Department of Infectious Diseases
The Sahlgrenska Academy at Göteborg University, Sweden

BLOOD CULTURE NEGATIVE ENDOCARDITIS

Maria Werner

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TO MY PARENTS

"HAVET BLIR STÖRRE AV EN ENDA DROPPE"

1 ABSTRACT

The lethal disease infective endocarditis (IE) is caused by microorganisms that attack heart valves. Early diagnosis and identification of the causative agents are important for the choice of treatment. Optimal treatment may be difficult to achieve if blood culture negative endocarditis (CNE) is present. This study was designed to estimate the prevalence of CNE, analyze clinical data from CNE patients, and to evaluate different diagnostic criteria. Further purposes were to evaluate the antibiotics used as treatment, to study serological evidence for fastidious bacterial infection and to assess the association of *Chlamydophila pneumoniae (C. pneumoniae)* antibodies with an increased risk of development for IE.

We analyzed data from presumptive IE patients in clinics at Borås (n=70) (Paper I) and Göteborg (n= 750) (Paper I, II, III) and at the Swedish Endocarditis registry (n=2509) (Paper IV). Serum samples from Göteborg IE patients were tested for the presence of *Bartonella*, *Coxiella burnetii* and *C. pneumoniae* antibodies. Samples from controls selected from the same geographic population were searched for antibodies to *C. pneumoniae*.

Twelve to 27% of all IE episodes were CNE with a mortality of 5-7 %. Antibiotic treatment preceded blood culturing in 45% of the episodes. Women died significantly more often than men with this disease (odds ratio 5.5). For establishing IE diagnosis, the Duke definite criteria were more sensitive but probably less specific than the Beth Israel criteria.

One patient had serologically verified Q-fever IE, but no *Bartonella* was detected. The proportion of *C. pneumoniae* antibodies did not differ significantly in patients with CNE from those with blood culture positive IE. However amounts of *C. pneumoniae* IgA and IgG were significantly higher in women with IE than in the female controls.

The mortality rate was significantly lower in CNE patients treated with aminoglycosides.

CNE occurred in 12-27% of IE patients reviewed here, but antibiotic treatment preceding blood culture diminished the validity of negative test results. Fastidious bacteria were identified mainly by testing with antibodies, yet interpretations of such results are difficult. Clearly, additional methods are needed for diagnosing CNE.

Key words: Infective endocarditis (IE), blood culture negative endocarditis (CNE), blood culture, diagnosis, mortality, *Bartonella, Coxiella burnetii, Chlamydophila pneumoniae*, prevalence, aminoglycoside.

LIST OF PUBLICATIONS

I. M. Werner, R. Andersson, L. Olaison, H. Hogevik. A clinical study of culture negative endocarditis. Medicine. 2003;82:263-73

II. M. Werner, P.-E. Fournier, R. Andersson, H. Hogevik, D. Raoult. *Bartonella* and *Coxiella* antibodies in 334 prospectively studied episodes of infective endocarditis in Sweden. Scand J Infect Dis. 2003;35:724-7

III. M. Werner, J. Gnarpe, A. Odén, R. Andersson, H. Hogevik. *Chlamydia pneumoniae* infection: a risk factor for infective endocarditis? (Submitted)

IV. M. Werner, R. Andersson, L. Olaison, H. Hogevik. A ten-year survey of blood-culture negative infective endocarditis in Sweden-Aminoglycoside therapy is important for survival. (Submitted)

CONTENTS

LIST OF ABBREVIATIONS

aOR	Adjusted odds ratio
CNE	Blood culture negative endocarditis
CPE	Blood culture positive endocarditis
FAN	Aerobic and anaerobic media composed of brain heart infusion broth and Ecosorb (contains absorbent charcoal and Fullers earth)
HACEK	*Hemophilus spp, Actinobacillus acinetocomitans, Cardiobacterium hominis, Eikinella, Kingella kingae*
ICD-9	International Statistical Classification of Diseases, Ninth revision
IE	Infective endocarditis
IVDU	Intravenous drug user
MIF	Microimmunofluorescence
NBTE	Nonbacterial thrombotic endocarditis
NVE	Native valve endocarditis
OR	Odds ratio
PCR	Polymerase chain reaction
PPV	Positive predictive value
PVE	Prosthetic heart valve endocarditis
RF	Rheumatoid factor
TEE	Transesophageal echocardiography
TTE	Transthoracal echocardiography

2 INFECTIVE ENDOCARDITIS (IE)

IE can result when microorganisms (bacteria and fungi) in the blood infect the heart valve(s) and form a vegetative growth consisting mainly of fibrin, platelets and the microorganisms. These vegetation constituents may embolize to such organs as the brain, skin, lung, central nervous system and kidney causing diverging symptoms. The bacteremia may cause septic symptoms. IE is lethal if not adequately treated. The incidence in Sweden of IE has been estimated at 5.9 /100,000 yearly (1). In spite of improved methods in blood culturing and echocardiography, and the introduction of increasingly accurate diagnostic criteria, IE is still difficult to diagnose (2, 3). Additionally, the diversified symptoms may attract the clinician's attention to other potential diseases including stroke, pneumonia, heart insufficiency, pulmonary embolism, meningitis, nephritis, urinary infection or collagenosis.

2.1 BLOOD CULTURE NEGATIVE ENDOCARDITIS (CNE)

Positive blood cultures are corner stones in diagnosing IE. However, CNE has been reported during recent decades in population-based epidemiological studies as well as hospital studies world wide ranging from 5 to 56% of all cases of IE (1, 4-13). When estimating the proportions of CNE, factors such as diagnostic criteria, demographic information, environmental factors and the health care structure must be considered. Numerous other factors may contribute to CNE such as antibiotic therapy started before blood culture (6, 11, 14, 15) and the presence of fastidious bacteria, for example *Bartonella, Coxiella, Legionella pneumophila* (16) and *Tropheryma whipplei* (17) all of which need diagnostic techniques other than blood culture. Nonbacterial thrombotic endocarditis (NBTE) in patients with cancer or autoimmune disease and hypercoagulability (18-20) is also a major reason for CNE.

2.2 CULTURE, HISTOLOGY AND MOLECULAR GENETIC METHODS

2.2.1 Blood culture

In the 1880s blood culturing in clinical practice was initiated by the French physician Jacques Dolores (21), advised by Louis Pasteur, who was the first to perform this procedure. The blood was collected by a pinprick on the index finger with a sterile technique. At that time, however, microbiologists had not overcome the technical problems of blood culturing and were not aware that the amount of blood from a pinprick provided too small a sample for culturing.

The modern microbiological laboratory uses automated systems analyzing 20 ml of blood from every puncture. The optimal volume for detecting the small concentration of bacteria in patients with IE has been estimated at three 20 ml samples of blood (60 ml) drawn before the initiation of antibiotic therapy (22-24). If no bacterial growth occurs, blood cultures from IE patients often are incubated for a longer period than the usual 7 to10 days in an attempt to detect bacteria from the *Hemophilus spp, Actino-bacillus acinetocomitans, Cardiobacterium hominis, Eikinella, Kingella kingae* (HACEK) group. However, recent studies have shown that prolonged incubation is no more sensitive for detecting these bacteria than the routine five days of incubation (25-27).The molecular genetic methods such as polymerase chain reaction (PCR) for detecting bacteria in blood are still not reliable in clinical practice (28).

2.2.2 Valvular culture, histology and molecular genetic methods

When diagnosing IE (2, 3), examination of the heart valve is considered as the gold standard. If present, pathogens can be identified in resected valves from IE patients with culture including Gram stain (29), histologic methods including histochemistry (30) and / or molecular genetic methods such as PCR (31-33). These procedures are often successful in identifying microorganisms in vegetations because of their large concentration. However, valvular culture has low sensitivity (13%) (32), (15%) (34), low negative predictive value for detecting microorganisms (56%) (32) and low specificity (35). Cultures may also have false-positive outcomes due to contamination of the resected valves at surgery (35). Regardless of the testing procedure(s) used,

making a clinical diagnosis that IE is or is not present during operative valve resection is important. Microorganisms detected in valves by PCR and histopathology may have persisted at that site months after the full recovery from IE (29, 36-38).

2.3   FASTIDIOUS BACTERIA IN CNE

2.3.1 *Bartonella*

Bartonella quintana causes trench fever, a five-day relapsing fever accompanied by severe pain in the shins. Although seldom fatal, this condition disables patients for a long time. Humans are the only proven reservoirs of the bacterium (39), and the body louse is the vector of the disease. Trench fever was endemic amongst the troops on all European fronts during World War I, then re-emerged as an epidemic disease mainly at the Eastern front during World War II (40). The last Swedish patient known to contract this disease was a farmer from Northern Sweden in close contact with Finnish refugees during World War II (41).

Trench fever emerged anew as CNE in homeless persons at the end of the 20th century in France (42) and North America (43) with *B. quintana* being isolated from these patients. Bacillary angiomatosis caused by *B. quintana* has been described in immunocompromised hosts (44). Moreover, antibodies to *B. quintana* have been found amongst homeless people in France (45).

Bartonella henselae causes cat-scratch disease, which is characterized by a skin lesion after a cat-scratch or cat-bite and regional lymphadenopathy. *B. henselae* has been found in patients with CNE , bacillary angiomatosis or peliosis hepatitis in immunocompromised hosts (46). The reservoir of this disease is probably the cat. The infection is spread directly from cat to human or indirectly via a vector, probably the cat flea (*Ctenocephalides felis*) (47). Sometimes *Bartonella* is detected in cultured blood when the incubation time is prolonged, but other diagnostic methods are more reliable such as the shell-vial technique (48), molecular methods or serology (26, 49).
 A genotypic variation of *B. henselae*, serotype *"Marseille"* has been isolated from patients with endocarditis and cat-scratch disease. Antibodies to this serotype have been shown in patients with cat-scratch disease who were seronegative to *B. henselae* (50,

51). At the start of our study, no cases of *Bartonella* endocarditis had been reported in Sweden.

