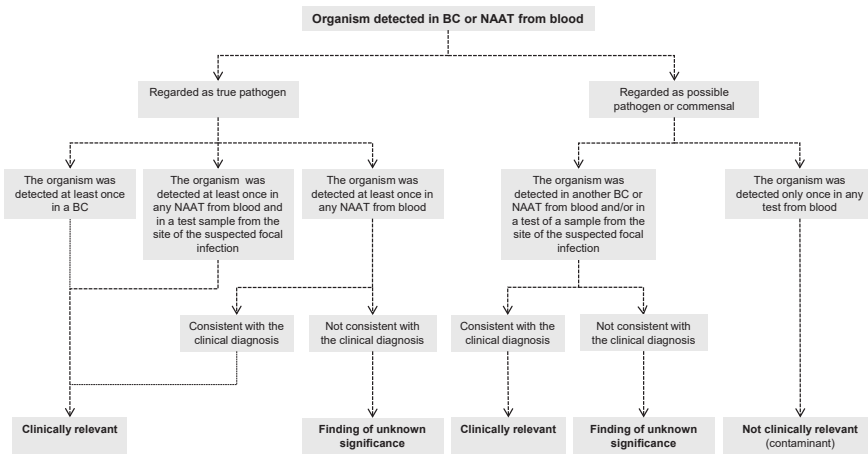


*Figure 6. Algorithm for deciding on clinical relevance of microbial findings in blood by blood culture [130] or NAAT. Other cultures were made from clinically relevant sites before administration of intravenous antibiotics. On suspicion of pneumonia or sepsis with unknown focus, a pulmonary X-ray was performed. Ultrasound, computed tomography scan, and magnetic resonance imaging were used when deemed necessary for diagnosing the site of infection. BC blood culture; NAAT nucleic acid amplification test. Adapted from [131].*

Blood culture yielded 43 clinically relevant findings. Of those, 22 were identified also by the multiplex PCR, but 21 were not. The main reason the multiplex PCR did not detect all bacteria found by blood culture may be the amount of blood used for the different methods, 1 ml for multiplex PCR and 40 ml for culture. On the other hand, the multiplex PCR identified 34 microorganisms not detected by blood culture. Of those, five were consistent with both the clinical diagnosis and bacteria cultured from the suspected site of the infection, and was therefore regarded as proven etiology. Ten were

regarded as possible etiology, since they were consistent with the clinical diagnosis, though not found in any other culture. The remaining 19 were regarded as "findings of unknown significance, since they did not correlate to the final clinical or bacterial diagnosis.

The conclusion was that the sensitivity of blood culture could be enhanced by adding the findings of a commercially available multiplex-PCR test, despite the much lower blood volume used. If the analytical sensitivity of the PCR is improved and if the DNA extraction methods are developed to allow testing on larger volumes of blood, findings by multiplex PCR will probably increase. However, since the multiplex PCR also identifies microbial agents that do not fit into the clinical picture, the results must be interpreted with caution and in light of the clinical diagnosis.

This multiplex PCR test was labor intense, but improved and automated versions are already on the market. Present drawbacks with the method are the lack of antibiotic susceptibility pattern and high costs.

## 4.3 Paper III. Respiratory viral infections

In this study, we evaluated the clinical significance of findings by two commercial multiplex PCR tests for respiratory viruses. The results were compared to the results of nasopharyngeal culture as well as to the clinical diagnosis. The laboratory work was performed by Unilabs Department of Clinical Molecular Biology.

During 13 weeks of the winter season, January – March 2012, we examined nasopharyngeal samples from 432 consecutive patients having a suspected respiratory tract infection or sepsis with unknown focus. By culture, bacteria were detected in 104 patients. By multiplex PCR, 166 viruses were detected in 158 patients and *Mycoplasma pneumoniae* was detected in another 5. In 50 patients, there was a mixed finding of both bacteria and respiratory virus. The most commonly found respiratory virus was influenza A virus (n=96), followed by human metapneumovirus (n=23), coronaviruses (n=19) and respiratory syncytial virus A and B (n=12).

