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Infective Endocarditis –

aspects of pathophysiology, epidemiology, management and prognosis

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To Stina, Gustav and Viktor

Patience is the key to an acceptable score.

Free from C. Montgomerie et al.

ABSTRACT

Infective endocarditis (IE) is a rare but complex disease that is fatal if untreated. With a modern combination of antimicrobial therapy and heart valve surgery, mortality is still 10-20 %. The structure of the endocarditis vegetation impedes the penetration of phagocytic cells such as monocytes and granulocytes. This leads to high bacterial counts inside the vegetation and the need for long treatment courses with a combination of intravenously administered bactericidal antibiotics.

The aim of this thesis was to study the changes in epidemiology, management, and mortality at our hospital between 1980 and 2001, and to identify prognostic factors associated with mortality. To assess the issue of referral bias, differences between referred episodes and episodes from our local community were studied. Additional aims were to study the occurrence of the pro-chemotactic cytokines IL-8 and TNF-α in heart valves and vegetations during the active phase of IE, and to study the effect of the glycopeptide antibiotic vancomycin in dense staphylococcal cultures in vitro. As it is a rare and complex disease, management of IE is usually complicated for non-specialists. For this reason a computerised decision support system for IE was developed and evaluated.

Between 1980 and 2001, the occurrence of Staphylococcus aureus IE and the use of early heart valve surgery increased significantly, regardless of whether the episodes were referred or of local origin. Glycopeptide antibiotics, mainly vancomycin, were used more frequently, especially among referred patients. Referred patients were younger, predominantly male, had more complications, and received surgical treatment more often than patients from our local community. The reason for the lower frequency of female patients in the referral cohort cannot be explained by more comorbidity or fewer complications. The differences between referred and local episodes seen in our study highlight the need for assessment and adjustment for referral bias in IE studies (Paper I).

In six patients who needed early heart valve surgery, the largest numbers of IL-8-containing cells, and the greatest amount of inflammation, were seen in patients with short preoperative antimicrobial treatment courses. No such relationships were seen with respect to TNF-α-containing cells. The IL-8-containing cells and the inflammatory cells were predominantly scattered in the heart valve stroma or in the margin of the vegetation (Paper II). The primary effect of IL-8 is to stimulate chemotaxis of polymorphonuclear neutrophil granulocytes. This indicates that there is no deficiency of IL-8 in the area close to the vegetation as a cause of the localised agranulocytosis often present inside the vegetation.

Our study revealed a need for computerised decision support systems (DSSs) in the field of IE, but to be used in clinical practice these DSSs need be part of knowledge bases covering larger domains (Paper IV). Some of our initial ideas described in Paper III, especially the use of Internet technology and the combination of rule-based advice and explanatory hypertext, will probably be included in these knowledge bases.

In vitro, there is a rapid reduction of free vancomycin in broth containing dense staphylococcal cultures. Consequently, there is a simultaneous increase in broth MICs, particularly in high inocula, which is not caused by a development of resistance (Paper V). These findings need further evaluation in vivo, but indicate that the dosing regimen of vancomycin is of particular importance in staphylococcal infections with dense inocula, e.g. infective endocarditis.

Diabetes mellitus and moderate to severe heart failure were independent risk factors for 6-month mortality in left-sided, Duke definite IE episodes, regardless of referral or local origin of the episodes. Early heart valve surgery had a positive impact on the 6-month mortality in the referral cohort of episodes, which may be due to referral bias (Paper VI).

LIST OF PAPERS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals.

I.	Ekdahl C, Broqvist M, Maller R, Stenestrand U. "Changes in left-sided infective endocarditis over two decades at a Swedish university hospital – and evaluation of the importance of referral bias." Manuscript.
II.	Ekdahl C, Broqvist M, Franzén S, Ljunghusen O, Maller R, Sander B. (2002) "IL-8 and tumor necrosis factor alpha in heart valves from patients with infective endocarditis." Scand J Infect Dis 34:759-62.
III.	Karlsson D, Ekdahl C, Wigertz O, Shahsavar N, Gill H, Forsum U. (1997). "Extended telemedical consultation using Arden Syntax based decision support, hypertext, and WWW technique." Methods Inf Med. 36: 108-14.
IV.	Ekdahl C, Karlsson D, Wigertz O, Forsum U (2000). "A study of the usage of a decision-support system for infective endocarditis." Med Inform Internet Med 25:1-18.
V.	Ekdahl C, Hanberger H, Hällgren A, Nilsson M, Svensson E, Nilsson LE (2005). "Rapid decrease of free vancomycin in dense staphylococcal cultures." Eur J Clin Microbiol Infect Dis 24:596-602.
VI.	Ekdahl C, Broqvist M, Maller R, Stenestrand U. "Prognostic factors for 6-month mortality in infective endocarditis – a retrospective study in a Swedish referral hospital." Manuscript.

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1 ABBREVIATIONS and DEFINITIONS

CI	Confidence interval
CME	Continuing medical education
CoNS	Coagulase-negative staphylococci
DM	Diabetes Mellitus
DSS	Decision support system, i.e. computer-based information systems that support decision making
NYHA	New York Heart Association functional classification system for heart failure
ICAM-1	Intracellular adhesion molecule-1
IE	Infective endocarditis
IL-8	Interleukin-8
IL-8$^+$cells	IL-8 containing cells
Hypertext	Often refers to text on a computer that will lead the user to other related information on demand through links and connections
HTML	Hypertext markup language, i.e. the language for web pages that combines text and extra information about the text
KB	Knowledge base
MCP-1	Monocyte chemotactic protein 1
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus
PVE	Prosthetic valve endocarditis
S aureus	Staphylococcus aureus
S viridans/bovis	Viridans group streptococci and S bovis/equinus
TEE	Transoesophageal echocardiography
TNF-α	Tumour necrosis factor alpha
TNF-α$^+$cells	TNF-α containing cells
TTE	Transthoracic echocardiography
VCAM-1	Vascular cell adhesion molecule-1
WWW	World Wide Web

2 INTRODUCTION

2.1 Infective endocarditis in summary

IE usually develops when gram-positive bacteria or fungi in the blood adhere to damaged endothelium on heart valves. The microbes are embedded in fibrin and platelets, thus forming the vegetation. Parts of the vegetation may embolise to the central nervous system, kidneys, spleen, skin or lungs. Embolisation, as well as congestive heart failure and abscesses in the heart, are feared complications in IE.

Despite the use of modern blood culture techniques and transoesophageal echocardiography, the diagnostic procedure in IE remains a challenge. The structure of the vegetation obstructs the penetration of phagocytic cells and antimicrobial substances, which leads to long treatment courses with high doses of intravenously administered bactericidal antibiotics. Early heart valve surgery is needed in approximately 25 % of cases due to congestive heart failure, abscesses, or risk of embolism from the vegetation.

The incidence of IE is low [1], but untreated it is fatal. With a modern combination of antimicrobial therapy and heart valve surgery, mortality is still 10-20 %.

2.2 The endocarditis vegetation

2.2.1.1 Non-bacterial thrombotic endocarditis

The development of endocarditis vegetation is a complicated mechanism that usually requires several independent factors related both to the host and the invading microbes [2, 3]. Valvular endothelial cells may be damaged as a result of haemodynamic factors, which leads to an exposure of extracellular proteins. A normal healing process is induced with deposition of platelets and fibrin, thus forming a sterile vegetation (non-bacterial thrombotic endocarditis, NBTE) [3, 4]. NBTE is quite common and has been found in 2.4 % of patients in an autopsy material, especially in elderly people with chronic wasting diseases, and in 19 % of echocardiographic examinations in patients with solid tumours [2].

2.2.1.2 Colonisation

Several microbes, especially viridans streptococci and staphylococci, obtain the ability to adhere to the NBTE through interaction of microbial surface components with extracellular endothelial molecules [3, 5]. Numerous bacterial surface proteins have been described, e.g. fibronectin-binding proteins among S aureus, and surface glucans in viridans streptococci [3, 5].

Bacteria may interact with human endothelial cells and stimulate the recruitment of phagocytic cells through induced surface expression of cell adhesion proteins such as vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin [5-7]. This may also lead to an increased secretion of chemotactic cytokines (chemokines) such as IL-8 and monocyte chemotactic protein (MCP-1), and proinflammatory cytokines such as TNF-α [8]. TNF-α has the capacity to further stimulate IL-8 production and to induce transendothelial migration of neutrophils, which is believed to be IL-8 dependent [9].

2.2.1.3 Further aggregation

After colonisation, the aggregation of platelets is a key factor in the maturation of the vegetation as they cover the microbes and allow them to avoid the host defence mechanisms. Tissue factor (TF), a membrane glycoprotein, is crucial in the formation and maintenance of the vegetation [3, 10]. TF is produced by monocytes and endothelial cells, and its production may be induced by staphylococci as well as viridians streptococci [3].

2.2.1.4 Host defence inside the vegetation

The growth of the vegetation caused by fibrin-platelet-microbe interactions in a cyclic fashion leads to embedding of bacteria in fibrin capsules at various depths in the vegetation [4, 11]. In addition, growth of the vegetation impedes the infiltration of phagocytes, which may result in a localised agranulocytosis [12]. In a study by Durack, aggregates of phagocytic cells were scattered in the vegetation only when the fibrin capsule was deficient [4]. It has also been shown that the phagocytic ability of macrophages may be reduced by fibrin barriers [4]. Together, these factors result in the development of dense bacterial inocula often reaching 10^9 to 10^{10} bacteria per gram of tissue [4, 12].

Knowledge of the role of phagocytic cells inside the vegetation is limited. Monocytes may contribute to the formation of the vegetation, such as by induction of TF production [10]. It has been contended that "the role of granulocytes in the vegetation is unknown" [2]. However, Meddens et al compared rabbits with or without monocytes and/or granulocytes in experimental IE in a series of studies in the early 1980s, and found that both cell types could contribute to the control of the infection, as measured by the number of bacteria in the vegetations [13-15].