2.3.2 Coxiella burnetii (C. burnetii)

Q-fever, a rickettsiosis caused by *C. burnetii,* is a zoonosis diagnosed worldwide. The bacterium is usually transmitted as an inhaled aerosol that reaches humans via inhalation of parturient fluid from infected animals. The bacteria can survive for long periods in areas where animals have been present and even the wind can transmit this infection to humans. Endocarditis is the most severe form of chronic Q-fever sometimes recognized in CNE patients. Patients with recurrent CNE in prosthetic valves may have chronic Q-fever (52). In Sweden sporadic cases of domestic Q-fever have been reported (53) but not in relation to IE. However, seroepidemiological studies from all over the country have revealed antibodies to *C. burnetii* (54, 55). This infection is diagnosed preferably with serology although valve culture, histopathology and molecular methods also detect *C. burnetii.*

2.3.3 Chlamydophila (Chlamydia) pneumoniae (C. pneumoniae)

Although *C. pneumoniae* has been described as an agent of CNE (56, 57), its role in IE remains debatable, because *Bartonella* and *C. pneumoniae* cross-react serologically. In addition, an association between *C. pneumoniae* and arteriosclerosis as well as valvular disease has been suggested (58-65). No validated reference test is universally agreed upon for the diagnosis of persistent *C. pneumoniae* infection. The microimmuno-fluorescence (MIF) test, considered to be the gold standard, is technically complex and diagnostic criteria can differ from laboratory to laboratory (66).

One can expect to detect IgG antibodies in serum for many years after an infection, and serum IgG of ≥512 may indicate either a possible chronic infection or a recent infection with the bacterium. IgA antibodies have a short half-life (about one week); therefore, high levels of this immunoglobulin more likely denote an acute or sustained infection, although this criterion is not a universally accepted (66). Tissue cell culture, molecular methods and histochemistry also detect *C. pneumoniae.*

2.4 ECHOCARDIOGRAPHY

After its invention in the 1970s, echocardiography was quickly introduced into medical practice to investigate suspected cases of IE (67). The first study of this method's impact in diagnosing CNE was published in 1981 (68). Transthoracal echocardiography (TTE) based on sound transmission through the thoracic wall may allow visualization of vegetations and heart function. The subsequent development of transesophageal echocardiography (TEE) with the sound-emitting probe in the esophagus closer to the heart improved such transmissions substantially. With its higher sensitivity TEE detects small vegetations, infection of prosthetic heart valves, infected pacemaker leads and perivalvular abscesses. However, TTE has improved with the second harmonic imaging (69-71). TEE is a cost-effective investigative tool (72) if the prior probability of IE is high. In a retrospective study, however, TTE was not effective in screening patients with little likelihood of having IE (73).

2.5 DIAGNOSTIC CRITERIA

IE is a difficult disease to diagnose because of its diverse manifestations. The Beth Israel criteria (3), introduced in 1981, were the first established guidelines for diagnosing IE on strictly defined clinical grounds. The introduction of echocardiography made it possible to visualize the heart, which was positive as a method was needed to enhance the diagnostic power for IE. Echocardiographic findings were added to the Beth Israel criteria in, for example, Göteborg, Sweden and France (modified Beth Israel criteria) (1, 5). The Duke criteria (2) published in 1994 combined echocardiographic findings with clinical criteria and are now established diagnosing IE. Numerous studies have compared the Beth Israel criteria (not modified) and Duke criteria in different settings (74-76), and most judge the Duke criteria as the more sensitive of the two. Li and coworkers revised the Duke criteria in the year 2000 (77).

2.6 ANTIBIOTIC THERAPY IN CNE

Treatment for CNE is difficult, and studies about antibiotic treatment for such patients are virtually nonexistent. The specialized diagnostic procedures described above are often time-consuming. Furthermore, the necessity of treating these patients with potentially harmful drugs such as aminoglycoside and the lack of knowledge about which microorganism to treat are frustrating for the responsible clinician. The Swedish Guidelines for IE (78) recommend the continuation of empiric therapy: for native valve endocarditis (NVE), penicillin-G for four weeks and aminoglycoside for two weeks and for PVE (prosthetic valve endocarditis), vancomycin, aminoglycoside and rifampicin for four to six weeks. The difficulties in treating CNE are also mirrored in the newly published consensus documents about IE from the US (79) and Europe (80). There are treatment suggestions for *Bartonella* IE (79) and chronic Q-fever (81), but comparative studies for treatment regimens in these diseases are missing .

No specific information about CNE in Sweden was available at the start of the present study; however, access to data registries for IE and collections of sera from IE patients gave us an opportunity to investigate this rare condition**.**

THE OBJECTIVES OF THE STUDY

1. Describe and analyze clinical data from CNE patients and estimate the prevalence of CNE in IE episodes (Papers I and IV).
2. Evaluate the Duke and the modified Beth Israel criteria in CNE patients. (Paper I).
3. Describe the antibiotic treatments used in CNE patients related to mortality and relapse in IE (Paper IV).
4. Analyze serological signs of *Bartonella, C. burnetii* or *C. pneumoniae* infection associated with CNE (Papers II and III).
5. Compare the assessed levels of *C. pneumoniae* antibodies in male and female IE with controls from the general population (Paper III).

3 PATIENTS AND METHODS

3.1 STUDY DESIGN

3.1.1 Paper I

To assess the incidence and characteristics of CNE in patients with IE, individuals (adults >18 years) were recruited at the Department of Infectious Diseases, Göteborg, Sweden between 1984 and 1996 and Department of Infectious Diseases, Borås, Sweden from 1989 to 1996.

For the purposes of this program, the term "episode" was defined as a hospital admission for the treatment of IE. Two hundred and thirty-three episodes of presumptive IE (217 patients) were prospectively and consecutively studied at the Göteborg clinic during 1984-88. One hundred and sixty-one of these episodes were diagnosed as apparent IE. For 517 episodes of suspected IE, 460 patients were recruited in Göteborg between 1989 and 1996. Of these, 145 episodes were classified according to the Duke criteria (2) as "definite," 111 as "possible" and 261 as "rejects." Antibiotic combination therapy started in 363 of the 517 episodes. Records of 237 episodes in patients discharged with a diagnosis classified as IE (ICD-9) at the Göteborg clinic, from 1989 through 1996, were also evaluated to seek CNE patients not registered in the prospective study. At Borås an IE diagnosis (ICD-9), was found in a patient group that included 70 episodes.

The information derived was used for a non-randomized descriptive study of consecutive patients with CNE treated as IE according to a protocol that included uniform antibiotic regimens, clinical evaluation procedures and the collection of specimens including blood cultures.

Routinely, patients underwent echocardiography during the first week of treatment and, if indicated, repeated at the fourth week. The location of IE was determined by surgery, autopsy or echocardiography.

Patients' demographic data, histories of suspected risk factors and other relevant events were extracted from records and registered in a standardized questionnaire. All CNE episodes were evaluated with the modified Beth Israel criteria (1) (Table 1). We categorized the CNE episodes according to the Duke criteria (Tables 2 and 3).

3.1.2 Paper II

Seven hundred and fifty episodes were considered as presumptive IE in the Göteborg clinic during the years 1984 to 1996 (Paper I). These patients' blood samples were collected at inclusion into the study, and sera were stored at -70°C. Sera from patients initially included in the study between 1984-1988 but later classified as "rejects" according to the modified Beth Israel criteria for IE were discarded (1). Some other samples were destroyed during storage. Finally, the 334 remaining sera from 334 episodes in 329 patients were examined. All records from these IE episodes were evaluated and classified according to the Duke criteria.

3.1.3 Paper III

Patient selection and blood sampling were done in the same manner as described in Paper II. Here, data for 314 episodes in 308 patients were analyzed for IE according to the Duke criteria and categorized as "definite," "possible" or "reject (2). Results from the 27 episodes classified as "reject" were excluded from further analysis. Serum samples were taken at the start of IE therapy from all but four patients.

The Longitudinal Gerontological and Geriatric Studies in Göteborg, Sweden, initiated in 1971/72 included 1148 70-year-old men and women who were randomly chosen from the same geographic population without regard of their state of health (82). The controls were a random sample from this group, which comprised of 102 men and 142 women whose sera were analyzed for *C. pneumoniae* antibodies. In 1992, another randomly selected sample of 753 70-year-olds was added to the study, and sera samples from 76 men and 106 women in this second group were also analyzed for *C. pneumoniae* antibodies (83).

3.1.4 Paper IV

During the ten-year period 1995-2004, 2546 episodes of IE were registered in a national Swedish registry (84) organized by the Swedish Society for Infectious Diseases. All 29 departments of infectious diseases in Swedish hospitals participated. For the present study, 37 episodes listed in this registry were excluded because of duplication, scarcity of data, lack of blood cultures or rejection by the Duke criteria. Data incorporated into this paper came from the remaining 2509 episodes (2410 patients).

Data were filled in standardized questionnaires that each patient's physician completed at the time of his or her hospital discharge. The form included questions about the patient's age, gender, clinical signs and predisposing factors for IE. Also required were data about the current episode of IE. At the follow-up visit, the physician filled in a second form answering questions about morbidity and mortality. The doctors treated their patients in accordance with local recommendations. The "Guidelines in Infective Endocarditis from The Swedish Society of Infectious Diseases" (78) included recommendations from 2004 regarding therapy for CNE.

Data from the questionnaires were entered into a computerized database and categorized according to the Duke criteria (2). Our analyses then answered questions about demography, predisposing factors, treatment and outcome.

Paper I
15 episodes
1989-96 Göteborg

Paper I
70 episodes
1989-96 Borås

Paper I
820 episodes
1984-96 Borås and Göteborg

Paper II
334 episodes
1984-96 Göteborg

Paper III
314 episodes
1984-96 Göteborg

Paper IV
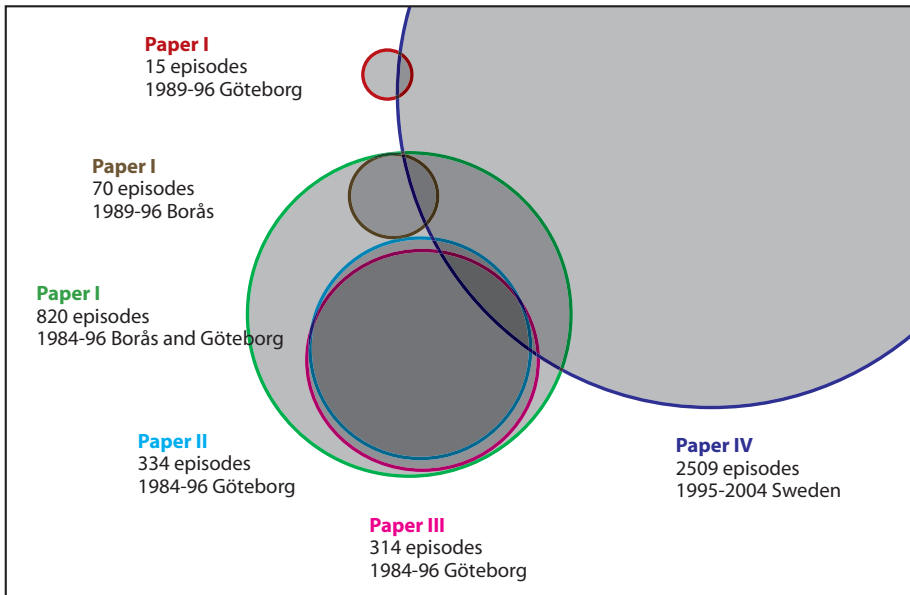2509 episodes
1995-2004 Sweden

FIGURE 1. *Shows the division between studies I, II, III and IV.*

3.2 METHODS

3.2.1 Definitions

We defined

- **An episode** as an admission to hospital for treatment of IE, regardless of the time interval since any previous episode and the first day of an episode as the first day of intravenous antibiotic combination treatment.
- **CNE** as IE without bacterial growth in blood culture.
- **Mortality** during treatment as death during parenteral antibiotic therapy calculated in episodes and
    - within the first month after completed treatment (Paper I)
    or
    - registered in questionnaire one (Paper IV).
- **Possible relapse** of IE as a new event of IE during the six months after completed IE treatment.
- **Acute surgery** as cardiac surgery performed during the period of parenteral antibiotic treatment.