We found respiratory viral infections to be more than twice as common as clinicians suspected. The clinical significance of this in individual cases is not presented in the paper, but could be described in terms of "cognitive errors". Some examples:

A middle aged man with sequels after a traumatic brain injury arrived with fever and bilateral interstitial pulmonary infiltrates on chest X-ray. CRP was moderately elevated. Initial suspicion: Pneumonia. Final diagnosis: Aspiration pneumonia, though there was no evidence of aspiration. Multiplex PCR: Bocavirus.

A woman in her forties undergoing chemotherapy for malignancy arrived one week after the latest treatment with chills and 40ºC temperature. Overt respiratory symptoms Initial suspicion: Neutropenic fever. Multiplex PCR: Influenza A virus.

An elderly woman who had two weeks previously started medical treatment against a hematological disease arrived with dyspnea, fever and bilateral interstitial pulmonary infiltrates. CRP was moderately elevated, no leukocytosis. Diagnosis: Adverse drug reaction. Multiplex PCR: Human metapneumovirus.

A man in his seventies on medical treatment for a hematologic disease arrived with fever since a few days, a mildly sore throat, and moderately elevated inflammatory parameters. The only finding was *E. coli* in the nasopharynx. Diagnosis: Pharyngitis caused by E coli. Treatment: Ciprofloxacin. Multiplex PCR: Respiratory syncytial virus.

As many as 75% of those with *S. pneumoniae* and 33% of those with *H. influenzae* in the nasopharynx having new infiltrates on chest X-ray, also had a respiratory viral infection. Co-infections are associated with more severe disease [51], and in our study, the only two patients under the age of 50 years with severe sepsis due to pneumonia needing treatment in the ICU, had bacterial-viral co-infections.

Since nasopharyngeal culture is generally regarded as a non-reliable method for etiological diagnosis of pneumonia, we had no *a priori* expectations of detecting any correlation. Therefore, it was an unexpected finding to see the seemingly convincing correlation between nasopharyngeal findings of *S. pneumoniae* or *H. influenzae* and X-ray verified pneumonia. In this cohort of patients with suspected sepsis, these bacteria were rarely found in patients not having pneumonia and not found at all in patients with no respiratory tract infection. Numbers were small and no far-reaching conclusions should be drawn, but the results do indicate an acceptable clinical value of nasopharyngeal culture for etiological diagnosis of pneumonia caused by *S. pneumoniae* and *H. influenzae*.

Asymptomatic carriage of *S. pneumoniae* in adults is uncommon. Gunnarsson [130] found *S. pneumoniae* in only 1% of Swedish adults. In a Swedish study on pneumonia etiology, Hedlund [131] found a high specificity of nasopharyngeal culture positive for *S. pneumoniae* to detection by other methods. In their study, 121 patients were diagnosed as having pneumonia caused by *S. pneumoniae* by other methods. Of those, 33 (27%) also had a positive culture of *S. pneumoniae* from the nasopharynx. If patients having been treated with antibiotics prior to culture were excluded, the diagnostic sensitivity increased to 36%. In another study on the etiology of pneumonia, Stråhlin [123] found that detection of *H. influenzae* in the nasopharynx showed good correlation to other diagnostic methods.

During the winter season, more frequent testing for respiratory viruses in patients admitted for infection with a respiratory focus or unknown focus could improve infection control measures and help doctors realize the contribution of viral infections to the many times complex and severe clinical picture. In patients arriving in hospital early in the course of the disease, antiviral treatment may also be beneficial.

## 4.4 Paper IV. Biomarkers in sepsis

This study was performed in co-operation with Unilabs and the Systems Biology Research Centre at the University of Skövde. Out of 2,196 patients evaluated in the epidemiological study, 1,637 gave a written consent in 1,887 episodes to participate in evaluation of biomarkers for sepsis. From those patients, 1.5 ml plasma had been drawn on admission, before start of intravenous antibiotic treatment, and stored at -80$^{o}$C. Plasma samples from the first 1,572 of those episodes were analyzed for procalcitonin and compared with study results on admission for C-reactive protein (CRP), the neutrophil to lymphocyte count ratio (NLCR) and lactate. Discriminant analysis was used to construct two composite biomarkers, one consisting of CRP + NLCR + lactate and the other of all four biomarkers. Results were evaluated according to the Swedish 2011 criteria (Sepsis-2), for Sepsis-3 criteria and for bacteremia.