2.2.1.5 Antimicrobial effect inside the vegetation

Bacteria deep within the vegetation may have reduced metabolic activity [16]. This affects the antimicrobial effect achieved by antibiotics that need cell wall synthesis for their effect, such as vancomycin [2, 17]. Several studies have shown that antimicrobial substances have problems penetrating the vegetation; for instance the glycopeptide teicoplanin does not penetrate the vegetation and is concentrated only at the periphery [18]. The limited host response inside the vegetation contributes to the need for long treatment courses with a combination of bactericidal antibiotics [19].

2.3 Changes in IE during recent decades

2.3.1 Clinical presentation and diagnostic routines

Several changes in IE epidemiology have been reported in developed countries during recent decades. The median age is increasing due to a decrease in rheumatic fever as an underlying cause of IE, an increase in the use of heart valve surgery for valve replacement, and increased average longevity in the population [2]. Traditionally, viridans streptococci were the main cause of IE, but S aureus is the most common IE pathogen reported in several current studies [20-22].

The time from the start of symptoms until the diagnosis of IE is established is decreasing, due in part to the use of transoesophageal echocardiography. In 1981 the Beth-Israel criteria for IE diagnosis were introduced [23]. They did not include echocardiography which, during the ensuing decade, was increasingly used in the IE diagnostic procedure. In 1994 the Duke criteria were published, in which typical findings in the blood cultures were combined with echocardiographic findings [24]. Since then several modifications of the Duke criteria have been suggested, including modifications from Duke University that stress a more rigorous approach in the use of modern transoesophageal echocardiography [25].

The general idea that the epidemiology of IE is undergoing major changes has recently been questioned by Tleyjeh et al. In a meta-analysis of 15 population-based studies from developed countries, they could identify only an apparent increase in PVE and the use of heart valve surgery, but no increase in staphylococcal IE. It should be noted, however, that only three of the 15 studies that were included covered any of the past 10 years [26].

2.3.2 Therapeutic management

2.3.2.1 Recommendations for antimicrobial therapy

Since their introduction during the 1940s, penicillins have been the basis for antimicrobial treatment of IE with or without being combined with aminoglycosides. Several other drugs have been evaluated for use in IE, but penicillins maintain their key place in IE treatment [27, 28].
Vancomycin, a glycopeptide antibiotic, was "fast-tracked" for approval in 1958 due to its effect on drug-resistant staphylococci. It was nevertheless not a success due to toxicity and the almost simultaneous introduction of antistaphylococcal penicillins and cephalosporins [29, 30]. However, since the early 1980s there has been a dramatic increase in the use of vancomycin world-wide, partly as a result of the widespread emergence of methicillin-resistant Staphyloccus aureus (MRSA) [30]. Since the early 1980s vancomycin has been recommended as an alternative for patients who are allergic to β-lactam antibiotics [31]. Today the main indications for vancomycin in IE treatment are β-lactam antibiotic allergy and/or resistance, and culture-negative PVE [27, 28]. Due to an increasing number of reports of therapeutic failure with glycopeptides, and the emergence of several types of glycopeptide resistance, the use of vancomycin and other glycopeptides has been increasingly debated [32, 33].

2.3.2.2 Early heart valve surgery

The use of heart valve surgery during active endocarditis has escalated since the first reports in the 1960s, and it is considered to be one of the major causes of the reduction in mortality during the past three decades [34]. In the beginning, heart valve surgery was avoided in early phases of the disease, as the risk of mortality was generally considered to be higher if antibiotic therapy had not been completed before surgery [35, 36]. During the past 15-20 years the time to surgery has decreased, based on a number of studies emphasising the importance of urgent heart valve surgery [34, 36]. In addition, several studies have demonstrated that the duration of antimicrobial therapy before surgery has no impact on operative mortality [34, 35]. Congestive heart failure is still a primary indication for heart valve surgery, followed by signs of local spreading in the heart and complicated PVE [28, 34, 37]. The frequency of surgery is constantly increasing [22, 26]. Roughly 25-30 % of IE patients require early heart valve surgery [2, 34]. Higher surgical frequencies have been reported, usually from tertiary care hospitals, and referral bias may be one explanation for these high rates [38]. However, the recent meta-analysis of population-based studies from developed countries showed evident "between-country heterogeneity" , with surgical frequencies of 12.8-49.0 % [26].

2.3.3 Mortality and prognostic factors for death

In the pre-antibiotic era, IE was fatal. With the introduction of penicillins during the 1940s, mortality decreased [39]. During recent decades, IE-related mortality has decreased further to 10-20 % [34, 39], which may be due chiefly to a more aggressive approach to early heart valve surgery [34]. Several factors have been associated with increased mortality, including heart failure [40], embolic events [41], a staphylococcal cause of infection [41, 42], age [38], diabetes mellitus [41, 43, 44], other comorbidity [40], and contraindications to surgery [38].

2.4 The need for computerised decision support systems in the management of IE

The need for easy-to-access and relevant information in a clinical situation is not controversial. The amount of biomedical knowledge doubles every 20 years [45], which makes it (almost) impossible for clinicians to keep up with knowledge that they "ought to know". There are many ways of handling this, from a total neglect of new knowledge and reliance on personal

experience, to anxiety about all the knowledge that is presented but cannot be assimilated, to reliance on "sorted" knowledge presented in guidelines or by expert consultants [46]. Furthermore, it is difficult for new medical knowledge to penetrate clinical practice [47]. In some ways this could be defined as a general problem in the continuing medical education (CME) of health care workers, which may reduce the quality of patient care and increase costs [48].

These issues apply to IE as well, because IE is a rare disease with which health care workers often have limited personal experience, a complicated disease with high mortality and morbidity if managed incorrectly, a disease undergoing constant change e.g. regarding diagnostic and therapeutic management, and a disease of great interest to the research community (a PubMed search on "Endocarditis"[Mesh] resulting in 19 556 items).

3 AIMS OF THE THESIS

1. To study the changes in epidemiology, diagnostic approach, therapeutic management and mortality in left-sided IE between 1980 and 2001, and to evaluate the impact of referral bias in this regard. (Paper I)

2. To study the chemotactic and pro-inflammatory response by investigation of the occurrence of IL-8 and TNF-α-containing cells in heart valves and vegetations during the active phase of IE. (Paper II)

3. To develop and evaluate the usage of a computer-based decision-support system for IE. (Papers III and IV)

4. To study the effect of a glycopeptide antibiotic, vancomycin, in dense staphylococcal cultures in vitro. (Paper V)

5. To identify prognostic factors for 6-month mortality in left-sided, Duke definite IE at our hospital, with special emphasis on referral bias. (Paper VI)

4 MATERIALS AND METHODS – summaries and comments

4.1 Changes in IE at our hospital (Paper I)

4.1.1 Summary

Clinical records of adult patients hospitalised for IE at our hospital from 1980 to 2001 were retrospectively reviewed. Patients were included if they fulfilled the Duke criteria for definite or possible endocarditis [24].

Our aim was to describe IE patients at our hospital, and not to do a population-based study. According to our local routines, the majority of IE patients are admitted to the Dept. of Cardiology, the Dept. of Infectious Disease, or the Dept. of Thoracic Surgery, and consequently IE episodes identified in the hospital database covering these three departments were included. In addition, IE episodes identified in the database for surgical procedures at the Dept. of Thoracic Surgery were included.
We chose to evaluate left-sided IE episodes, since our strictly right-sided and pacemaker IE episodes were few in number (Paper I). Furthermore, the right-sided and pacemaker IE episodes presented with different clinical pictures compared to the left-sided IE episodes (data not shown).

The IE episodes that were included were divided into three groups based on time of admission. For the first group the time period ended when heart valve surgery was introduced at our hospital on April 1, 1989. Groups two and three were divided into groups of equivalent size. To assess referral bias, changes in epidemiology, management and mortality in the three study groups were identified for referred episodes and episodes from our local community, respectively. For definitions of clinical data, please see Paper I.

4.1.2 Comments

4.1.2.1 Statistical methods used

The Kruskal-Wallis one-way analysis of variance was used for continuous variables. For comparison of categorical variables between the three time periods studied, the Fisher-Freeman-Halton test was used, since some groups contained small samples. For comparison of dichotomous data between two study groups (e.g. males vs. females) the two-tailed Fisher's exact test was used.
Significance was defined as $p < 0.05$.

4.1.2.2 Comments and limitations of the study

The choice of the original Duke criteria according to Durack et al [24] was based on the long time period studied. The modified Duke criteria suggested by Li et al [25] are probably more exact for recent patients with high-quality echocardiographic data, but transoesophageal echocardiography was not done in any of the episodes in our early study group.
The retrospective approach is an obvious limitation owing, for example, to lack of standard definitions and the risk of missing data or obtaining inaccurate information [26]. Nonetheless, due to the low incidence of IE, retrospective designs have commonly been used in clinical IE studies [49, 50].

Causes of death were obtained through the National Cause of Death Register in Sweden. Unfortunately, these data were hard to interpret due to limited details, and in some cases incorrect information as compared to that in patient charts, which were possible to assess in detail. Because of this, causes of death were not available with certainty for patients who had died after discharge from our hospital, and this valuable information had to be excluded.

4.2 Paper II and simultaneous examination of IL-8, TNF-α, and CRP in plasma

4.2.1 Summary of Paper II

Heart valve tissue was obtained from six consecutive patients with IE who needed early heart valve surgery. By using immunohistochemistry, paraffin-embedded sections of the heart valves were stained for the amount of IL-8-containing (IL-8^{+}) and TNF-α-containing (TNF-α^{+}) cells. Tissue sections were also stained to examine the amount of inflammatory cells, healing, and microbes.