3.2.2 Diagnostic criteria

The modified Beth Israel criteria (1, 3), and Duke criteria (2) were applied in Paper I. (Tables 1, 2 and 3)

TABLE 1. *Modified Beth Israel criteria (1, 3), further modified (only patients without bacterial growth at blood culture were accepted as culture negative).*

Definite infective endocarditis.

Episodes in patients from whom the diagnosis was verified at surgery or autopsy by culture, microscopy

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Probable infective endocarditis.

At least 2/3 positive blood cultures and one of the following factors characterize culture-positive endocarditis: A new cardiac murmur, predisposing heart disease[1] with vascular phenomena[2] and/or sonographic evidence of vegetation.

Culture-negative endocarditis is characterized by negative blood cultures plus fever, new cardiac murmur and vascular phenomena[2] or sonographic evidence of vegetation

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Possible infective endocarditis.

Culture-positive endocarditis is characterized by at least 2/3 positive blood cultures and one of the following: Predisposing heart disease [1] and /or vascular phenomena [2]

Culture-negative endocarditis is characterized by negative blood cultures and all of the following: Fever, predisposing heart disease[1] and vascular phenomena [2] alternatively sonographic evidence of vegetation

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Rejected

(A). Endocarditis unlikely, alternative diagnosis generally apparent

(B). Endocarditis likely, empirical antibiotic therapy warranted

(C).Culture-negative endocarditis diagnosed clinically, but excluded by postmortem examination

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[1] Valvular or congenital heart disease or valvular prosthesis.

[2] Petechiae, splinter hemorrhages conjunctival hemorrhages, Roth spots, Osler's nodes, Janeway lesions, aseptic meningitis, glomerulonephritis and pulmonary, central nervous system, coronary or peripheral emboli

TABLE 2. *Duke criteria (2)*.

Definite infective endocarditis.

Pathological criteria: Microorganisms demonstrated by culture or histology in a vegetation, or in a vegetation that has embolized, or in an intracardiac abscess, or pathological lesions: vegetation or intracardiac abscess present, confirmed by histology showing active endocarditis

Clinical criteria, using specific definitions (listed in Table 3):

Two major criteria, or one major and three minor criteria, or five minor criteria

Possible infective endocarditis. Findings consistent with infective endocarditis that fall short of "definite", but not rejected

Rejected.

Firm alternative diagnosis for manifestations of endocarditis, or resolution of manifestations of endocarditis with antibiotic therapy for four days or less, or no pathologic evidence of infective endocarditis at surgery or autopsy after antibiotic therapy for four days or less

TABLE 3. *Definitions of terminology used in the Duke criteria* **(2).**

Major criteria

Positive blood culture for infective endocarditis

Typical microorganism for infective endocarditis from two separate blood cultures:

(i) *Streptococcus viridans* [3], *Streptococcus bovis*, HACEK[4] group, or

(ii) community-acquired *Staphylococcus aureus* or enterococci, in the absence of a primary focus, *or*

Persistently positive blood culture, defined as recovery of a microorganism consistent with infective endocarditis from:

(i) blood cultures drawn more than 12 h apart, or

(ii) all of three or a majority of four or more separate blood cultures, with first and last drawn at least one hour apart

Evidence of endocardial involvement

Positive echocardiogram for infective endocarditis:

(i) Oscillating intracardiac mass on valve or supporting structures, or in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation or

(ii) abscess or

(iii) new partial dehiscence of prosthetic valve, or

New valvular regurgitation (increase or change in pre-existing murmur not sufficient)

Minor criteria

- Predisposition: predisposing heart condition or intravenous drug use
- Fever: ≥38.0°C (100,4 °F)
- Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhage, Janeway lesions
- Immunologic phenomena: glomerulonephritis, Osler's nodes, Roth spots, rheumatoid factor
- Microbiologic evidence: positive blood culture but not meeting the major criterion noted previously[5] or serologic evidence of active infection with organism consistent with infective endocarditis
- Echocardiogram: consistent with infective endocarditis but not meeting the major criterion noted previously

[3]Including nutritional variant strains. [4]HACEK: *Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis, Eikenella* spp., and *Kingella kingae*. [5]Excluding single positive culture for coagulase-negative staphylococci and organisms that do not cause endocarditis

3.2.3 Blood culture

Papers I, II and III

According to the protocol, a standardized series of three blood cultures were drawn with three separate venous punctures with intervals of at least 20 minutes. In Göteborg, one aerobic and one anaerobic bottle (the biphasic model modified Castaneda system) were used at every blood culture when the study began in 1984. The bottles contained 2.5 ml of blood each in the first 21 months of the study (altogether 5 ml). This procedure changed to an increased blood volume of 5 ml per bottle (altogether 10 ml) in 1986.

The BACT / ALERT SYSTEM (Organon Teknika Corp, Durham, NC, US) was introduced for specimens from most Göteborg patients in the study in 1993. 20 ml of blood was divided into one aerobe and one anaerobe bottle at every blood culture. FAN (aerobic and anaerobic media composed of brain heart infusion broth and Ecosorb (contains absorbent charcoal and Fullers earth) media were introduced in 1995 instead of standard media. All blood cultures collected in Göteborg were incubated for 10 days. Blood samples from the Borås patients (1989-96) were cultured in the SIGNAL system of OXOID (Basingstoke, United Kingdom). In this system, only one bottle containing 10 ml of blood was used on every blood culture occasion. The incubation time was 7 days.

Paper IV

The local departments of microbiology, most often situated at the same hospital as the Infectious Diseases Departments, analyzed the blood cultures, which were sent to them. The automated blood culture systems most often used during the period 1995-2004 were the BacT/Alert and BACTEC systems. About 20 ml blood in every draw was divided into one aerobe and one anaerobe bottle at least twice but usually three times in every episode. The blood was incubated for 7 to10 days before test results were confirmed as culture negative.

3.2.4 Serology

Paper II

Bartonella* and *C. burnetii

One serum sample collected from each IE patient before therapy began was tested for antibodies to *B. quintana, B. henselae*, *C. burnetii*. *B. quintana*, Oklahoma strain, and *B. quintana,* Marseille strain isolated from a French patient (ATCC 49882T). *B. henselae*, serotype Marseille B, and *B. henselae*, Houston-strain were used as antigens. The bacteria taken between the fourth and seventh passages in a human endothelial cell line were harvested, pelleted and used as crude antigen in a MIF assay (85, 86). The current cut-off levels for diagnosis of *Bartonella* endocarditis has been estimated at $\geq$ 800 IgG antibodies with a positive predictive value (PPV) of 0.955 in patients with IE (87). Patients with titers between 50 and 200 may have been in contact with cats and have a residual positive serology (86). Q-fever serology was performed with a MIF assay to identify antibodies to *C. burnetii* antigen [29] . The cut-off level for diagnosing chronic Q-fever was phase I IgG $\geq$ 800 with a PPV of 0.98.

Paper III

C. pneumoniae

For analysis with a modified MIF (88), patients' sera were diluted 1:16 (for IgM and IgA antibodies) and 1: 32 (for IgG antibodies) in phosphate buffered saline pH 7.4, and tested for IgG, IgA and IgM antibodies on 21- well antigen slides containing elementary body preparations of *C. psittaci*, *C. pneumoniae* and *Chlamydia trachomatis* in each well (Lab Systems Oy, Finland), as previously described (88). Sera positive in screening tests for IgG were rediluted and tested in doubling dilutions. Sera positive in screening tests for IgA and/or IgM were mixed with Gullsorb (Gull Laboratories, US) at a dilution of 1:16 to remove all IgG and then titrated in doubling dilutions with PBS (89). The same investigator blinded to case/control status read all slides. Control sera routinely used in the laboratory were included in every test run, and test results were accepted only when the control sera were within one titer step of the mean calculated earlier. The last dilution step to give a specific fluorescent pattern was reported as the reciprocal titer (88).

A *C. pneumoniae* titer of IgG antibodies ≥512 and a *C. pneumoniae* IgA ≥64 antibody titer were chosen as cut-offs for presumptive acute reinfection or persistent infection (89).

3.2.5 Echocardiography

Paper I

The Göteborg patients were examined at the Department of Clinical Physiology, Sahlgrenska University Hospital and the Borås patients at the Department of Cardiology, Södra Älvsborgs Hospital. The investigators used exclusively the TTE method until the end of the 1980s when TEE was introduced. This latter method was added increasingly to the diagnostic procedures during the study period until, by the program's end, most patients underwent TEE.

Paper IV

Echocardiography was performed with TTE and/or TEE at the local hospital according to local and general recommendations from the *Guidelines in Infective Endocarditis* from The Swedish Society of Infectious Diseases (78).

3.2.6 Statistics

Values of quantitative variables were expressed as means, medians and range (in brackets []) in all papers.

Paper I

Confidence limits (95% level) were given in brackets [95% CI]. The Chi square test was used to test the difference between proportions. A significance level of 0.05, two-tailed test was used. Yates correction was applied when applicable.

Paper II

No calculations performed.

Paper III

The probability of *C. pneumoniae* IgG ≥512 and *C. pneumoniae* IgA ≥64 in persons who are 70 years old was estimated by using a logistic regression analysis. Adjusted

odds ratios (aOR) were calculated taking age into account by using the multiple logistic regression. Confidence limits (95% level) were given in brackets [95% CI].