Using the Swedish 2011 criteria for proven bacterial infections, the NLCR showed the best performance in detecting sepsis of any severity, having an area under the receiver operating curve (AUC) of 0.68 (95% CI 0.65-0.71). This was equal to the composite biomarkers but better than the other single biomarkers.

Using Sepsis-3 criteria for proven bacterial infections, procalcitonin showed the best performance in detecting sepsis. The AUC for procalcitonin was 0.68

(95% CI 0.65-0.71), which was comparable to the AUCs for the both composite biomarkers.

Using the Swedish 2011 criteria for severe sepsis, the composite biomarkers performed better than any of the single biomarkers. The three-part biomarker had an AUC of 0.85 (95% CI 0.82-0.88) and the four-part biomarker had an AUC of 0.86 (95% CI 0.83-0.89). Thus, the additional value of procalcitonin to the three-part biomarker was small. One reason the composite biomarkers performed so well was probably due to the fact that lactate is part of the diagnostic criteria for severe sepsis. Another reason is that sepsis is a complex biochemical event where combined biomarkers may improve diagnostic accuracy compared to single biomarkers.

For bacteremia, procalcitonin had the highest AUC 0.74 (95% CI 0.70-0.78), though not significantly higher than the NLCR, AUC 0.71 ($p = 0.17$; 95% CI 0.67-0.75). The three-composite biomarker had an AUC of 0.75 (95% CI 0.71-0.79). The four-composite biomarker had an AUC of 0.78 (95% CI 0.74-0.81), which was significantly better (all $p < 0.001$) than all markers but PCT ($p = 0.06$). In several previous studies, PCT has shown the best performance for bacteremia, for example in the study by Gille-Johnson [71]. This was so even in this study, though procalcitonin was not significantly better than the NLCR.

Advantages with the NLCR are that it reacts faster from disease onset than either procalcitonin or the CRP and is more sensitive and specific for acute bacterial infection than the leukocyte count. It is easily and rapidly analyzed at no or a very low extra cost from a normal blood count and is therefore suited to be part of the sepsis screening toolbox in the emergency departments.

The combined three-biomarker using CRP, NLCR and lactate, improved diagnosis of the most critically ill sepsis patients. The combined biomarkers were designed using discriminant analysis, a method that could easily be applied in the laboratory output systems and evaluated in further studies. Combinations of biomarkers, vital signs and clinical data could be used to even further improve sensitivity and specificity of a screening tool for early sepsis diagnostics.

## 4.5 Paper V. Symptoms of sepsis

Upon reviewing almost 3,000 episodes of suspected sepsis in the epidemiological study, it became obvious that there were six symptoms recurring over and over, regardless of the focus of the infection or the causing pathogen. These symptoms were characterized by sudden onset, in minutes or

a few hours, and so pronounced that they prompted the patients to seek medical care. *Figure 7.*These symptoms were: Sudden onset of

- fever and/or rigors
- dyspnea
- confusion or lowered level of conscience
- vomiting and/or diarrhea
- severe pain, related or unrelated to the focus of the infection.
- pronounced muscle weakness.