The amount of cytokine-containing cells, inflammatory cells, healing, and microbes were scored semiquantitatively in a regular light microscope. The presence of bacteria was also based on microscopy of tissue samples sent to the Dept. of Clinical Microbiology for culture, and on culture of the heart valves. For a definition of the scoring system and further details, please see Paper II.

Infiltration of polymorphonuclear neutrophil granulocytes (PMN) was defined as acute inflammation, and the presence of lymphocytes, mononuclear cells and/or plasma cells was defined as chronic inflammation [51]. Healing was defined as endothelialisation of the surface, infiltration and organisation by fibroblasts, and/or phagocytosis of bacterial debris [4].

4.2.2 Cytokines and CRP examination in plasma

(not previously reported)

4.2.2.1 Assay of cytokine levels in plasma

Blood samples for cytokine examination were obtained in a standardised way before start of antibiotic treatment and until clinical improvement was observed (Figure 3). If possible, they were taken between 8 and 9 am. Blood samples were also obtained during the heart valve surgery procedure. Blood was aseptically collected in Endotube ET, 4-mL endotoxin-free vacuum tubes with sodium heparin as an anticoagulant (Chromogenix AB, Mölndal, Sweden), transported on ice, and centrifuged within 15 minutes at 3000g for 10 minutes. The supernatants were frozen and stored at -70°C.

All analyses were done with commercially available ELISA kits according to the instructions of the manufacturers. IL-8 (Immunotech International, Marseilles, France) and TNF-∝ (R&D Systems, Minneapolis, MN, USA) were measured. The plates were read on a Dynatech 7000 spectrophotometer (In Vitro AB, Stockholm, Sweden).

4.2.2.2 Assay of CRP in serum

Blood for CRP determination was obtained at the same time as the plasma cytokine samples were secured from the patients. Analysis was performed using the routine procedure at the Dept. of Clinical Chemistry, University Hospital, Linköping, using the Tina-quant® CRP kit (Roche Diagnostics, Bromma, Sweden), and a Hitachi 917 (Roche Diagnostics).

4.2.3 Comments

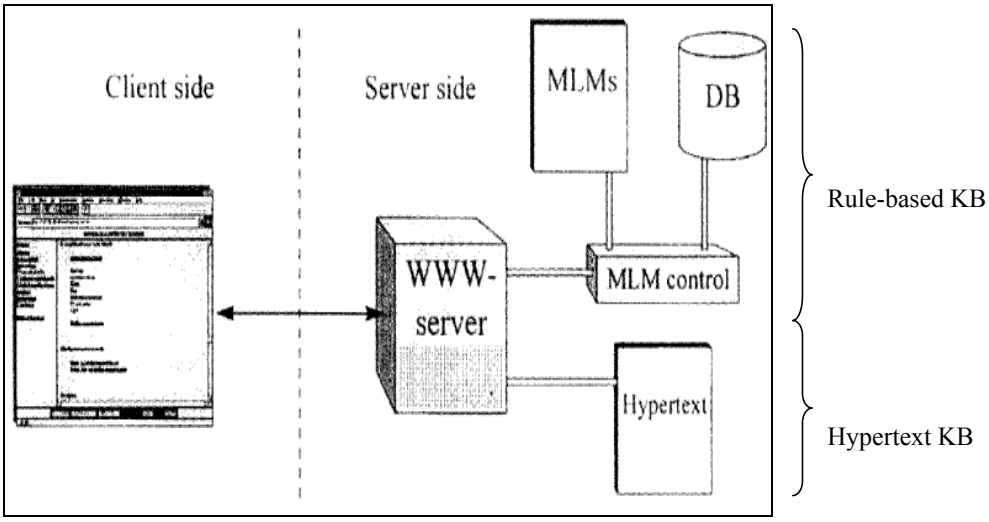
Immunohistochemistry has been used for the detection of cytokines in situ in other endocarditis studies [7, 52], and the same scoring system was also used by Muller et al [7]. Earlier studies using in vitro activated cells have shown good correspondence between detection of individual cytokine-producing cells by in situ hybridisation for mRNA and cytokine staining by immunofluorescence [53]. Litton et al have demonstrated good correspondence between the amount of mRNA and detectable cytokines during immune stimulation in vivo, indicating that the cytokines are produced at the place of detection [54].

4.3 Development of a DSS for IE (Paper III)

4.3.1 Summary

A rule-based decision support system (DSS) using medical logic modules (MLMs) was integrated with hypertext using WWW technology in order to provide expert consultation in the field of IE (Figure 1). Arden Syntax, a language designed for representing procedural medical knowledge, was used for the MLMs. WWW technology was used in order to provide explanations for the advice given, efficient geographic distribution of the expert knowledge and decision-support, and to facilitate easy access to specialists and expert knowledge sources in the field when the limit of the system was reached. The technical features of the system are described in detail in Paper III.

Figure 1: General architecture of the system

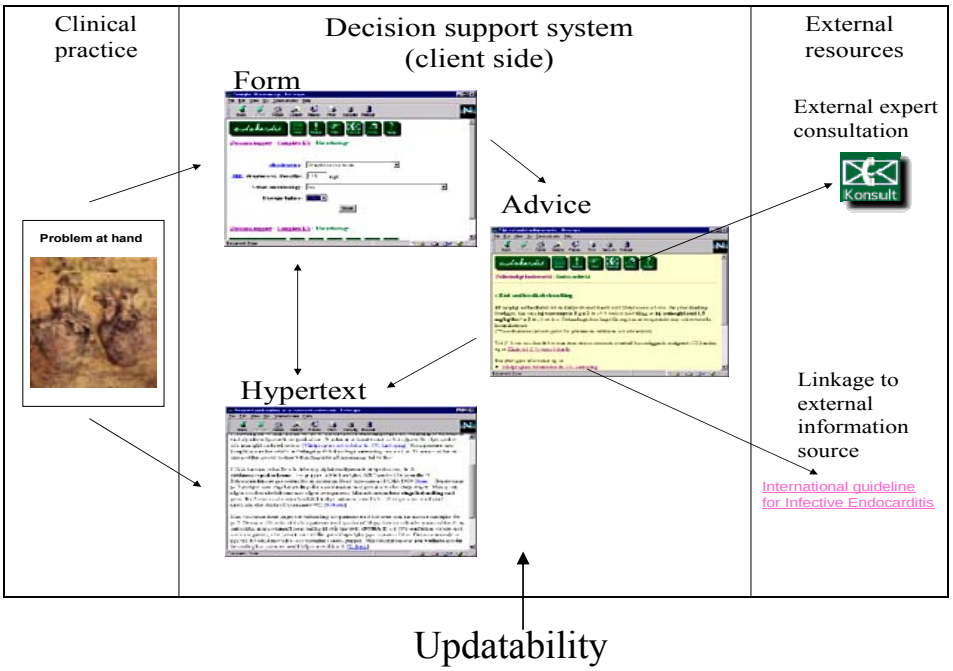


The system was designed to be user-driven in order to make it less dependent on the user's background knowledge, i.e. the user could reach his/her own level of information need. Another key issue was the lack of a computer-based infrastructure for patient records and laboratory data systems in Sweden at the time the system was designed, which prohibited the automatic entry of sufficient data into the system.

In short, the user could choose to enter the system through the hypertext KB, or through the rule-based KB (Figure 2). The hypertext KB worked in a traditional way, with links to related pages.

When entering patient data and a question, the rule-based KB generated advice in a single hypertext page. This page included a short explanation for the advice given, and links to adjacent explanatory hypertext pages, links to external knowledge sources, and e-mail connections to expert consultants (Figure 2). In this way the user could utilise the rule-based KB to easily access specific information in the hypertext KB regarding the problem at hand. A centralised server provided technical feasibility for easy updates of the knowledge base and the ability to guarantee the authority of the information provided (Figure 1).

Figure 2: General architecture of the system from a client's point of view



4.3.2 Comments on the use of Arden Syntax and WWW technology

At the time of development, Arden Syntax was a well established language for the representation of modular medical logic. It is a standard adopted by the American Society for Testing and Materials (ASTM) (in the Summer of 1998 it was moved to Health Level Seven (HL7)) [55].
At the same time, in early 1995, the World Wide Web and global Internet started to provide technology for further development and distribution of computerised DSSs, with the introduction of HTML 2.0 [56, 57].

4.4 Evaluation of a DSS for IE (Paper IV)

4.4.1 Summary

The aim of the study was to evaluate how the system could meet information needs arising in clinic-like situations and to gain better understanding of the qualities of the usage of the system in relation to these information needs. The aim was not to validate the system or its knowledge base. In order to achieve our aim, a qualitative study was performed.

Six potential users of the system, all specialists in internal medicine, each worked in a laboratory setting with extracts from medical records from two patient cases concerning IE or other Gram-positive septicaemia. When needed by the users, one member of the research group (CE) acted as an expert consultant in the field of IE. The sessions were videotaped, including the text presented on the computer screen. About a week after the session each physician and an interviewer (CE) looked at the videotape. Using the stimulated recall technique [58], spontaneous comments, and the answers to a limited number of open-ended, general questions posed at the end of the interview, were audio-taped.
The analysis was based on a Grounded Theory approach influenced by Strauss [59] and Starrin [60].Three members of the research team individually examined transcripts of the interviews line by line. The initial codes were sorted into categories, and relations between categories were examined until stable categories and relations were established. Based on these categories and their relations, a theory of the system usage was created.

4.4.2 Use of a qualitative method

Several methods have been suggested when addressing the difficult question of DSS evaluation [61, 62]. Qualitative methods have been used previously in DSS evaluation and they have been recommended as a part of the design process [63]. In creating a basis for further system development, attitudes of end-users, their expected information needs, and their ways of using the prototype have been of great importance. To get access to this information, the data obtained using qualitative methods was found to surpass that which could be achieved using quantitative techniques [64]. The strength of quantitative methods lies in their "numbers", which may lead to a conclusion that is reliable and can be generalised with questions like: "What proportion of smokers have tried to quit?" Qualitative research, on the other hand, aims at validity ("closeness to the truth") with questions like: "What prevents people from giving up smoking?" [64].