Paper IV

Odds ratios (OR) were calculated with 95% confidence limits [95% CI] or exact confidence limits when needed (StatCalc, EpiInfo-6). The differences in mortality in patients with or without aminoglycoside therapy were compared with an aOR (adjusted for age and gender) using the multiple logistic regression analysis (EpiINFO). A significance level of 0.05, two-tailed test was used. Yates correction and Fishers exact test were used when appropriate.

4 RESULTS AND DISCUSSION

4.1 THE PROPORTION OF CNE IN SWEDISH IE PATIENTS, A DESCRIPTION AND AN ANALYSIS OF CLINICAL DATA IN CNE PATIENTS (PAPERS I AND IV)

The proportions of CNE in episodes of IE in Sweden appear in Table 4. Paper I records 116, and Paper IV encompasses 304 such episodes. Studies from several countries world wide estimating the proportion of CNE in IE episodes are documented in Table 5, and Table 6 depicts patients' demographic and clinical features as well as comparing blood culture-negative and -positive IE. The mortality rates in men and women are compared in Table 7.

TABLE 4. *The estimated proportions of CNE in different Swedish populations (Papers I and IV).*

PLACE	TIME	ALL IE EPISODES	CNE EPISODES (%)	95% CI (%)
Göteborg	1984-88	161 (Paper I)	34 (21)	[15-28]
Göteborg	1989-96*	256 (Paper I)	48 (19)	[14-24]
Borås	1989-96	70 (Paper I)	19 (27)	[18-37]
Sweden	1995-2004*	2509 (Paper IV)	304 (12)	[9-15]

IE (infective endocarditis), CNE (blood cultures negative for IE), CI (confidence interval),

*Duke definite and possible episodes only.

TABLE 5. *The proportion of CNE in IE patients in different study populations.*

	IE EPISODES (N=)	PERCENTAGE CNE OF ALL IE EPISODES	YEAR (S)	DIAGNOSTIC CRITERIA
Göteborg, Sweden secondary and tertiary referral center (1)	99	12	1984-1988	Von Reyn (modified with echocardio-graphic findings)
France (multi-center) (5)	620	10	1990-1991	Von Reyn (modified with echocardio-graphic findings)
France (multi-center) (11)	390	5	1999	Duke definite
Beirut, Lebanon (9)	91	23	1986-2000	Duke definite and possible
Athens, Greece (7)	101	18	1997-2000	Duke definite and possible
Cape Town, South Africa (tertiary referral center) (8)	60	55	1997-2000	Duke definite
Copenhagen, Denmark (tertiary referral center) (13)	132	18	1998-2000	Duke definite and possible
Marseille, France (secondary and tertiary referral center) (6)	170	10	1994-2000	Duke definite
Argentina (multi-center) (4)	390	11	2001-2002	Duke definite
Alger, Algeria (secondary and tertiary referral center) (10)	110	56	2000-2003	Duke definite and possible
Turku, Finland, (tertiary referral center) (12)	155	20	1995-2004	Duke definite and possible
Göteborg, Sweden prospective (secondary and tertiary referral center) (90), Paper I	256	19	1989-1996	Duke definite and possible
Sweden (multi center), Paper IV	2509	12	1995-2004	Duke definite and possible
Sweden (multi-center), Paper IV	1952	4	1995-2004	Duke definite

TABLE 6. *Patients' demographic and clinical features: comparison of blood culture negative (CNE) and blood culture positive endocarditis (CPE).*

PATIENTS	CNE GÖTEBORG, BORÅS - PAPER I (N=116)	CNE SWEDEN PAPER IV (N=304)	CPE SWEDEN PAPER IV (N=2205)
Females	50%	42%	35%
Age in years, median [range]	67 [19-85]	68 [17-89]	66 [14-100]
Prosthetic valve	24%	19%	18%
Previous endocarditis	13%	10%	10%
Injection drug use	1%	3%	12%
Aortic valve infection	42%	50%	38%
Mitral valve infection	30%	29%	34%
Aortic and mitral valve infection	8%	7%	7%
Tricuspid valve infection	0%	5%	11%
Duke criteria (Definite/ Possible/ Reject[1])	20 (17%)/ 80 (69%)/ 16 (14%)	83 (27%)/ 221 (73%)	1869 (85%)/ 336 (15%)
Echocardiography	98%	99%	99%
TTE	91%	66%	69%
TEE	33%	82%	78%
Vascular phenomena (emboli)	16%	21%	35%
Mortality during treatment	7%	5%	11%
Registered possible relapses	3%	2% (3/152[2])	2% (23/1116[2])
Deceased during follow up	No data	6% (4/152[2])	7% (39/1116[2])
Antibiotic treatment and surgery	(15%)	15% (46/297[3])	21% (453/2152[4])

[1]Reject episodes not studied (paper IV). [2]Follow-up visit. [3]Data about antibiotic treatment missing for seven episodes. [4]Data about antibiotic treatment missing for 53 episodes.

In the Swedish IE registry study the mortality for patients with CNE and CPE was 5% vs. 11%, respectively, a disparity that reached statistical significance with an odds ratio of 0.38 [95% CI 0.21- 0.68].

TABLE 7. *Comparison of mortality in CNE and CPE in Sweden (1995-2004) (Paper IV).*

	CNE	CPE	ALL IE
Mortality	5 % (n=304))	11 % (n=2205)	10.3 % (n=2509)
Mortality Women/Men	9 % (n=127)/ 2 % (n=177)	13 % (n=764)/ 10 % (n=1441)	12.1% (n=891)/ 9.4% (n=1618)
Female vs. male episodes. Odds ratio(OR)	5.5 [95% CI 1.40-31.2]	1.26 [95% CI 0.95-1.67]	1.33 [95% CI 1.02-1.74]

We studied the influence of antibiotic pre-treatment on blood cultures, as described in Paper I (Figure 2). In 45% [(95%CI) 36-55] of the episodes antibiotic therapy might have influenced the results from blood cultures. In 16% of all episodes, such therapy stopped less than 60 days [median 7 [1-41] before blood was cultured.
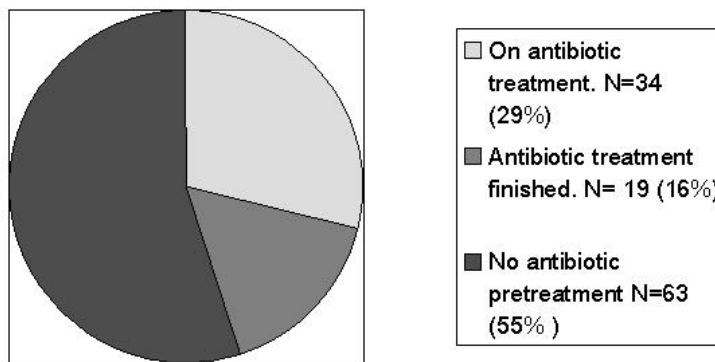

FIGURE 2. *Antibiotic pre-treatment for CNE episodes.*

CNE was found in 12 to 27 % of all Swedish IE episodes (Table 4). The lowest of these percentages is an estimate from data in the Swedish registry (n=2509 episodes) and the highest percentage is from the smallest data set collected retrospectively in a second-line hospital (Borås) (n=70 episodes). The estimates calculated for CNE were 19 to 21% in the prospectively collected IE episodes in the Göteborg study. Although the large size of the Swedish registry study increases the precision of estimated values, the validity of data may be influenced by selection bias (91). A disease like CNE may be difficult to diagnose and therefore not registered. The proportion of Duke "definite" CNE in the Swedish registry study was 27% compared to 17% in the Göteborg IE study, a difference that points out that the CNE episodes which were reported could

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have been more obvious IE cases. The absence of positive blood cultures may be one reason not to perform echocardiography, which in turn may cause some CNE episodes to remain undetected. Although IE is not a self-limiting disease, it may be cured by short-term treatment with intravenous antibiotics. In fact, the Duke criteria consider this possibility when differentiating between the "possible" and "reject" groups.

The high proportion 27%, of CNE found in the Borås study may be a chance estimate, since the confidence limits were wide. Undoubtedly, the estimate that about 20% of patients with IE could be classified as having CNE among the prospectively analyzed Göteborg group is the most trust worthy calculation in this comparison. Our results coincide with other modern studies of CNE in Europe, measured at 5 to 20% of IE episodes (6, 9, 11-13, 90), (Table 5). The lowest figures emerged in IE patients who belonged to the Duke "definite" group (11).

Patients with CNE studied here had a mortality rate of 5 to 7% (Table 6), which is lower than the 10.3% for patients with IE in Sweden during 1995-2004. Moreover, the mortality rate for individuals with IE in Sweden is low compared with studies of IE recently published in other countries (14 to 26%) (4, 11, 92). The centralized treatment of patients with IE at infectious diseases departments and the low prevalence of methicillin-resistant *S. aureus* (93) during the period studied have contributed to this low death rate.

Mortality was lower for those with CNEs than for the CPE group (5% vs. 11%) in the Swedish study (Paper IV, Table 7). The relatively low mortality and low prevalence of acute valvular surgery in these patients with CNE may indicate that a milder variant of CNE may prevail in Sweden. Additionally, preparedness for early parenteral antibiotic treatment may have affected this positive outcome. Also of importance is that no episodes of Q-fever or infections with *Bartonella* were reported (94); if present; such infections could have worsened the outcome of IE.

A fatal outcome was significantly more common in females with CNE, in females with IE (culture positive and -negative episodes) but not in women with positive blood cultures (Table 7). This increased risk indicates that treatment and diagnostic procedures for women with IE need improvement.

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Data from CNEs are in relatively good agreement with episodes of blood culture-positive patients (Table 6). The absence of positive blood cultures explains the low proportion of Duke "definite" episodes in those groups compared to the CNE groups (5, 52, 90, 95), because blood culture findings are one of the cornerstones of the Duke criteria (2). Few intravenous drug users (IVDU) were counted in the CNE group correlating with the low prevalence of tricuspid IE, which is a common manifestation of IE in IVDU.

We used the term *possible relapse* as a new event of IE during the six months after completed IE therapy. If confirmational testing by molecular analysis is not performed, the terms relapse and reinfection are inadequate (96). Obviously, the term reinfection is difficult to use in patients with CNE.