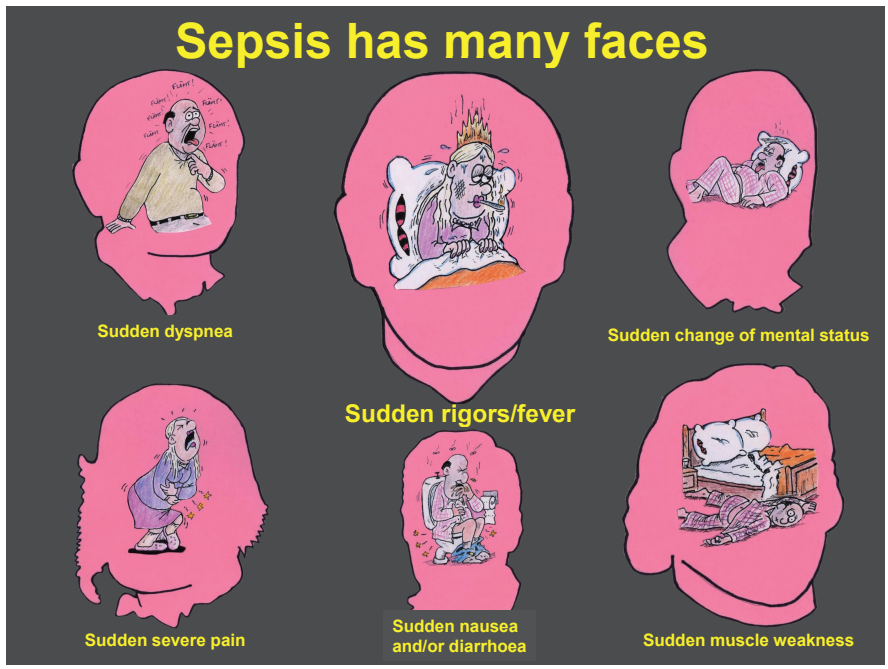


*Figure 7. Systemic symptoms seen in patients with sepsis. These symptoms vary between patients, giving rise to many different clinical pictures. Thus, "Sepsis has many faces". The silhouettes are those of historical persons who have made great contributions to our abilities to identify, prevent and treat sepsis. These are: Ignaz Semmelweis, Alexander Fleming, Louis Pasteur, Joseph Lister, Robert Koch and Edward Jennings. Illustrations by Lars Duvander.*

In a joint venture within Region Västra Götaland, these symptoms were evaluated in a study performed at Södra Älvsborg Hospital in Borås. As in the epidemiological study, all adult patients who, during the month of March 2012, had received intravenous antibiotic treatment for suspected sepsis were retrospectively examined for these symptoms.

Out of 289 consecutive patients, 90 fulfilled any of the Swedish criteria for severe sepsis. Among those, presence of ≥3 of the suggested symptoms; fever, dyspnea, altered mental status, vomiting/diarrhea, severe pain or muscle weakness, significantly correlated to presence or development of severe sepsis. This was true even when the results were corrected for altered mental status as a confounder, since altered mental status is also a criterion for severe sepsis. In patients with ≥3 of the symptoms there was also a tendency towards higher frequency of positive blood cultures and to higher in-hospital death. *Figure 8.*

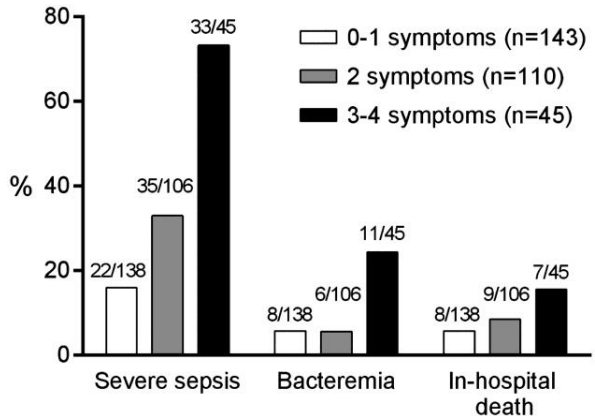


*Figure 8. Frequency of severe sepsis, bacteremia, and in-hospital death within sub-groups based on number of systemic symptoms*

Of individual symptoms, acute change of mental status and dyspnea, significantly correlated to severe sepsis. *Figure 9*.

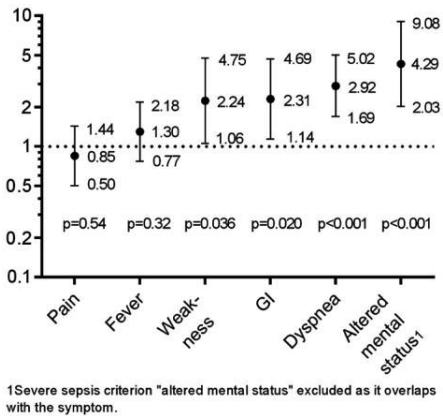


*Figure 9. Odds ratios for severe sepsis for individual symptoms with 95% CI, adjusted for age, gender and comorbidities as confounders. Weakness=muscle weakness.*

Though some symptoms of sepsis have been documented in previous studies, it was not until 2017 that there was a systematic study on the symptoms of sepsis, published by Wallgren [132]. The study was a mixed methods analysis of presentations of septic patients as documented in Emergency Medical Services (EMS) records in the Stockholm area, Sweden. The symptoms most commonly found were: fever, pain, acute alteration of mental status, weakness of the legs, breathing difficulties, loss of energy, and gastrointestinal symptoms. These are almost identical with the symptoms we found by observation in patient records and that were used in our study, thus verifying our basic presumptions.