4.4.3 Methods used for data collection

The methods used for data collection in Paper IV and their definitions are summarised in Table 1.

Table 1: Methods used for data collection in the evaluation of the system (Paper IV)

Method	Suggested definition
Participant observation	• Observation in which the researcher also occupies a role or part in the setting, in addition to observing[64]
In-depth interviews	• Face-to-face conversation for the purpose of exploring issues or topics in detail. Does not use preset questions, but is shaped by a defined set of topics [64].
Stimulated recall	• Reviving the memories of a respondent after a session so that the respondent recalls the thoughts that occurred during the session [58]

4.4.4 Method used for data analysis (Grounded Theory)

Grounded theory (GT) is a research method developed by Barney Glaser and Anselm Strauss during their research on dying in hospitals. In this work they developed the constant comparative method later known as Grounded Theory. GT was developed as systematic methodology for the generation of theory from data. The first publication of grounded theory methodology, "The Discovery of Grounded Theory: Strategies for Qualitative Research" [65], was in 1967. The purpose of writing this book was to offer research instruments for the generation of theories that

were grounded, i.e. “generated and developed through interplay with data collected during research projects” [66, p. 277]. With this approach they wanted to contribute to “closing the embarrassing gap between theory and empirical research”. They also wanted to legitimise “careful qualitative research”, which at that time “was not believed capable of adequate verification” [66].
The approach to achieving these goals was to systematically gather and analyse data. The original publication, “The Discovery of Grounded Theory”, considered GT to be a general methodology, suitable for both quantitative as well as qualitative studies. Mostly, however, GT has been used for the study of qualitative data [66].
The data sources when using a GT approach could be interviews, field observations, documents, videotapes, etc. [66]. Initial data are coded, the open codes generated from the original data are compared, and coding schemes are constructed to generate categories. Relationships between these categories form the basis for the grounded theory. These different steps are repeated until stable categories and relations are created, and the phenomenon that is to be studied can be explained.

4.4.5 Limitations

One clear limitation was our usage of a laboratory setting, i.e. that we never reached the level of evaluation of the system in clinical routine practice. Several problems have been described during the evaluation of health information systems, such as unclear or changing evaluation goals during the study, the major efforts needed to prepare and execute the study, and complex and contradictory results [61]. Nevertheless, evaluation was essential at this time in the design process in order to guide our continued development strategies.

4.5 Vancomycin in dense staphylococcal cultures (Paper V)

4.5.1 Summary

Five ATCC strains, two S epidermidis and three S aureus strains, were studied at different inocula (~10^5-10^9 bacteria/ml). All the tested strains, with one exception, were methicillin-susceptible, and all the strains were vancomycin-susceptible.

Bacterial numbers were determined by bioluminescence assay of intracellular bacterial ATP. Vancomycin MICs were determined for all the strains that grew after 24 h of incubation in Mueller-Hinton broth (MHB). MIC was defined as the lowest concentration of vancomycin that prevented an increase in bacterial ATP. Regrowth was also assessed visually for inocula 10^5 and 10^6. Free vancomycin concentrations in the broth cultures were determined with an agar well bioassay after filtration or centrifugation of the bacterial cultures. Free vancomycin concentrations and bacterial numbers were also determined after ½, 2, 4, and 8 hours for one S epidermidis and one S aureus strain.

To examine whether dead bacterial cell products could bind vancomycin, broth cultures were heated to 100°C before exposure to vancomycin. To examine whether it was possible to release vancomycin from its binding sites, broth cultures at different inocula exposed to vancomycin were heated to 100°C or enzymatically treated for lysis of the bacterial cell walls. Vancomycin concentrations were determined before and after lysis.

To exclude the emergence of resistance, E-test MICs were determined before and after vancomycin exposure. To exclude enzymatic destruction of vancomycin, broth cultures were filtered, and the cell-free supernatants were exposed to vancomycin.

4.5.2 Comments

4.5.2.1 Bioluminescence assay of intracellular ATP for MIC

Bioluminescence assay of intracellular bacterial ATP has previously been shown to correlate to bacterial counts [67].

4.5.2.2 Accuracy of the agar well bioassay

The coefficient of variation (CV) was calculated for ten measures of four different vancomycin concentrations. The CV is defined as the standard deviation divided by the mean value. The mean recovery was defined as the mean value divided by the set-up value (Table 2).
We evaluated the high pressure liquid chromatography (HPLC), enzyme multiplied immunoassay (EMIT), and fluorescence polarization immunoassay (FPIA) techniques, but obtained the best accuracy at low vancomycin concentrations with the agar well bioassay.

Table 2: Accuracy of the agar well bioassay method for determination of vancomycin concentrations.

n	Set-up value (µg/ml)	Mean ± SD (µg/ml)	Coefficient of variation (%)	Mean recovery (%)
10	0.75	0.76 ± 0.063	8.3	100.7
10	1.5	1.44 ± 0.049	3.4	95.8
10	6	6.13 ± 0.28	4.6	102.1
10	12	11.88 ± 0.59	4.9	99.0

For one S epidermidis and one S aureus strain the broth cultures were centrifuged at 3000xG for 10 min or filtered through 0.22µm pore filters in parallel. No difference in vancomycin concentrations was seen, and filtration was subsequently used.

4.6 Prognostic factors in IE at our hospital (Paper VI)

4.6.1 Summary

Using multivariable logistic regression, predictors of all-cause mortality 6 months after start of treatment were identified for left-sided, Duke definite IE episodes referred to us and from our local community, respectively.

For definitions of patients selected and clinical data, please see section 4.1.1 and Paper I.

4.6.2 Comments

4.6.2.1 Statistical methods used

To compare data between two study groups, the two-tailed Fisher's exact test was used for dichotomous variables and the Kruskal-Wallis one-way analysis of variance for continuous variables.

Multivariable backward stepwise logistic regression was used to identify variables independently associated with 6-month mortality. The variables included were based on an estimation of importance for the outcome, of which a majority have been reported earlier as risk factors for mortality in IE.
The multivariable logistic regression used may be summarised as

$$\text{Risk of death after 6 months} = \frac{e^{\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_4 x_4+\cdots}}{1+e^{\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 x_3+\beta_4 x_4+\cdots}}$$

where x_i is the different risk factors for 6-month mortality
x_1 = S aureus IE
x_2 = early heart valve surgery
x_3 = year of admission, etc.

β is the regression coefficient for each risk factor (x_i). In the example above this is
β_1 = the effect of S aureus
β_2 = the effect of early heart valve surgery
β_3 = changes over time, etc.

Changes in the frequency of x_i, e.g. an increased frequency of S aureus IE, or an increased use of early heart valve surgery over time, does not induce bias in the estimated regression coefficients [68]. To adjust for general changes in epidemiology and management over time, e.g. improvement in surgical techniques, year of admission was used as a proxy and was included throughout the stepwise backward multivariable logistic regression.

Significance was defined as $p < 0.05$.

4.6.2.2 Limitations of the study

The long time frame may be a limitation in the prognostic evaluation, despite the fact that it has been used in earlier IE studies presented during the last decade [69, 70], and regardless of the adjustment made with year of admission as a surrogate for changes over time. Propensity scoring has been suggested in prognostic IE studies in order to avoid bias in patient characteristics, referral status, and treatment selection [49, 50, 71]. Nevertheless, despite the use of propensity scoring, disparity has been reported regarding the prognostic impact of early heart valve surgery in IE [49, 71]. The size of our patient material did not allow us to use propensity scoring which, combined with the retrospective design and the long time frame, limits the generalisability of our prognostic results.

5 RESULTS AND DISCUSSION

5.1 Changes in referred and local IE episodes at our hospital (Paper I)

Some of the changes seen in our study (Table 3), such as an increased use of TEE, have been shown earlier and are not controversial. The increase in S aureus IE and the increased use of early heart valve surgery are discussed below.

Table 3: Significant changes between the three time periods studied for the referred and local cohorts of IE episodes, respectively (extract from Paper I).

	Changes over time									
	1980-1989		1989-1995		1996-2001		*p* value	Total cohort	
	n	(%)	n	(%)	n	(%)		n	(%)
Referred episodes									
Episodes	21		46		64			131	
Duke definite	14	(66.7)	44	(95.7)	57	(89.1)	0.006	115	(87.8)
Known heart failure[a]	6	(28.6)	5	(10.9)	5	(7.8)	0.048	16	(12.2)
S aureus IE	3	(14.3)	9	(19.6)	24	(37.5)	0.044	36	(27.5)
Non-cerebral embolic events	0		10	(21.4)	8	(12.5)	0.045	18	(13.7)
Echocardiography									
Transthoracic	19	(90.5)	21	(45.7)	11	(17.2)	<0.001	51	(38.9)
Transoesophageal	0		25	(54.4)	52	(81.3)	<0.001	77	(58.8)
Early heart valve surgery	5	(23.8)	25	(54.4)	35	(54.7)	0.037	65	(49.6)
Local episodes									
Episodes	39		40		42			121	
S aureus IE	4	(10.3)	9	(22.5)	14	(33.3)	0.044	27	(22.3)
Echocardiography									
Transthoracic	37	(94.9)	20	(50.0)	6	(14.3)	<0.001	63	(52.1)
Transoesophageal	0		20	(50.0)	36	(85.7)	<0.001	56	(46.3)
Early heart valve surgery	1	(2.6)	3	(7.5)	12	(28.6)	0.002	16	(13.2)

[a] NYHA class before the IE episode. All these episodes were NYHA class II or III.