Antibiotic treatment preceded blood culture in 45% of all CNEs (Paper I). Since, as stated earlier, the influence of antibiotic pretreatment is very important (15), all episodes in patients with ongoing or completed antibiotic treatment preceding testing have been analyzed together in relation to blood culture. The median time from withdrawal of antibiotic treatment to blood culture was seven days, and outcomes of patients in that post-treatment group accounted for 16% of the episodes. The length of antibiotic pretreatment has been proposed to have a great influence on the inhibition of bacterial growth in subsequent blood cultures (97). The median duration of antibiotic therapy was 10 days in our study. Initially, the volume of blood used for cultures was rather small altogether 5 ml per puncture. In 1993, the cultured blood volume changed from 10 to 20 ml (90) and the amount presently used is 20 ml per culture. In spite of this increased volume, however, no significant change followed in the proportion of CNE among IE patients in Göteborg during the period 1989-96 (86). We did not assess the issue in blood culturing of the time from venepuncture to start of incubation, but the length of that interval could have had an impact on the outcomes of testing for bacterial content. For example, the growth of viridans streptococci in blood cultures may be inhibited by antibiotic pretreatment (1, 15, 29, 98).

Finally, to improve diagnostic accuracy in patients with suspected IE, it is important that excised valves in IE patients are subjected to histological examination, culturing (32), microbiology Gram staining (29) and, in selected cases, PCR investigation (32).

4.2 A COMPARISON OF THE DUKE CRITERIA AND THE MODIFIED BETH ISRAEL CRITERIA (PAPER I)

One hundred and sixteen CNEs were evaluated with the Duke criteria and the Beth Israel criteria. Twenty episodes were assigned to the Duke "definite" and 13 to the Beth Israel "definite" categories (Table 8). Sixteen episodes were classified as Duke "rejects" compared to 61 episodes to the Beth Israel "reject" group.

TABLE 8. *Duke and Beth Israel criteria for IE compared.*

BETH ISRAEL	DUKE			
	DEFINITE	*POSSIBLE*	*REJECT*	*TOTAL*
Definite	5	8	0	*13*
Probable	7	8	0	*15*
Possible	6	21	0	*27*
Reject	2	43	16	*61*
Total	*20*	*80*	*16*	*116*

As shown in Table 8 and described in Paper I (90) (1, 2), 20 episodes fulfilled the criteria for definite IE in Duke's classification. Valvular culture and histological investigation were not performed for four of the 17 patients who underwent surgery. The Duke criteria classified episodes as possible IE, even though resected valves from that patients showed macroscopic signs of IE. Consequently, the Duke criteria for "definite" may have been insensitive for assessing this group.

The largest classification of this population was Duke "possible" IE, accounting for 80 of the 116 CNEs. This group was heterogeneous, including patients with a high likelihood of IE but also patients with very low likelihood despite four or more days of treatment for IE, because no other diagnosis seemed applicable. Thirty-nine episodes in the Duke "possible" group fulfilled the requirement for one major criterion and two minor criteria. In diagnosing CNEs, the major endocardial criterion is very important in the absence of positive blood cultures. Only 38 of the CNEs in Paper I were investigated with TEE. This sensitive method might have detected more episodes with the major endocardial criterion. Additionally, no investigation of immunological phenomena such as rheumatoid factor was done according to the study protocol,

although positivity for rheumatoid factor would certainly have classified more episodes as definite IE (99).

In 27 of the 80 possible CNEs, only two minor criteria were present (i.e., fever >38.0°C and a predisposing heart condition). Valvular prosthesis, an earlier episode of IE, atherosclerotic valves or congenital heart disorder and fever were noted. Clearly, classifying patients with a valvular prosthesis and fever as Duke "possible" IE is too simplistic. We agree with proposed modifications of the Duke criteria to increase the specificity of the "possible" class and concur that at least three minor criteria or one major and one minor criterion are necessary to classify an episode as possible IE (77). TEE should be used to enhance the ruling of "possible" and separate that group from the "reject" group via the Duke criteria (100). If the Duke criteria become more specific, they could be used as tools in bedside decision-making to determine whether or not antibiotic therapy should be administered.

We found that the Duke criteria were more sensitive than the modified Beth Israel criteria in identifying "definite" episodes of IE (20 vs. 13). However, it is more useful comparing the modified Beth Israel criteria "definite" and "probable" groups (the latter group included patients who neither underwent surgery nor died) with the Duke definite group evincing an increased sensitivity for 28 vs. 20 episodes.

The modified Beth Israel criteria distinguished 61 episodes as rejects, indicating a better specificity compared to 16 rejected episodes with the Duke criteria. This better discriminating ability results from the fact that the Beth Israel criteria disregard the time of IE treatment (more or less than four days) and do not require a firm alternative diagnosis to reject IE, which sometimes is impossible in a clinical situation.

4.3 ANTIBIOTIC THERAPY IN CNE (PAPER IV)

We studied the influence of aminoglycoside treatment for the outcome of CNE (Table 9) and found that CNE patients who received aminoglycoside treatment had a significantly decreased risk of mortality, aOR 0.26. Alternative antibiotic therapies used in the treatment of CNE and CPE are listed in Table 10.

TABLE 9. *Effect of aminoglycoside on mortality from CNE.*

	DEATHS AMINOGLYCOSIDE THERAPY N= (%)	DEATHS NO AMINOGLYCOSIDE THERAPY N= (%)	aOR [95%CI][1]	p=[1]
CNE N=297	8/249 (3)	6/48 (13)	0.23 [0.08-0.85]	0.03

[1]adjusted for age and gender

TABLE 10. *Antibiotic therapy in 297 CNE episodes and 2152 CPE IE episodes.*

	ANTI-BIOTIC THERAPY	β-LACTAM	AMINO-GLYCO-SIDE	AMINO-GLYCO-SIDE AND β-LACTAM	VANCO-MYCIN	RIFAM-PICIN
ALL CNE n=(%), treatment days median [range]	297[1] (100) 28 [1-101]	261 (88) 28 [2-77]	249 (84) 14 [2-63]	232 (78) 14 [1-49]	81 (27) 21 [2-83]	12 (4) 27 [6-56]
NVE n=(%), treatment days median [range]	240 (100) 28 [1-101]	216 (90) 28 [2-77]	201 (84) 14 [2-56]	192 (80) 14 [1-40]	57 (24) 15 [2-83]	8 (3) 21 [6-43]
PVE n=(%), treatment days median [range]	57 (100) 28 [1-89]	45 (79) 25 [2-80]	48 (84) 14 [3-63]	40 (70) 14 [1-27]	24 (42) 30 [2-56]	4 (7) 42 [21-56]
ALL CPE n=(%), treatment days median [range]	2152[2] (100) 30 [1-133]	2005 (93) 28 [1-133]	1831 (85) 14 [1-61]	1758 (81) 14 [1-61]	494 (23) 14 [1-98]	190 (9) 18 [1-90]

[1]No data in seven other episodes, [2]No data about antibiotic treatment in 53 other episodes

The higher rate of mortality in patients without aminoglycoside therapy (p=0.03) described in Paper IV and confirmed in Table 9 supports the inclusion of aminoglycoside in the treatment regimen for CNE, as suggested in recently published guidelines from the US (79) and Europe (80). This finding is particularly important,

because the impact of aminoglycoside treatment for IE has attracted a great deal of attention in recent years (101, 102).

Seventy-eight percent of all CNE patients studied here received combination therapy with an aminoglycoside and a beta-lactam antibiotic, but fewer patients had this combination in the PVE group (70%) Table 10. Vancomycin was the predominant treatment for CNE PVE compared to that for NVE (42% vs. 24%, respectively). Only twelve patients received treatment with rifampicin, four (7%) with CNE PVE and eight (3%) with NVE; therefore, the impact of rifampicin treatment for CNE warrants further study. Overall, we documented good agreement with respect to antibiotic treatment received by patients with culture negative and -positive IE.

4.4 SEROLOGICAL SIGNS OF *BARTONELLA, C. BURNETII* AND *C.PNEUMONIAE* (PAPERS II, III)

Q-fever and *Bartonella*

Three hundred and thirty-four blood samples were analyzed with *Bartonella* and *C. burnetii* serology (Paper II). In no case was the cut-off antibody level for *Bartonella* serology IgG ≥800 reached. *C. burnetii* serology consistent with Q-fever endocarditis with phase I antigen IgG 1000 was detected in one patient.

C. pneumoniae

No significant differences appeared when *C. pneumoniae* antibodies were compared in patients with blood culture and -positive episodes of IE (Table 11).

TABLE 11. *C. pneumoniae antibodies in patients with blood culture negative and -positive episodes of IE.*

	MEN		aOR[1] [95 % CI]	WOMEN		aOR[1] [95 % CI]
	CNE%	CPE %		CNE%	CPE %	
C. pneumoniae IgG ≥512	42	30	1.69 [0.84-3.33]	40	29	1.69 [0.83-3.45]
C. pneumoniae IgA ≥64	53	41	1.61 [0.79-3.23]	40	29	1.61 [0.79-3.23]

[1] age adjusted

The screening of sera from 334 episodes of IE did not disclose any undiagnosed cases of Q-fever or *Bartonella* infection. During the period studied in Göteborg (103, 104), body lice were seldom found, and none of the investigated patients was homeless. The only patient with an increased level of phase I antibodies to *C. burnetii* had a previously diagnosed and treated Q-fever endocarditis (105, 106) probably acquired in Crete, Greece. Although this was the only patient with Q-fever endocarditis found here, we suggest that CNE patients should be screened for Q-fever. Many travelers go to countries where this infection is endemic, and the treatment for Q-fever differs from that recommended for CNE.

 Despite the published case reports of *C. pneumoniae* IE (56, 57), considerable doubt remains that *C. pneumoniae* is the causes. Reinvestigation of patients diagnosed with *C. pneumoniae* IE a showed serologic cross reactions between *Bartonella* and *C. pneumoniae,* and the former was deemed to be the etiologic agent (107). Furthermore, no significant differences were seen in the proportions of patients with increased levels of *C. pneumoniae* antibodies when comparing CNE and CPE episodes (Paper III). Nearly all patients were screened for *Bartonella* by serological assays without finding any with IE. Consequently, our results do not support the hypothesis of *C. pneumoniae* as a cause of CNE.

4.5 *C. PNEUMONIAE* ANTIBODIES IN IE AND POPULATION CONTROLS (PAPER III)

Since increased amounts of *C. pneumoniae* antibodies are frequently found in IE patients, we compared the titers in their blood with those from the control populations (Table 12).

TABLE 12. *C. pneumoniae antibodies in IE patients and population controls*.

	MEN		aOR [95 % CI]	WOMEN		aOR [95 % CI]
	IE%[1]	CONTROLS%		IE%[1]	CONTROLS%	
C.pneumoniae IgG ≥512	33	26	1.37 [0.83-2.27]	32	17	2.21 [1.32-3.70]
C.pneumoniae IgA ≥64	45	31	1.80 [1.12-2.91]	32	10	3.98 [2.26-7.01]

[1] age adjusted

Our results documented significantly higher C. *pneumoniae* IgG and IgA antibody levels in the females with IE than in female controls. *C. pneumoniae* IgA antibody levels were also increased in males with IE compared to their controls. However, these results should be judged with caution. Although adjusted for age and gender, the results were not adjusted with respect to smoking, hypertension or obesity, which are proposed confounding factors in studies of aortic valve sclerosis (108, 109).