Do symptoms of sepsis relate to disease severity? One model for understanding the symptoms of sepsis is that the more serious the disease, the more pronounced the symptoms of the dysregulated immune response than of the focal infection. In the example of the patient with erysipelas (p. 11), the patient only complained of upper abdominal pain, not of pain in the leg though that was the focus of the infection. One study supporting this theory is a French study by Denis [133], who found that respiratory symptoms were common in patients with *E. coli* pyelonephritis, and significantly more common in those having *E. coli* bacteremia than in those with no bacteremia.

Interestingly, in Borås Hospital, the initially suspected focus of infection has been registered for more than a decade in all patients receiving antibiotic treatment. The most commonly found bacteria in patients with clinically suspected community-acquired pneumonia is - *E. coli*! (A Lundquist, personal communication).

As illustrated by the example of James Hudson Taylor, symptoms alone could in 1852 be used by a doctor to both diagnose and give a prognosis of sepsis. Maybe it is time to recapture this forgotten knowledge, to use it in medical education and to integrate it into algorithms designed to identify patients with possible sepsis, not least in a pre-hospital setting.

# 5 CONCLUSIONS

Using the Swedish 2011 criteria, we found an incidence of severe sepsis, of 276/100,000 in this population based study of patients with suspected sepsis,

The incidence of Sepsis-3 was three times that of severe sepsis, 878/100,000, mainly due to a high rate of patients having respiratory dysfunction.

The incidence of bacteremia was high, 203/100,000/year, higher than in most previous studies, despite being in community onset bacteremia only. The rate of blood cultures before start of antibiotic treatment was >99%.

Risk factors for severe sepsis were age ≥75 years, cardiovascular disease, diabetes mellitus, and "other" co-morbidities.

The overall 28-day CFR in the whole study population was 8.6%. Among 429 patients with severe sepsis the 28-day CFR was 25% and in those with non-severe sepsis it was 4%. Among 1,362 patients with Sepsis-3, the 28-day CFR was 12%, and in those with non-Sepsis-3 it was 2%.

The 28-day CFR in patients with bacteremia was 13%, as in similar Scandinavian studies. However, the 28-day CFR was linked to severe sepsis or not, and was not higher than in either patients with severe sepsis or non-severe sepsis but without bacteremia.

Independent risk factors for 28-day case fatality in those with severe sepsis were; age ≥75 years, cerebral dysfunction, renal dysfunction, respiratory dysfunction, and temperature on arrival. The lower the temperature on arrival, the higher the risk.

Adding the results of commercial multiplex PCR on whole blood to the results of blood culture increased the detection rate of clinically relevant etiological findings. However, the multiplex PCR did on the one hand not identify all bacteria found by blood culture and on the other hand yielded findings that did not correlate to either the clinical or the bacteriological diagnosis.

Respiratory viral infections were more common in patients with suspected sepsis and a respiratory focus or unknown focus than clinicians were aware of. The most often found virus in patients with respiratory tract infections was influenza A, followed by metapneumovirus and respiratory syncytial virus.

For detecting severe sepsis, a combination of the commonly used biomarkers, CRP, NLCR and lactate, had the best performance. For identifying verified bacterial infections of any severity, the NLCR and procalcitonin exhibited equal performance.

Sudden onset of; fever and or rigors, dyspnea, altered mental status, vomiting/diarrhea, severe pain or severe muscle weakness, are symptoms that can be used to identify patients with suspected sepsis, especially in a pre-hospital setting.