5.1.1 Staphylococcus aureus IE

There was a shift towards S aureus as the dominant cause of endocarditis in both the referred and local cohorts of IE episodes. This trend has been well described previously [1, 20-22, 72], but has recently been debated by Tleyjeh et al. They could not detect any increase in S aureus IE in their own population-based study [73], or in a meta-analysis of this and 14 other well-defined population-based studies [26]. They stated that an increase in S aureus IE may be due to a high prevalence of injecting drug users or referral bias. Our low cohort of IDU contradicts the first contention (Paper I), and this trend towards an increase in S aureus IE was seen in both the referred and local cohorts (Table 3).

Fowler et al state that S aureus IE often is health care-associated (nosocomial IE) [20], but, regrettably, our study was not designed to address this important aspect.

5.1.2 "Referral bias"

Differences between referred and local episodes are presented in Table 4. The proportion of referred episodes increased significantly (35.0 %, 53.5 %, 60.4 %; p =0.007). Most patients were referred to us for evaluation for surgical therapy, and as expected they were significantly younger and had more complications (Table 4). They probably represent a selection of complicated IE episodes, which in part may explain the high mortality rate seen in this cohort, although the surgical frequency was high.

It is also feasible that several patients who needed surgery, but had contraindications to surgery such as comorbidity and high age, were never referred to us. These patients are represented in our local cohort, and probably contribute to the mortality figure and the lower surgical frequency in this cohort of IE episodes.

As expected, the frequency of early heart valve surgery was significantly higher among referred episodes (Table 4), even though the surgical frequencies increased significantly in both the referred and local cohorts (Table 3). The increased use of early heart valve surgery during recent decades is a well-known fact [26]. However, the surgical frequencies have differed to a great extent between different studies [26]. This makes it hard to estimate the "true" frequency of surgery that is needed in a population.

Considered together, our data presented in Table 3 and Table 4 underscore the need for adjustment of referral bias in IE studies, and for caution when interpreting data from IE studies if such adjustment is not presented.

Table 4: Significant differences between referred and local IE episodes and 6-month mortality (Paper I).

	Referred IE episodes[a]	Local IE episodes[a]	p value
Episodes	131	121	
Duke definite	115 (87.8)	86 (71.1)	0.002
Age (Median years)	60 (19-86)	70 (18-86)	<0.001
Gender (Female distribution)	36 (27.5)	48 (39.7)	0.045
Coagulase-negative staphylococcal IE	18 (13.7)	7 (5.8)	0.037
Complications			
Moderate to severe heart failure	58 (44.3)	33 (27.3)	0.006
Cerebral embolic events	39 (29.8)	19 (15.7)	0.011
Echocardiography			
Transthoracic	51 (38.9)	63 (52.1)	0.043
Early heart valve surgery	65 (49.6)	16 (13.2)	<0.001
Death within 6 months	24 (18.3)	21 (17.4)	0.871

[a] Data presented as N (%) of total in each cohort if not stated otherwise

5.1.3 Gender differences

There were significantly fewer women in the referral cohort of IE episodes (Table 4). Female gender has been stated as being predictive of medical management without the use of heart valve surgery [74]. Contrary to this study by Aksoy et al [74], there was no overrepresentation in comorbidity among females in our study, and no gender differences regarding complications such as moderate to severe heart failure and embolic events were seen in any of the cohorts in our study (Paper I). The only significant gender difference observed in our study was a higher median age among female patients in the total cohort of IE episodes (69.5 vs. 63 years; p = 0.003). This

trend was also seen in the referral cohort (females 66.5 vs. men 59 years; $p = 0.102$) and in the local cohort (females 73 vs. 67 years; $p = 0.091$) of episodes. Comorbidity and complications are most certainly of great importance when deciding who to refer for surgical evaluation, but age may also be an explanation for the gender difference between the referral cohort and the local cohort in our study.

5.2 IL-8 and TNF-α in active IE

5.2.1 Summary of Paper II

The results from Paper II are summarised in Table 5. As expected, there was an apparent relation between signs of acute inflammation and time of antimicrobial treatment prior to surgery. Interestingly, there was no association between signs of acute inflammation and the microbes found in blood cultures before start of treatment (Table 5), even though the patients presented with either sub-acute or acute symptoms.

Morphological signs of healing were seen in all the patients (Table 5). The amount and stage of healing varied between different parts of the heart valves and vegetations. The grade of healing mainly depended on the length of preoperative treatment and the total time of the disease process. For example, penicillin has been shown to greatly accelerate the healing process in endocarditis [4]. Even the patient with septic shock (patient 3) showed signs of healing in some parts of his infected tissue. The picture presented by patient 6 corresponded to complete healing.

Bacteria were visualised in heart valve tissue from four patients, mainly those with short treatment courses before heart valve surgery. In only one of the patients, with only four days of antimicrobial therapy before surgery, was the heart valve culture positive. It takes months for dead bacteria to be removed from sterile vegetations [75]. When studying the factors of importance for successful reduction of the time for postoperative antimicrobial therapy, Morris et al concomitantly based their decisions on the heart valve culture, and not on the microscopy of heart valve tissue [76].

IL-8 and TNF-α were detected in both PMN and mononuclear cells, which were primarily distributed in the stroma of the heart valves or close to the margin of the stroma. It was possible to detect cytokine-containing cells deep inside some of the vegetations, but generally there were few IL-8^+cells or TNF-α^+cells inside the vegetations.
The largest amount of IL-8^+cells was seen among patients with the most signs of acute inflammation, and with the shortest antimicrobial therapy before surgery. No such relationships were seen with respect to the amount of TNF-α^+cells.

Table 5: Endocarditis presentation, time of antimicrobial therapy, morphological findings, and amount of cytokine-containing cells.

Patient	Endocarditis presentation	Microbe in blood culture before start of treatment	Days until initiation of antimicrobial therapy	Days of antimicrobial therapy prior to surgery
1	Sub-acute	CoNS[d]	17	2
2	Sub-acute	CoNS[d]	14	2
3	Acute	S aureus	3	4
4	Acute	S aureus	3	19
5	Sub-acute	Betastrep Group G	30	24
6	Acute	H. parainfluenzae	6	43

Patient	Inflammation[a]		Healing[a]	Presence of bacteria[a]	IL-8$^+$ cells	TNF-α^+cells
	Acute	Chronic				
1	+++	+	++	++++[b]	+++	+++
2	++++	++	++	++++[b]	++++	++++
3	++++	+	+	++++[c]	+++	+++
4	+	++	++++	++	-	+++
5	++	++++	++	-	++	++++
6	-	+	++++	-	+	-

[a] As assayed by morphology from paraffin-embedded heart valve tissue
[b] Positive microscopy from biopsy material for culture
[c] Culture of heart valve material
[d] Methicillin-sensitive
For definition of the scoring systems, please see Paper II.

5.2.2 Cytokines and CRP in plasma

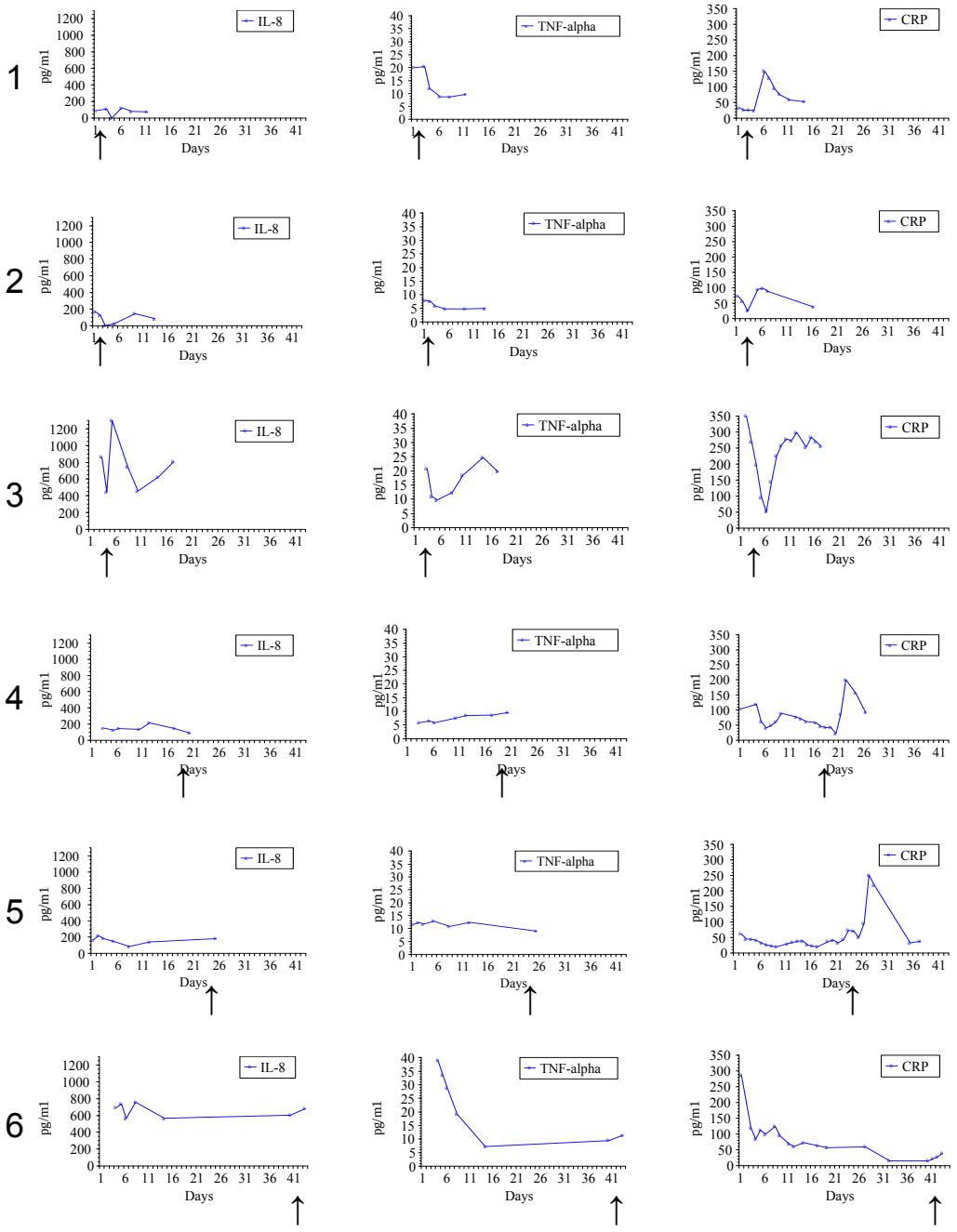
(not previously reported)

Most clinical cytokine-IE studies have focused on measuring serum or plasma levels [77-83].