Detection of IgG antibodies in serum can be expected years after the initial infection, and a serum IgG of ≥ 512 may indicate either a chronic infection and/or earlier infection with *C.pneumoniae*. Distinguishing between these two options might have been possible by analyzing sequential sera samples. However, it is uncertain whether the results of antibody tests in our patients mirror true *Chlamydia* infections in heart valves, since we did not have the opportunity to study tissues from our patients. The association between *C. pneumoniae* antibodies and valve sclerosis is not proven (61). Although *C. pneumoniae* has been detected in calcified aortic valves with immunohistochemistry and/or PCR (59, 60, 62, 110), results from other such studies disagreed (61, 63, 65). Moreover, the correlation of raised *C. pneumoniae* IgG and IgA antibody content in sera and of *C. pneumoniae* infection in heart valves is not fully

investigated (61, 62, 111). *C. pneumoniae* has been found in atherosclerotic lesions, so this bacterium could be either an innocent bystander or a co-pathogen in the pathogenesis of endocarditis.

5 CONCLUSIONS

1. The proportions of CNE in IE episodes were estimated at 12% in the Swedish IE registry study and 19 to 27 % at Göteborg and Borås clinics. The mortality during treatment was 10.3% in all registered IE episodes in Sweden and 5 to 7% in the CNEs. There was a significant higher death rate in women with CNE than in men with CNE. Antibiotic treatment preceded blood culture in 45% of all CNEs.

2. For establishing IE diagnosis, the Duke definite criteria were more sensitive but probably less specific than the Beth Israel criteria.

3. Aminoglycoside treatment was associated with a significantly lower mortality in CNE patients.

4. *Bartonella* and Q-fever-related IE were rare in Sweden, and this study does not support the hypothesis that *C. pneumoniae* causes CNE.

5. We found a higher prevalence of *C. pneumoniae* antibodies in women with IE compared to female population controls.

6   ASPECTS FOR THE FUTURE

The study of CNE needs access to new tools for accurate diagnoses of bacterial infections from patients' blood samples. Although the PCR method for detecting microorganisms in the blood have been considered promising for the past 15 years, no real breakthrough has occurred in the study of bacteria and fungi. Serological methods may be difficult to interpret and are often not specific. Antibiotic pretreatment before testing blood cultures is still common. For that reason, the practice of culturing and analyzing blood from outpatients with fever despite a rather small suspicion of IE should be further encouraged. Patients given parenteral antibiotic therapy should always undergo blood culture analysis before antibiotic administration. An important adjunct to these conclusions is the continued development of new blood culture media with improved inhibitors to the effect of antibiotics.

We still rely on analysis of heart valve tissue for a definite diagnosis of IE. Therefore, when a patient with CNE undergoes valvular surgery or dies after a CNE, it is of utmost importance that the resected valves are sent for histopathology, microbiological Gram staining, valvular culture and, in selected cases, PCR investigation. Although some series of resected valves from IE patients have been handled in this way, the incidence is small in view of the far larger number of patients involved and further studies are wanted.

Our data shows that, to some extent, clinicians are overdiagnosing CNE. If all patients with fever not clearly related to a specific disease are screened with echocardiography, considering its current high sensitivity and specificity, we believe that many patients with vegetations or other suspicious valve alterations will be suspected as having IE. However, echocardiography is not a screening method for IE in patients with fever of short duration and no clinical signs of heart disease.

Clearly, the role of aminoglycosides in IE therapy needs to be studied further, because of its potential for harm.

The study indicates higher mortality in women with IE than men. Therefore, future studies should be addressed studying the influence of gender in the diagnosis, treatment and outcome of IE.

Although the improvements in diagnostic procedures facilitate the exclusion of the diagnosis of CNE in suspected IE patients, CNE occurs in about one fifth of all cases of IE. Antibiotic treatment preceding blood culture diminishes the value of a negative test result. Fastidious bacteria are mainly diagnosed with antibody determinations, which may be difficult to interpret and take a long time to get. Consequently, clinicians still need better methods for discriminating CNE from other diseases.

7 ACKNOWLEDGEMENTS

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I

A Clinical Study of Culture-Negative Endocarditis

Maria Werner, MD, Rune Andersson, MD, PhD, Lars Olaison, MD, PhD, and Harriet Hogevik, MD, PhD

Abstract: Culture-negative infective endocarditis (CNE) is a diagnostic problem in spite of improved echocardiographic and blood culturing techniques. We conducted the present study to estimate the proportion of CNE in patients with infective endocarditis, to investigate data regarding risk factors, and to evaluate the Duke and the modified Beth Israel criteria in patients with CNE.

We evaluated 820 consecutive suspected episodes of infective endocarditis in adults at the Departments of Infectious Diseases in Göteborg and Borås, Sweden (1984–1996). All patients were diagnosed and treated according to a protocol; 487 episodes were identified as infective endocarditis. Episodes with absence of bacterial growth at blood culture were defined as CNE and were classified with the Duke and the modified Beth Israel criteria.

We identified 116 CNE episodes (median age, 67 yr). Mortality was 7%, and in 15%, cardiac surgery was performed. The Duke criteria classified 20 definite, 80 possible, and 16 reject episodes. The modified Beth Israel criteria distinguished 13 definite, 15 probable, 27 possible, and 61 reject episodes. The proportion of CNE among patients with infective endocarditis varied from 19% to 27% at the 2 departments. Antibiotic treatment preceded blood culture in 45% of the CNE episodes.

About 20% in a Scandinavian population of infective endocarditis patients have CNE. Antibiotic pretreatment explains less than 50% of all CNE episodes. The Duke criteria are more sensitive but less specific than the modified Beth Israel criteria in classifying patients with CNE.

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INTRODUCTION

Positive blood cultures are keystones in diagnosing infective endocarditis. However, blood culture-negative endocarditis (CNE) is detected in both population-based epidemiologic studies and hospital studies of infective endocarditis. The proportion of CNE among infective endocarditis patients in different studies has been estimated at 1%-55% (6, 18, 20, 30, 38, 40). The marked difference between studies has been explained by inadequate blood culturing techniques, antibiotics preceding blood culture, and different criteria for diagnosis.

To our knowledge, only 1 clinical epidemiologic study (18) has concentrated on patients with CNE during the last decade, a nationwide survey of infective endocarditis patients in France describing 88 patients with CNE. Previous antibiotic treatment was found in 48% of all cases, mortality was 15%, and a causative agent was found in 17% of all episodes. Fifty-one percent of the episodes were defined as definite according to the Duke criteria (11).

Infective endocarditis has been prospectively studied at the Department of Infectious Diseases, Göteborg University, Sweden, since 1984. The proportion of CNE was estimated earlier (1984–1988) to be from 12% to 21% (20, 27), depending on which inclusion criteria were used. Antibiotic treatment preceded blood culture in 85% of the CNE episodes, compared with 35% of the episodes in the control group of blood culture-positive episodes. The study was expanded to include the Department of Infectious Diseases, Borås, Sweden, since 1989.

The Duke criteria (11) for classifying patients with infective endocarditis have been established during the last few years and have nearly replaced the Beth Israel criteria (von Reyn criteria) (39), although the latter have been modified to include findings at echocardiography (20, 27). These classification systems have not been compared in consecutively collected CNE episodes, to our knowledge.

We conducted the present study with the following objectives: 1) to estimate the proportion of CNE in a group of patients with infective endocarditis recruited in a secondary and a tertiary hospital in Sweden; 2) to describe and analyze data from patients with CNE regarding suspected risk factors and demographic data; and 3) to evaluate the Duke and modified Beth Israel criteria in CNE patients.

PATIENTS AND METHODS

Setting

The study took place at the Departments of Infectious Diseases at Sahlgrenska University Hospital, Göteborg, and Borås Hospital, Borås, Sweden. The former is a university clinic and a tertiary referral center and serves an urban population of about

600,000 inhabitants. The latter is a second-level referral hospital that serves a mixed urban and rural population of about 250,000 inhabitants.

Patients

The patients were prospectively recruited at the Göteborg clinic between 1984 and 1996 and at the Borås clinic from 1989 through 1996. The patients were transferred from other departments as cases of suspected infective endocarditis or fever of unknown origin; were referred by their general practitioner; or attended the outpatient clinics. All patients were adults (>18 yr).

We prospectively studied 233 consecutive episodes (217 patients) at the Göteborg clinic between 1984 and 1988. Of these episodes, 161 (149 patients; 81 male, 80 female episodes) were diagnosed as apparent infective endocarditis (20). Suspected infective endocarditis was identified in 517 episodes (460 patients; 267 male and 250 female episodes) in Göteborg between 1989 and 1996: 145 episodes (89 male, 56 female episodes) were classified according to the Duke criteria (11) as definite, 111 (59 male, 52 female episodes) as possible, and 261 (119 male, 142 female episodes) as rejects. Antibiotic combination therapy was initiated in 363 of the 517 episodes. Records from 237 episodes, in patients discharged with the diagnosis of infective endocarditis (International Statistical Classification of Diseases, ninth revision [ICD-9]) at the Göteborg clinic from 1989 through 1996, were also evaluated to find CNE patients not registered in the prospective study. In Borås, 70 episodes in 39 men and 31 women with an infective endocarditis diagnosis (ICD-9) were found.

Study design

The study was a nonrandomized descriptive study of consecutive patients with CNE treated as infective endocarditis according to a study protocol with uniform antibiotic treatment regimens, clinical evaluation procedures, and collection of specimens. Echocardiography was regularly done in the first week and, if indicated, repeated at the fourth week of treatment. Transesophageal echocardiography, available since 1991, was used when applicable. The location of infective endocarditis was determined by surgery, autopsy, or echocardiography. Patients who attended the Göteborg clinic were regularly examined by a dentist but those in Borås, only sporadically. Demographic data and data concerning suspected risk factors and other preceding events were extracted from records and registered in a standardized questionnaire.

All CNE episodes were evaluated with modified Beth Israel criteria (20, 28) further modified to define only patients with the absence of bacterial growth at blood culture as culture negative (Table 1). The episodes were also categorized according to the Duke criteria (11) (Tables 2 and 3). Only lesions verified by pathologic and anatomic diagnosis (PAD) or culture were accepted as definite by the Duke pathologic criteria.