# 6 FUTURE PERSPECTIVES

Sepsis constitutes a large proportion of hospitalized patients with high costs and high case fatality rates. Sepsis incidence, characteristics and outcomes should therefore be monitored continuously or at regular intervals in Swedish hospitals. The online-based national Swedish "infection tool", with mandatory registration of all intravenous antibiotic prescriptions, will facilitate identification of patients to be evaluated and make future studies easier to conduct. Thus, patients with hospital-acquired sepsis can also more easily be found. Electronic protocols linked to a hospital database could greatly simplify data collection and evaluation.

New techniques for rapid identification of patients with sepsis or patients with infection at risk of developing organ dysfunction are under development. In a near future we may be using molecular diagnostics and other biomarkers than today, like heparin binding protein, or micro-RNAs [134], combinations of biomarkers, or combinations of biomarkers and other markers of infection. Using microchip techniques, results may be available within few minutes and presented to the clinician already in the emergency department.

Maybe in a near future, immunotyping, as described by Kaczorowski [135], will allow for identification and continuous follow-up of patients at risk of both acquiring severe infections and for having poor outcomes?

## 6.1 Personal reflections

I view sepsis very much as a disease of age, reflecting the ageing immune system, where varying parts of our native defense systems are deteriorating at different pace in different individuals, causing imbalances within the system. One result is the increased incidence of sepsis in the elderly. The changes in symptoms, vital signs and biochemistry in the individual person may to some extent depend on this imbalance. They may also depend on pre-existing co-morbidities or simply aged organ systems with diminished abilities to withstand the assault of an infection. Sepsis in the elderly adds dysregulation of the immune system to an already impaired immune system as well as to several of our essential organ systems, resulting in increased in-hospital case fatality rates, long term disabilities and increased long term case fatality rates. Early identification and early effective treatment can halt the infection and slow down the immune reaction before organ dysfunction has become too pronounced, thereby reducing the harmful effects of sepsis. This is probably in analogy with acute myocardial infarction, which affects the ageing heart that

is predisposed to myocardial infarction, but where early intervention saves life and reduces muscle dysfunction, thereby reducing morbidity and even long term case fatality rates.

There is yet no biomarker or vital sign for a priori assessment of an ageing and dysfunctioning immune system, but maybe the lack of fever (>38ºC) in sepsis is a proxy for an immune system that is incapable of mobilizing enough defense to combat the infection?

In contrast, in younger adults, below the age of 50, without severe co-morbidities, having a healthy immune system, sepsis is a rare event. They may spend long time in hospital and may have debilitating sequelae, but they rarely die from sepsis.

For early identification of sepsis, there is no single symptom, biomarker, or vital sign that identifies all persons with sepsis. However, extreme values in known markers of sepsis should alert every doctor to include sepsis as a possible or contributing cause of these changes. Of symptoms, the more of fever, dyspnea, altered mental status, severe pain, vomiting/diarrhea, or muscle weakness, the more likely sepsis is. Of vital signs, though not specific for sepsis, low oxygen saturation, low systolic blood pressure, acutely altered mental status or level of consciousness, are indicators of organ dysfunction that demand urgent evaluation also for possible sepsis. Increased respiratory rate, >24/min or definitely >30/min, is probably the fastest and most significant alert system for possible sepsis. Low temperature, in the presence of organ dysfunction due to infection, is another sign of severe disease with high risk of case fatality. Of biomarkers, extreme levels of leukocytes, neutrophils, lymphocytes (low), thrombocytes (low), CRP, procalcitonin, or lactate should also raise the suspicion of sepsis. The more of these markers of sepsis that are present at different times during the course and the more extreme the values, the more likely the patient has sepsis or is about to develop sepsis.

Any doctor familiar with these markers of sepsis, their different presentation in different individuals and their variation over time, will by and by learn to discern the sometimes diffuse picture of sepsis, even though not all the pieces of the puzzle are at hand and not at every moment of the disease.

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# APPENDIX

**The natural history of a case of severe sepsis in 1852:**

At the age of 17, John Hudson Taylor (1832-1905) was called by God to become a missionary to inland China, in those days a country closed to foreigners. One way to be accepted in China was through becoming a doctor. In 1852 he started studying medicine at the Royal College of Surgeons in London, where he was staying with an uncle.