In our study the plasma levels of both IL-8 and TNF-α were associated with endocarditis presentations with some extent of complications, especially for patient 3, who developed septic shock and eventually died during treatment. Patient 4 also had complications, but low levels of IL-8 in plasma. This could be explained by treatment with betamethasone intravenously for five days because of a spondylitis with partial paralysis, as glucocortocoid treatment may suppress IL-8 production [84]. The CRP levels in serum followed the severity of the infection of the patients, with a clear elevation seen postoperatively.

We could not detect any association between plasma levels of IL-8 and TNF-α, and the amount of IL-8$^+$ and TNF-α^+ cells inside the vegetations (Table 5 and Figure 3). This has previously been shown by Azzawi et al when studying biopsies at various times after cardiac transplant [85]. Our findings indicate that the plasma levels of the cytokines studied do not reflect the occurrence in heart valve tissue, and in that sense they seem to be of limited value.

Figure 3: Dynamics of IL-8 and TNF-α in plasma, and CRP in serum, from the first day of antimicrobial therapy. Arrows indicate day of surgery. (not previously reported)



5.2.3 Infiltration of phagocytic cells in the endocarditis vegetation

As mentioned in the introduction, infiltration of phagocytic cells in the vegetation is limited [2, 4]. Adhered and internalised bacteria in endothelial cells may stimulate the recruitment of phagocytic cells by means of induced expression of adhesive molecules such as VCAM-1 and ICAM-1 from endothelial cells [6]. In experimental S aureus endocarditis, fibronectin-binding proteins seem to be of vital importance in this regard, through increased IL-8 secretion from endothelial cells [5]. Müller et al demonstrated a marked expression of the adhesive molecules E-selectin and VCAM-1 on inflamed valves, but also on degenerative valves without inflammation [7]. Söderquist et al also demonstrated that patients with S aureus IE showed significantly higher serum levels of E-selectin and VCAM-1 than did patients without IE. TNF-α levels in serum were significantly correlated to levels of E-selectin, ICAM-1, and VCAM-1, but there was no correlation with serum levels of IL-8. However, in another study by Beekhuizen et al S aureus-infected endothelial cells produced considerable amounts of IL-8 and MCP-1, and IL-8 significantly enhanced transendothelial migration of granulocytes [86]. It is noteworthy that many of the studies performed by Beekhuizen et al have been done on mere endothelial cells, thus mimicking "the early phase of the pathogenesis of S aureus endocarditis when the valvular endothelium is still undamaged, and fibrin depositions have not yet been formed" [5].

In our study, IL-8^{+}cells and TNF-α^{+}cells were found primarily inside the heart valve stroma, but also deep inside some of the vegetations, although sparse and asymmetrically distributed. However small, our study suggests that IL-8 is produced in the heart valve stroma and to some extent even inside the vegetations. Theoretically, this is thought to stimulate chemotaxis of PMN, but very few granulocytes were found inside the vegetation. The upregulation of adhesive molecules and the stimulated expression of IL-8 described in several of the papers mentioned above do not seem to have a real impact deep inside the vegetation.
The most probable reason for this is the "diffusion barrier" composed of fibrin and platelets suggested by David Durack [4]. Several authors have tried to modify this barrier by means of adjuvant treatment with aspirin or ticlopidine, which has proven to decrease the size of the vegetation [3]. These studies have been convincing on an experimental level, and have also indicated a synergistic effect with respect to valve sterilisation with the combination of aspirin and vancomycin [87] However, when aspirin therapy was tested clinically, there tended to be an overrepresentation of haemorrhagic complications among patients receiving aspirin [88].

In conclusion, the difficult issue of the diminished host response inside the endocarditis vegetation needs extensive research.

5.3 Decision support systems for endocarditis (Papers III and IV)

5.3.1 Development of a DSS for IE (Paper III)

There is a need for easy-to-access and useful information in clinical situations such as the management of IE. A much-quoted definition of usefulness of information that was presented by Slawson et al is as follows

$$\text{Usefulness of medical information} = \frac{\text{relevance x validity}}{\text{work}}$$

i.e. the information must be relevant to everyday practice, it must be correct, and it must require little work to obtain [46].

Different computerised decision support systems have tried to meet this need for useful information, as well as to overcome the deficiency in health care in adjusting to new medical knowledge [47]. Several key issues need to be successfully resolved if new systems are to be accepted and used in clinical practice, and/or as tools for continuing medical education. Those addressed in our prototype development (Paper III) are presented in Table 6.

Table 6. Several key issues described as essential regarding the usability of DSSs and the theory behind our design that is presented in Paper III.

Parts of the DSS	Problems to be solved	References
Rule-based knowledge base	Deal with real-life clinical problems ("Relevance [a]")	[89]
	Time-saving and easy-to-use, i.e. by directing the user to specific information about the problem at hand ("Work [a]")	[90]
Hypertext knowledge base	Explain advice given ("Validity [a]")	[91]
	Support the user in verifying given advice ("Validity [a]")	[91]
	Provide the opportunity for the user to reach his/her level of information need ("Relevance [a]")	[89]
	Provide support for information-seeking in external knowledge sources ("Work [a]")	[57]
WWW technology with a centralised server	Provide efficient geographic distribution of expert knowledge	[57]
	Easy access to system updates ("Validity [a]")	[57]
	Control of the knowledge in the system ("Validity [a]")	

[a] according to Slawson et al [46]

5.3.2 Evaluation of our DSS prototype (Paper IV)

The evaluation of our prototype displayed several features regarding how the system was used as well as factors of importance for subjective usefulness (Table 7). The users expressed a number of opinions and demonstrated several ways of using the system that supported most of our general ideas during prototype development (Table 6), such as the ability to find specific information about the problem at hand, the capacity to use and appreciate the linkage to the hypertext KB for verification of advice given, the opportunity to reach the user's level of information need, and the ability to highlight actions that were not initially expected by the user (Table 7). They also expressed a need for regular updating of the system, which was one of the reasons for the use of WWW technology.
As expected, they also exposed several shortcomings of the system, such as by wanting to understand the technology or logic behind given advice, and the need for psychological support and shared responsibility with another human being (Table 7).

5.3.3 Decision support for endocarditis

With respect to IE management, the users expressed a need for extended decision support, as well as CME, both of which are feasible in the system according to the users (Table 7). However, the most important limitation of the system was the need for an extension of the knowledge base, which was very well expressed by one user:

" ... when you actually see an endocarditis, you may have forgotten that the system exists."

There is a clear need for the depth of information presented in this system, especially as IE is a rare, complex, and serious illness [2], and the users often used rather specialised information during management of the patient cases. However, to be utilised in real clinical practice, breadth of coverage seems essential as well.

An obvious limitation of the study is the fact that the system development ended at the development phase, and was never further developed for real-life use. Regardless of the attractive technical opportunities provided by DSSs, many systems have failed to improve clinical practice [47]. Recent studies identified some key factors for the success of DSSs in clinical practice. An automatic provision of decision support, provided as a part of the clinical workflow, was considered essential in all the studies [47, 92, 93]. Other factors identified in order to achieve success included decision support delivered at the time and location of decision making, provision of recommendations rather than just assessments, computer-based decision support [47], automatic data retrieval from electronic medical records, and support regarding drug prescriptions and ordering diagnostic tests, for example [93].

At the time of development, the only system available for managing patient data was a laboratory data system that supplied only a small minority of the variables needed for decision support. Consequently, we had to exclude the feasibility of automatic presentation of decision support. However, in a health care system with a computer-based infrastructure for the management of the majority of patient-related data, our system has the technical facilities to provide most of the factors identified for the success of DSSs in clinical practice. The hardest feature to achieve may be the automatic retrieval of sufficient data to give high-quality decision support. Many decisions in IE management depend on data stored in free-text format, which is hard to categorise in a way that is retrievable for a computer without loss of the nuance that is required.