Definitions

Patients with absence of bacterial growth in blood culture and apparent infective endocarditis (1984–1988, Göteborg) were defined as having CNE.

Patients with absence of bacterial growth in blood culture and classified as definite or possible according to the Duke criteria (1989–1996, Göteborg) were defined as having CNE.

Patients with absence of bacterial growth in blood culture and discharged with an infective endocarditis diagnosis (ICD-9) and not registered in the prospective studies were defined as having CNE.

An episode was defined as an admission to hospital for treatment of infective endocarditis, irrespective of the time interval since any previous episode.

The first day of an episode was the first day of intravenous antibiotic combination treatment.

Heart failure was defined as an increased demand for diuretics.

TABLE 1. Modified Beth Israel criteria*, further modified so only patients with absence of bacterial growth at blood culture were accepted as culture negative

1. Definite infective endocarditis
   Episodes in patients for whom the diagnosis was verified at surgery or autopsy by culture, microscopy.
2. Probable infective endocarditis
   Culture-positive endocarditis is characterized by at least 2/3 positive blood cultures and 1 of the following: new cardiac murmur, predisposing heart disease† with vascular phenomena‡, and/or sonographic evidence of vegetation.
   Culture-negative endocarditis is characterized by negative blood cultures and all 3 of the following: fever, new cardiac murmur, and vascular phenomena.‡
3. Possible infective endocarditis
   Culture-positive endocarditis is characterized by at least 2/3 positive blood cultures and 1 of the following: predisposing heart disease† and/or vascular phenomena.‡
   Culture-negative endocarditis is characterized by negative blood cultures and all 3 of the following: fever, predisposing heart disease†, and vascular phenomena‡, alternatively sonographic evidence of vegetation.
Rejected
   A. Endocarditis unlikely, alternative diagnosis generally apparent.
   B. Endocarditis likely, empirical antibiotic therapy warranted.
   C. Culture-negative endocarditis diagnosed clinically, but excluded by postmortem.

*References 20, 28.
†Valvular or congenital heart disease or valvular prosthesis.
‡Petechiae; splinter hemorrhage; conjunctival hemorrhages; Roth spots; Osler nodes; Janeway lesions; aseptic meningitis; glomerulonephritis; and pulmonary, central nervous system, coronary, or peripheral emboli.

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TABLE 2. Duke criteria*

Definite infective endocarditis
    Pathologic criteria: microorganisms demonstrated by culture or histology in a vegetation, or in a vegetation that has embolized, or in an intracardiac abscess, or pathologic lesions: vegetation or intracardiac abscess present, confirmed by histology showing active endocarditis.
    Clinical criteria, using specific definitions listed (see Table 3): 2 major criteria, or 1 major and 3 minor criteria, or 5 minor criteria
Possible infective endocarditis
    Findings consistent with infective endocarditis that fall short of "definite" but not rejected.
Rejected
    Firm alternate diagnosis for manifestations of endocarditis, or resolution of manifestations of endocarditis with antibiotic therapy for 4 days or less, or no pathologic evidence of infective endocarditis at surgery or autopsy after antibiotic therapy for 4 or fewer days.

    *Reference 11.

Treatment mortality was defined as all deaths during treatment and up to 1 month posttreatment.

Blood culture and serology

According to the protocol, a standardized series of 3 blood cultures was drawn with 3 separate venous punctures with intervals of at least 20 minutes. In Göteborg, 1 aerobic and 1 anaerobic bottle (the biphasic model modified Castaneda system) were used at every blood culture. The bottles contained 2.5 mL of blood each in the first 21 months of the study (altogether 5 mL).

This procedure was changed to an increased blood volume of 5 mL per bottle (altogether 10 mL). The BACT/ALERT System (Organon Teknika Corp., Durham, NC) was introduced for specimens from most Göteborg patients in the study in 1993. At every blood culture, 20 mL of blood was divided into 1 aerobe and 1 anaerobe bottle. FAN–aerobic and anaerobic media composed of brain heart infusion broth and Ecosorb (contains absorbent charcoal and Fullers earth)–media were introduced in 1995 instead of standard media. All blood cultures collected in Göteborg were incubated for 10 days.

TABLE 3. Definitions of terminology used in the Duke criteria*

Major criteria
    A. Positive blood culture for infective endocarditis
        1. Typical microorganism for infective endocarditis from 2 separate blood cultures:
            a. viridans streptococci[†], *Streptococcus bovis,* HACEK[‡] group, or
            b. Community-acquired *Staphylococcus aureus* or enterococci, in the absence of a primary focus, or
        2. Persistently positive blood culture, defined as recovery of a microorganism consistent with infective endocarditis from:
            a. Blood cultures drawn more than 12 h apart, or
            b. All of 3 or a majority of 4 or more separate blood cultures, with first and last drawn at least 1 h apart
    B. Evidence of endocardial involvement
        1. Positive echocardiogram for infective endocarditis:
            a. Oscillating intracardiac mass, on valve or supporting structures, or in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation, or
            b. Abscess, or
            c. New partial dehiscence of prosthetic valve, or
    C. New valvular regurgitation (increase or change in pre-existing murmur not sufficient)
Minor criteria
    A. Predisposition: predisposing heart condition or intravenous drug use
    B. Fever = 38.0 °C (100 °F)
    C. Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhage, Janeway lesions
    D. Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, rheumatoid factor
    E. Microbiologic evidence: positive blood culture but not meeting the major criterion noted previously[§] or serologic evidence of active infection with organism consistent with infective endocarditis
    F. Echocardiogram: consistent with infective endocarditis but not meeting the major criterion noted previously

    *Reference 11.
    [†]Including nutritional variant strains.
    [‡]HACEK: *Haemophilus* spp, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella* spp, and *Kingella kingae.*
    [§]Excluding single positive culture for coagulase-negative staphylococci and organisms that do not cause endocarditis.

Blood from the Borås patients was cultured in the SIGNAL system of OXOID (Basingstoke, UK). In this system, only 1 bottle containing 10 mL of blood was used on every blood culture occasion. The incubation time was 7 days. *Coxiella burnetii* serology was performed in some suspected cases, but not regularly.

Statistical methods

Values of quantitative variables were expressed as means, medians, and ranges. Confidence limits (95% CI) were given. The chi-square test was used to test the difference between proportions. A significance level of 0.05, 2-tailed test, was used. Yates correction was used when applicable.

RESULTS

Patients

During the study period there were 116 episodes of CNE in 115 patients (mean age, 63 yr; median age, 67 yr; range, 19–85 yr). Nineteen episodes were identified at the Borås clinic and 97 episodes (95 patients) at the Göteborg clinic. Three patients were referrals to the Göteborg clinic from other parts of western Sweden. The series consisted of 58 episodes in men (mean age, 63 yr; median age, 64 yr; range, 38–83 yr) and 58 episodes in women (mean age, 63 yr; median age, 70 yr; range, 19–85 yr). The actual proportions of CNE in the prospectively studied populations with infective endocarditis are presented in Table 4. Fifteen episodes identified retrospectively from records in Göteborg from 1984 through 1996 were not included in this calculation. The male/female ratio was 1.0 in the CNE group (Duke reject group excluded).

Classification

The 116 episodes of CNE were divided into 20 definite, 80 possible, and 16 reject according to the Duke criteria (11) and were also evaluated with the modified Beth Israel criteria (20, 28) (Table 5). The alternative diagnoses in the Duke reject episodes are shown in Table 6.

The proportion of CNE was estimated to be 6.4% (16/251) (95% CI 3.8–9.9) in the Duke definite group and 40% (65/163) (95% CI 33–47) in the Duke possible group (1984–1996, Göteborg prospective episodes). The distribution of major and minor criteria in the Duke possible group is presented in Figure 1. All consecutive prospective episodes of suspected infective endocarditis in Göteborg, 1989–1996, were subdivided into an annual incidence and sorted with the Duke criteria (Figure 2).

Presentation

Data concerning symptom duration, doctor's delay, and time to treatment are presented in Table 7. The median highest recorded temperature before infective endocarditis treatment was 39 °C (range, 36.8°-40.3 °C).

Predisposing factors and risk factors

Data concerning suspected risk factors are presented in Table 8. Thirty percent of the episodes occurred after previous open heart surgery. The median time between surgery and the start of infective endocarditis symptoms was 26 months (mean, 62 mo; range, 0–385 mo).

Twenty-four percent of the episodes occurred in prosthetic valve carriers. The median time from surgery to first symptom of infective endocarditis was 23 months

TABLE 5. Duke* compared with modified Beth Israel† criteria in 116 episodes of culture-negative infective endocarditis

	Duke Criteria			
	Definite	**Possible**	**Reject**	**Total**
Beth Israel Criteria				
Definite	5	8	0	13
Probable	7	8	0	15
Possible	6	21	0	27
Reject	2	43	16	61
Total	20	80	16	116

*Reference 11.
†References 20, 28.

TABLE 4. The proportion of CNE in the studied IE populations

Place	Years	All IE Episodes No.	CNE Episodes No. (%)	95% Confidence Interval
Göteborg	1984–88	161	34 (21)	15–28
Göteborg	1989–96	256[1]	48 (19)	14–24
Borås	1989–96	70	19 (27)	18–37

Abbreviations: IE = infective endocarditis; CNE = blood culture-negative infective endocarditis.
[1]Duke definite and possible episodes only.

TABLE 6. Alternative diagnosis in 16 CNE patients classified as reject by the Duke criteria*

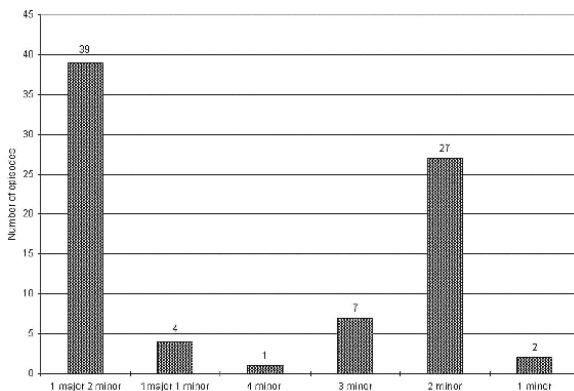| Diagnosis	No.
Marantic endocarditis	3
Systemic lupus erythematosus	2
Wegener disease	1
Papillary muscle rupture	1
Myxoid degeneration	1
Pneumonia	2
Other (dental infection, lymphoma, etc.)	6
Total	16

*Reference 11.

FIGURE 1. Distribution of major and minor criteria in 80 Duke possible episodes.