This recollection of the natural history of the course of a septic infection is found in the diary of John Hudson Taylor, cited in the "Biography of John Hudson Taylor" by Dr. and Mrs. Howard Taylor. China Inland Mission 1965. Pages 43-45: Published with permission of the OMF, Overseas Missionary Fellowship www.omf.org. OMF is since 1964 the successor of CIM, China Inland Mission, founded by James Hudson Taylor and his wife in 1865.

"Very soon after this, possibly the same evening, while sewing together some sheets of paper on which to take notes of lecture, I accidentally pricked the first finger of my right hand, and in a few moments forgot all about it.

The next day at the hospital I continued dissecting as before. The body was that of a person who had died of fever, and was more than usually disagreeable and dangerous. I need scarcely say that those of us who were at work upon it dissected with special care, knowing that the slightest scratch might cost our lives.

Before the morning was far advanced I began to feel weary, and while going through the surgical wards at noon was obliged to run out, being suddenly very sick – a most unusual circumstance with me, as I took but little food and nothing that could disagree with me.

After feeling faint for some time, a draught of cold water revived me and I was able to rejoin the students. I became more and more unwell, however, and during the afternoon lecture on surgery I found it impossible to hold the pencil and continue taking notes. By the time my next lecture was over, my whole arm and right side were full of pain, and I was both looking and feeling very ill.

Finding that I could not resume work, I went into the dissecting-room to bind up the portion I was engaged upon and put away my apparatus, and said to the demonstrator, who was a skillful surgeon:

‘I cannot think what has come over me’, describing the symptoms.

‘Why’, said he, ‘what has happened is clear enough. You must have cut yourself in dissecting, and this is a case of malignant fever.’

All at once it occurred to me that I had pricked my finger the night before, and I asked him if it were possible that a prick from a needle at that time could have been still unclosed. His opinion was that this was probably the cause of the trouble, and he advised me to get a hansom, drive home as fast as I could and arrange my affairs forthwith:

‘For,’ said he, ‘you are a dead man’.

My first thought was one of sorrow that I could not go to China; but very soon came the feeling, ‘Unless I am greatly mistaken, I have work to do in China and shall not die’. I was glad, however, to take the opportunity of speaking to my medical friend, who was a confirmed sceptic, of the joy that the prospect of soon being with my Master gave me, telling him at the same time that I did not think I should die, as unless I was much mistaken I had work to do in China, and if so, however severe the struggle, I must be brought through.

‘That is all well’, he answered, ‘but get a hansom and drive home as fast as you can. You have no time to lose, for you will soon be incapable of winding up your affairs’.

I smiled a little at the idea of riding home in a hansom, for by this time my means were too exhausted to allow for such a proceeding, and I set out to walk the distance if possible. Before long, however, my strength gave way and I felt it was no use to attempt to reach home by walking.

On going into the house I got some hot water from the servant, and charging her very earnestly – literally as a dying man – to accept life as the gift of God through Jesus Christ, I bathed my hand and lanced the finger, hoping to let out some of the poisoned blood. The pain was very severe. I fainted away,

and was so long unconscious that when I came to myself, I found I had been carried to bed.

My uncle sent for his own medical man, an assistant surgeon at the Westminster Hospital. When the surgeon came and learned all the particulars he said,

'Well, if you have been living moderately you may pull through, but if you have been going in for beer and that sort of thing there is no manner of chance for you'.

I thought that if sober living was to do anything, few could have a better chance.

'But now, he said, you must keep up your strength, for it will be a pretty hard struggle'. And he ordered me a bottle of port wine every day and as many chops as I could consume. I smiled inwardly, having no means for the purchase of such luxuries. This difficulty, however, was met by my kind uncle, who sent me at once all that I needed……

Days and nights of suffering passed slowly by; but at length, after several weeks, I was sufficiently restored to leave my room; and then I learned that two men, though not from the London Hospital, who had had dissection wounds at the same time as myself, had both succumbed, while I was spared in answer to prayer to work for God in China.

After some months of recovery James Hudson Taylor was able to resume his studies. He did survive and became a missionary to inland China, where he died at the age of 73 years.