Table 7: Main results of the evaluation of the DSS prototype (Paper IV)

Main results	Comments by the users
Factors of importance for subjective usefulness	
Patient-specific decision support	
Relevance	
In everyday clinical work	*"This diagnosis occurs so seldom that we always need some kind of support..."*
Need for information outside the system	*"..You often have a suspicion of endocarditis, but ... before that stage, when you deal with a fever of unknown origin ..."*
	"The more information stored in the system, the more I would use it."
Psychological and nuanced support	*"... when you have a seriously ill patient, you want to talk to someone ..."*
	"When I call I have the opportunity to ask so much more."
Validity	
Confirmation of advice by means of previous knowledge	*"I would never use the system ... without the corresponding medical knowledge."*
Trusting the authority behind the information	*"... a person who I know is reliable ... , i.e. the same person I talk to when I call for advice."*
Ability to understand given advice	*"(I trusted the system...) because reasons were given ..."*
Wanting to understand the system	*"Have I typed everything correctly?"*
Updatability	Advantages compared with often dated, paper-based guidelines
Work to access	
Time to information may be faster than trying to contact an expert	*" ... You don't have unnecessary phone consultations, ... wait until you have really important problems ..."*
Free-text/index search	Functionality requested by the users
Continuing medical education (CME)	
Relevance	
IE rare disease	*"We ... are left with what we once learned"*
In everyday clinical work	*"... excellent educational tool, working with real cases."*
Validity	
Easier to assess the validity of information due to extended explanatory information about a given problem with less time pressure	*"When you call ... the referral hospital, you get an answer such as "Do this, do that, etc. ... There is no time for learning…"*
Work required to access system	
Knowledge acquisition in daily practice	*"After you've managed some cases, you'll enter the system much later."*
Observed ways of using the system	
Confirmation	
Verification of knowledge	*"... confirm my decisions"*
	"... check ... so there'd be nothing I missed"
Higher level support	
Applied new information for patient management	*"... certain things ... which I wouldn't have done without access to the system"*
Use of hypertext for extended explanation	*"...I wanted to see if the vegetation was large enough for surgical treatment..."*
Usage as with an expert	*"If I hadn't had access to the system, I would probably have had to ask someone else..."*
Support for consultation	
More and better questions to an expert	*"Before I talked to the thoracic surgeon, I checked ... some things which I should have done"*
Initiate contact with an expert	*"... the system stressed that in the presence of emboli you should contact a cardiologist ..."*

5.3.4 DSSs in Sweden today

There is an ongoing debate in Sweden today about the need for DSSs and their potential positive effects in clinical practice [89, 90, 94, 95]. Several important issues regarding DSSs in Sweden were expressed at a seminar in Stockholm on June 13, 2007. These included poor adjustment to clinical needs and low cost-benefit for the effort required to obtain information [94]. In a recent survey, Gustavsson et al studied DSSs in Sweden, and only 19 fulfilled their criteria for patient-care decision support [89]. The systems "are not working or contain a lot of disturbing factors, or the users do not see the benefit of them". Many of the systems just run at one place, "which shows how hard it can be to export successful solutions". Only a third of the systems have been scientifically evaluated, because such "studies are costly to perform" [89, 90].

These facts may seem depressing, but the general impression among attendees at the seminar in Stockholm was that there will be a breakthrough in DSSs in clinical practice in the next five years [94]. This will be brought on by patient demands, and the need for more cost-efficient and safer health care, especially concerning the prescription of drugs [94, 95]. To accomplish these goals, a well-established computerised infrastructure in the health care organisation, with generally distributed computer-based patient records and laboratory data systems, among other things, is essential. A survey among Swedish health care organisations 2006 showed that computer-based patient records existed in 95 % of primary health care facilities, but in only 69 % of hospitals. There were many different systems that did not communicate with one another [96]. Consequently, much work remains to be done.

5.3.5 Concluding remarks concerning DSSs for IE

The general impression is that there is an obvious need for improved tools for decision support and CME in the field of endocarditis that are not based on mere (hyper)text. With improved computerised infrastructures, well-performing endocarditis DSSs in clinical practice will probably exist in the future as parts of knowledge bases covering large medical domains. Some of our initial ideas, especially on the use of Internet technology and the combination of rule-based advice and explanatory hypertext, will probably constitute part of these DSSs.

5.4 The use of vancomycin in IE treatment (Papers I and V)

5.4.1 Changes seen in the use of vancomycin in IE (Paper I)

There has been increased use of glycopeptides in endocarditis treatment between the three time periods studied (3.3 %, 17.4 %, 14.2 %; $p = 0.035$), primarily due to an increased use in referred patients (Paper I). Glycopeptides were given as the main antimicrobial therapy in 32/252 (12.7 %) of the episodes. In two of these episodes teicoplanin was used, and in all the other episodes vancomycin was the glycopeptide of choice.

Five (15.3 %) of these episodes were caused by methicillin-resistant CoNS, and eleven (34.4 %) were culture-negative episodes. In sixteen (50.0 %) of the episodes in which glycopeptides were used the episodes were PVE, of which six (37.5 %) were culture-negative. Only one of the S aureus episodes was treated with glycopeptides.

Vancomycin has been recommended for β-lactam antibiotic treatment failures since the early 1980s [31], and an increased use of vancomycin for this indication is not controversial. An increase in MRSA does not explain the increased use of glycopeptides seen in our study, since none of the S aureus strains were methicillin-resistant. With the global occurrence of methicillin-resistant CoNS, mainly among PVE cases that recently had their initial heart valve surgery ("Early PVE"), the inclusion of glycopeptides was recommended in the treatment regimen for these cases during the 1990s [97]. This may in part explain the increased use of glycopeptides seen in our study.

5.4.2 Decrease in vancomycin in high staphylococcal inocula (Paper V)

5.4.2.1 Inoculum effect on MICs

Broth MICs increased 2- to 4-fold when the inocula increased (Table 8). Cultures with the highest inocula (~$10^{9\text{-}10}$ bacteria/ml) did not grow, making it impossible to assess MIC.

Table 8. Range of broth MICs (μg/ml) at different inocula, based on 2-3 experiments.

	Inoculum (bacteria/ml)			
Strain	~10^5	~10^6	~10^7	~10^8
S epidermidis ATCC 14990	2	2	4-8	8
S epidermidis ATCC 35984	2	2	2-4	8
S aureus ATCC 25923	1-4	2-4	2-4	4-8
S aureus ATCC 29213	1	2	2	4
S aureus ATCC 6538	1	2	2	4

Vancomycin inhibits the synthesis of the bacterial cell wall peptidoglycan by binding to the end of a pentapeptide cell wall precursor. It has been suggested that there is a need for increased amounts of vancomycin to saturate these binding sites in dense bacterial cultures, thus causing an inoculum effect [98].

5.4.2.2 Rapid and inoculum-dependent decrease of vancomycin regardless of growth

The inoculum-dependent increase in MIC levels seen in our study is probably caused by an increased binding of vancomycin in dense cultures, thus causing a reduction in free vancomycin concentrations. This decrease in vancomycin concentrations was primarily seen in the cultures with very high bacterial ATPs reaching -5 to -6 $\log_{10}$M (~$10^{9\text{-}10}$ bacteria/ml). As in the study by Sanyal et al, the vancomycin reduction was fast [99], usually within 30 min in the broths with the highest inocula. The vancomycin reduction was growth-independent, and dead bacterial products showed the same ability to reduce vancomycin concentrations. It has been suggested that the loss of glycopeptides from the growth medium is caused by an excessive binding to the cell wall or cell-wall material [99-101], and that dense bacterial cultures may bind more drug [99].

Our strains showed no increase in E-test MICs after vancomycin exposure, which contradicts an emergence of resistance as a cause of growth at high vancomycin concentrations in the broth cultures. Enzymatic destruction is also contradicted by the fact that filtered broth cultures before vancomycin exposure showed no decrease in vancomycin concentrations, which was also shown by Sanyal et al [99].

5.4.3 Therapeutic failures with vancomycin in staphylococcal IE

In an early study on rabbit staphylococcal IE, vancomycin killed bacteria at a rate similar to the combination of penicillin and gentamicin [102]. Anti-staphylococcal penicillins still remained standard therapy, mainly due to their low failure rates in clinical practice [33]. More recent studies on experimental staphylococcal endocarditis have reported an inferior effect for vancomycin compared with anti-staphylococcal penicillins, e.g. with high relapse rates [103] and slower killing rates in the vegetations [104]. In clinical studies in recent decades several authors have reported vancomycin failure rates of 37-50% for staphylococcal endocarditis [33, 105, 106], with a longer duration of bacteraemia seen when vancomycin was used [105]. In a recent prospective multicenter study of S aureus bacteraemia, multivariate analysis showed that only endocarditis and vancomycin were significantly associated with relapse [107].

Regardless of these facts, interest in vancomycin and other glycopeptides in staphylococcal endocarditis has flourished, mainly as a result of an increase in methicillin-resistant staphylococcal endocarditis [33, 108]. Similar to methicillin-susceptible S aureus (MSSA), an increased relapse rate in experimental IE [103] and a slow clinical response have also been reported for MRSA IE [32].

The following are suggested explanations for the deficient efficacy of vancomycin in (staphylococcal) endocarditis:

- Teicoplanin does not penetrate the vegetation and is concentrated only at the periphery [109]. Poor tissue penetration in the vegetation is described for vancomycin as well, although these findings were based on homogenisation of vegetations in rabbits [110]. On the other hand, it has been stated that the vancomycin concentration in heart valves after a bolus injection is sufficient and remains above a S aureus MIC of 1 μg/ml for five hours [111]. This study was also done on homogenised heart valve material and probably does not reflect possible variations in drug concentration in the vegetation due to poor penetration.
- The stationary phase of bacteria deep inside the endocarditis vegetation [16] is known to restrain the effect of vancomycin [17, 112].
- In vitro, vancomycin has demonstrated a delayed killing at high S aureus inocula [113, 114].

A recent additional problem is, of course, the emergence of staphylococcal strains with increased MICs for glycopeptides (e.g. glycopeptide-intermediate S aureus (GISA)) [33, 108]. In a recent study on staphylococci and enterococci from different ward levels in Northern Europe, 1.6 % of S aureus and 49 % of CoNS were oxacillin resistant, whereas 0.9 % of S aureus and 14 % of CoNS had decreased susceptibility to glycopeptides [115].

In conclusion, the efficacy of vancomycin in the treatment of staphylococcal infections has been increasingly questioned, especially in staphylococcal IE therapy [33].

5.4.4 Papers I and V regarding vancomycin therapy in staphylococcal IE

Our data in Paper V suggest that the free vancomycin concentrations decrease at high staphylococcal inocula, leading to an increase in MICs. The endocarditis vegetation present with high bacterial inocula, and penetration problems for vancomycin, have been described. Bacteria deep inside the vegetation often have a reduced metabolic activity, which limits the effect of vancomycin. In our study, dead cell material decreased vancomycin concentrations as efficiently as bacteria in an exponential growth phase, and bacteria in a stationary phase will probably bind glycopeptides and reduce the free vancomycin concentrations in the vegetation as well.
Our epidemiological study (Paper I) showed an increased use of vancomycin that was not based purely on resistance. As in cases with methicillin-resistant IE, vancomycin is recommended when treatment failures or side effects of β-lactam antibiotic therapy are present [27, 28]. For example, daptomycin and linezolid have been suggested as alternative drugs, but the clinical data are limited and both drugs have several shortcomings [19, 116].

Although based on in vitro data, our findings in Paper V indicate that there is a need for careful evaluation of the indications for vancomycin in IE therapy, e.g. in culture-negative cases and when β-lactam antibiotic side effects are present. However, until superior alternatives to vancomycin exist, these findings also indicate that the dosing regime, and thorough monitoring of plasma concentrations, are of great importance when vancomycin is used for the treatment of ascertained or supposed staphylococcal IE.

5.5 Risk factors for 6-month mortality among referred and local left-sided Duke definite IE episodes (Paper VI)

The independent risk factors for 6-month mortality for the referred and local left-sided, Duke definite IE episodes are presented in Table 9 and Table 10, respectively.

Table 9. Independent risk factors for 6-month mortality for 115 referred, left-sided, Duke definite IE episodes

	aOdds ratio[a]	*p* value	95 % CI
Diabetes Mellitus	14.52	0.002	(2.65-79.51)
Moderate or severe heart failure	13.09	<0.001	(3.11-55.18)
Non-cerebral emboli	5.69	0.024	(1.25-25.87)
Cerebral embolism	5.35	0.007	(1.59-17.98)
Early heart valve surgery	0.10	0.002	(0.02-0.42)
Year of admission[b]	0.96	0.417	(0.86-1.06)

[a] Adjusted odds ratio for changes over time
[b] For adjustment of changes over time

Table 10. Independent risk factors for 6-month mortality for 86 local, left-sided, Duke definite IE episodes

	aOdds ratio[a]	*p* value	95 % CI
Diabetes Mellitus	15.21	0.005	(2.25-102.75)
Charlson comorbidity index without DM ≥ 2	10.85	0.002	(2.34-50.37)
Moderate or severe heart failure	4.29	0.040	(1.07-17.22)
Year of admission[b]	0.92	0.169	(0.82-1.04)

[a] Adjusted odds ratio for changes over time
[b] For adjustment of changes over time

5.5.1 Diabetes Mellitus

The prognostic impact of DM in IE has been debated [41, 117, 118], but recent large studies have identified DM as an independent risk factor for mortality [43, 44, 71]. DM has been associated with an increased risk of death from infectious diseases in several [119-121], but not all, studies [122].

The causes of the negative impact of DM in infectious diseases are probably multifactorial. Hyperglycaemia impairs several factors in the immune system, such as chemotaxis, phagocytosis, and the bactericidal activity of neutrophils and macrophages [123-125]. Hyperglycaemia may also stimulate coagulation through activation of the tissue factor pathway [126, 127]. Theoretically, these factors may further impair the local host defence inside the vegetation (see Introduction).

Our diabetic patients were significantly older (73 vs. 60 years; $p = 0.004$), and the frequency of a Charlson comorbidity index without DM ≥ 2 was significantly higher in episodes in diabetic patients compared to patients without DM (38.1 % vs. 16.1 %; $p = 0.031$). Early heart valve surgery was performed in six (28.6 %) of the episodes in the diabetic patients, and two of them died within six months.
Two recent, large IE studies suggest that the poor prognosis for diabetic patients may be due to poorer overall health [43, 44]. Nevertheless, DM was an independent risk factor for 6-month mortality in the multivariable logistic regression which included other comorbidity.

5.5.2 Heart valve surgery in moderate to severe heart failure

Refractory heart failure (HF) is one of the most common causes of death in IE [2]. Consequently, HF has been one of the major indications for early heart valve surgery [2, 34], which was also the case in our study (Paper VI).
Several studies have shown a beneficial effect of early heart valve surgery on moderate to severe HF in IE [34]. In two previous Swedish studies the respective mortality rates for HF patients treated surgically vs. non-surgically were 9 % vs. 20 % [36] and 10 % vs. 27 % [128]. In our study the 6-month mortality was 18.9 % (10/53) among surgically treated patients with moderate to severe HF compared with 46.9 % (15/32) for non-surgically treated patients ($p = 0.008$). This disparity in comparison to other Swedish studies may be caused in part by a longer time frame covered, the assessment of 6-month mortality instead of in-hospital mortality, and maybe different definitions of HF. Of the 15 patients with moderate to severe HF who died without surgery, 11 had contraindications to surgery, and it seems that surmounting contraindications to surgery in such cases is of vital importance.

5.5.3 Charlson comorbidity without DM

The Charlson comorbidity index was presented in 1987 [129]. A more recent index has been suggested that includes age [130]. We chose to use the older index due to its previous use in prognostic IE studies [40, 131], and have addressed age separately in our multivariable analysis. Comorbidity was an independent risk factor for 6-month mortality, particularly in the study by Hasbun et al [40], and it seems essential to include this variable in prognostic IE studies.

5.5.4 Early heart valve surgery in IE

The increased use of early heart valve surgery seen in our study, and its beneficial effect on the decreased overall mortality seen in IE over recent decades, has been generally accepted [2, 34]. Nevertheless, two of the most recent studies on this issue demonstrate contradictory results concerning the positive effect of early heart valve surgery in IE [49, 71]. Of special interest is the afterword to the study by Tleyjeh et al [49] stating:

"*Whether to recommend valve surgery in a patient with left-sided infective endocarditis (IE) can be one of the most difficult management conundrums in the treatment of infectious diseases. Because of the uncommon occurrence of IE, no randomized controlled trials have been conducted to better clarify its role in IE management.*

……

Given the disparity between the results of this study and those of other observational studies, well-designed prospective investigations that address the methodological limitations are needed to further evaluate the role of valve surgery in IE management and to define the group of patients that would benefit from this intervention."

This highlights the difficulties with studies on the impact of early heart valve surgery in IE and the need for large, multicenter-based studies that re-address areas such as the indications and timing of surgery, how to handle contraindications to surgery, and the unresolved issue of the length of antimicrobial therapy after heart valve surgery.

5.5.5 "Referral bias"

Our patients with referred episodes were significantly younger ($p = 0.002$), had a higher frequency of CoNS ($p = 0.020$), moderate to severe heart failure ($p = 0.043$), cerebral embolism ($p = 0.038$), and early heart valve surgery ($p < 0.001$), and a lower frequency of therapy with betalactam antibiotics ($p = 0.008$).

More importantly, however, only DM and moderate to severe heart failure were independent risk factors for 6-month mortality in both the referral and the local cohort of IE episodes. The other risk factors presented in Table 9 and Table 10 must be considered with extra caution, since referral bias is a well-known problem when undertaking endocarditis prognostic studies, especially when dealing with the impact of heart valve surgery [71, 132].

6 CONCLUSIONS

1. Between 1980 and 2001 the microbiological cause of IE has shifted towards S aureus at our hospital, regardless of referred or local origin of the IE episodes. The use of transoesophageal echocardiography has increased. Glycopeptide antibiotics, mainly vancomycin, are now used more frequently, especially among referred patients. The most important therapeutic change is the increased use of early heart valve surgery, both in S viridans/bovis and S aureus IE (Paper I).
   Referred patients were younger, predominantly male, had more complications, and received surgical treatment more often than patients from our local community. The reason for the lower frequency of female patients in the referral cohort cannot be explained by more comorbidity or fewer complications (Paper I). The differences between referred and local episodes seen in our study highlight the need for assessment and adjustment for referral bias in IE studies.

2. In six patients who needed early heart valve surgery, the largest numbers of IL-8-containing cells, and the greatest amount of inflammation, were seen in patients with short preoperative antimicrobial treatment courses. No such relationships were seen with respect to TNF-α-containing cells. The IL-8-containing cells and the inflammatory cells were predominately scattered in the heart valve stroma or in the margin of the vegetation (Paper II). The primary effect of IL-8 is to stimulate chemotaxis of polymorphonuclear neutrophil granulocytes. This indicates that there is no deficiency of IL-8 in the area close to the vegetation as a cause of the localised agranulocytosis often present inside the vegetation.

3. Our study displayed a need for computerised decision support systems in the field of IE, but to be used in clinical practice these DSSs need to be part of knowledge bases covering larger domains (Paper IV). Some of our initial ideas described in Paper III, especially the use of Internet technology and the combination of rule-based advice and explanatory hypertext, will probably be included in these future knowledge bases.

4. Vancomycin is now used more frequently at our hospital, especially in PVE and culture-negative IE episodes (Paper I). In vitro, there is a rapid reduction of free vancomycin in Mueller-Hinton broth containing dense staphylococcal cultures. Consequently there is a simultaneous increase in broth MICs, particularly in high inocula, without development of resistance (Paper V). These findings need further evaluation in vivo, but indicate that the dosing regimen of vancomycin is of particular importance in staphylococcal infections with dense inocula, e.g. infective endocarditis. Together with the increased number of reports of vancomycin failures in the treatment of IE, the indications for vancomycin in each case of IE must be critically evaluated.

5. Diabetes mellitus was an independent risk factor for 6-month mortality in both referred and local left-sided, Duke definite IE episodes. Moderate to severe heart failure was the most common indication for heart valve surgery, but was nevertheless an additional independent risk factor for 6-month mortality in both cohorts. Early heart valve surgery had a positive impact on the 6-month mortality in the referral cohort of episodes, which may be due to referral bias. (Paper IV).

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