(mean, 34 mo; range, 0–162 mo). Six of the episodes could be defined as early prosthetic valve endocarditis (<2 mo after surgery), and 23 as late prosthetic valve endocarditis (>2 mo after surgery).

Noncardiac surgery had been performed less than 90 days before the first symptom of infective endocarditis in 6 episodes. The surgical procedures were hip surgery, neural decompression, fasciotomy of the thigh, incision of a vaginal hematoma, urethra dilatation, and drainage of a peritoneal abscess. Four patients developed infective endocarditis during hospital treatment; the reasons for their initial admission were valvular surgery, ventricular septum

defect repair, coronary by-pass, and childbirth, respectively. In 9 episodes, patients presented with wounds or abscesses. Two patients had postoperative infections after thoracotomy, 6 had superficial wounds, and 1 had a deep abscess. One patient had a urinary tract infection preceding the first symptoms of infective endocarditis.

Dental status

In 22 episodes (19%), the patient had visited the dentist less than 90 days before the first day of illness (see Table 8). The dentist examined the patients during 74 episodes and found abnormal dental status in 39 (52%).
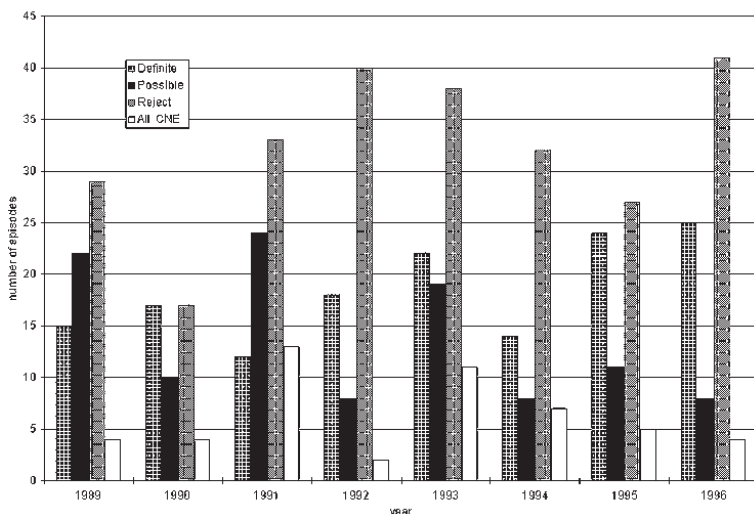

FIGURE 2. Göteborg, Sweden, 1989–1996: 517 consecutive episodes of suspected infective endocarditis evaluated with the Duke criteria (CNE = blood culture negative-endocarditis).

TABLE 7. Days with symptoms until hospitalization and start of IE treatment

	Median (**d**)	Mean (**d**)	Range (**d**)
Symptom duration until hospitalization (n = 111)*	10	23	0–247
Time to IE treatment after hospitalization (doctor's delay) (n = 109)†	2	5	0–32
Total symptom duration until IE treatment (n = 114)‡	14	27	0–266
Fever duration until start of IE treatment (n = 112)§	9	16	0–180

Abbreviations: IE = infective endocarditis.
*Five episodes excluded because of nosocomial infection.
†Seven episodes excluded (episodes without IE treatment, 5 episodes with nosocomial IE).
‡Two episodes without IE treatment.
§Missing data in 4 episodes.

Cardiac murmurs, heart failure, and embolism

Data concerning heart failure are shown in Tables 8 and 11. In 46 (40%) episodes heart failure was noted at the start of infective endocarditis therapy, with deterioration in 27 (23%) of the episodes. In another 15 (13%) of the episodes, heart failure developed during infective endocarditis therapy (see Table 11). Data concerning murmurs are presented in Table 9; 89% of patients presented a cardiac murmur at admission.

In 19 episodes (16%) clinical embolism was present at the start of infective endocarditis therapy (see Table 8); 11 of the emboli passed to the central nervous system. In another 9 (8%) episodes emboli occurred after the initiation of infective endocarditis therapy (see Table 11).

Location

Left-sided infective endocarditis was diagnosed in 94 episodes (aortic valve, 49; mitral valve, 35; combined aortic and mitral valve, 9; and combined aortic and patch infection, 1) (Table 10). One patient had a pacemaker infection, and 2 patients had mural infections. In the remaining 19 episodes no apparent location was found at echocardiography. Transesophageal echocardiography was used in 6 of these episodes.

Echocardiography

Transesophageal echocardiography was performed in 39 episodes and transthoracic echocardiography in 106 episodes. Altogether 114 episodes were investigated with echocardiography during the first week of treatment. The use of transesophageal echocardiography and transthoracic echocardiography was studied especially in the Duke possible group since transesophageal echocardiography became available in 1991. Five (38%) of the 13 episodes with 2 minor criteria were investigated with transesophageal echocardiography, compared with 19 of 24 (79%) in the 1 major and 2 minor criteria group during this period (p = 0.03).

TABLE 8. Predisposing factors in 116 episodes of culture-negative infective endocarditis

	(%)
Valvular factors	
Prosthetic valve	24
Previous IE	13
Valvular surgery	30
Atherosclerosis	10
Rheumatic fever	14
Congenital heart disease	6
No valvular disease (except IE at diagnosis)	30
Concomitant diseases	
Connective tissue disease	9
Diabetes mellitus	10
Malignancy	9
Drug use and medication	
Treatment with neuroleptic drugs	2
Habitual alcohol drinker	8
Intravenous drug addict	1
Events preceding start of IE therapy	
Embolism	16
Heart failure	40
Visit to the dentist <90 d before first symptom of disease	19
Noncardiac surgery <3 mo before IE	5

Abbreviations: IE = infective endocarditis.

TABLE 9. Cardiac murmurs (systolic and/or diastolic) (n = 116)

	(%)
Murmur at admission	89
New murmur at admission	37
Changed murmur at admission	7
New murmur during treatment	2

TABLE 10. Location of infective endocarditis determined by echocardiography, surgery, or autopsy (n = 116)

	(%)
Aortic valve	42
Mitral valve	30
Aortic and mitral valve	8
Aortic valve and patch infection	1
Mural vegetation	2
Pacemaker wire	1
No exact location	16

Blood culture, serology, and antibiotic therapy

A median of 3 blood cultures was done (mean, 5; range, 2–16). The exact number of blood cultures before infective endocarditis treatment was unknown in 7 patients. One patient had serologically verified Q-fever endocarditis. In 34 (29%) episodes the patients were on antibiotic treatment, and in another 19 (16%) episodes antibiotic pretreatment was given less than 60 days (mean, 11 d; median, 7 d; range, 1–41 d) before the first blood culture was done. The median duration of antibiotic treatment in the pretreated episodes was 10 days (mean, 9 d; range, 1–33 d).

The proportion of episodes with ongoing or previous antibiotic treatment at the time of the first blood culture in all CNE episodes was 45% (95% CI 37–55). The proportion of antibiotic pretreatment at blood culture was 45% (95% CI 24–69) in the definite group, 46% (95% CI 35–57) in the possible group, and 44% (95% CI 20–64) in the reject group (Duke criteria). The Duke possible group was further divided into 6 subgroups. Antibiotic pretreatment at blood culture was present in 43% (17/39) of episodes in the 1 major and 2 minor criteria group, in 100% (4/4) of episodes in the 1 major and 1 minor criteria group, in 14% (1/7) of episodes in the 3 minor criteria group, and in 55% (15/27) of the episodes in the 2 minor criteria group. There was no antibiotic pretreatment in the groups with 1 minor or 4 minor criteria.

Valvular culture was performed in 9 of 16 surgically removed valves. One valve was culture positive (*Capnocytophaga canimorsus*). One valve was cultured postmortem with growth of *Klebsiella* and *E. coli* (see Table 11).

Surgery

Surgery data are presented in Table 11. The median time to surgery from start of infective endocarditis therapy was 9 days (mean, 15 d; range, 0–40 d). Seventeen patients underwent early open heart surgery in 18 episodes, with a male/female ratio of 0.89 (8/9).

Mortality and follow-up

Eight patients (7%) died during infective endocarditis therapy or less then 1 month posttreatment (see Table 11). The median time from start of infective endocarditis therapy to death was 29 days (mean, 38 d; range, 5–80 d). One of the deceased was surgically treated. Three patients had autopsies, and all had PAD-proven endocarditis. Four of the 8 patients who died were defined as Duke definite, including the 3 autopsied cases.

Four patients suffered a new episode of culture-positive infective endocarditis during follow-up. Ninety-seven of 106 surviving patients had a median follow-up time of 42 months (mean, 49 mo; range, 1–180 mo).

DISCUSSION

Patients

The rather high proportion of CNE in the studied population corresponds with studies from Finland (Duke criteria, definite 23% versus possible 66%) (16), Denmark (Beth Israel definite and possible 12%) (3), and Sweden (Duke criteria, definite and possible 23%) (23). A study from the Netherlands found 1% CNE in the studied infective endocarditis group (38). This low proportion is due to selection bias, the participants being collected on the basis of positive blood cultures. Studies from developing coun-

TABLE 11. Clinical and diagnostic events and surgery during IE treatment (n = 116)

	(%)
Increasing heart failure	36
Embolism during antibiotic therapy	8
Valvular surgery during therapy and 1 mo follow-up	15
Aortic valve surgery	8*
Mitral valve surgery	5†
Aortic and mitral valve surgery	2‡
Removal of pacemaker wire	1
PAD performed at surgery	5§
Valvular culture	9¶
Death during therapy and 1 mo follow-up	7
Autopsy	3
Valvular culture at autopsy	1**
PAD performed at autopsy	3††

Abbreviations: IE = infective endocarditis; PAD = pathologic and anatomic diagnosis.
*In 2 episodes mechanical valves and in 7 episodes biological valves.
†In 4 episodes mechanical valves and in 2 episodes vegectomy.
‡In both episodes 1 mechanical and 1 biological valve.
§Two episodes of infective endocarditis, 1 myxoid degeneration, 1 sclerosis (not IE), 1 vegetation (later diagnosis of systemic lupus erythematosus.
¶*Capnocytophaga caninimorsus* was cultured in 1 episode.
**Klebsiella* spp and *E. coli* were cultured in 1 episode.
††Three episodes of IE.

tries such as India and Morocco present much higher proportions of CNE (55%-58%) (4, 6). The relatively high rate of CNE in the cited studies from developed countries does not support the statement that CNE is a rare diagnosis (<5%) in modern infective endocarditis studies (37). The low proportion (6.4%) of CNE among Duke definite episodes in the present study may be an underestimation due to the classification system.

There was no trend in the annual incidence of CNE among the patients with infective endocarditis, even though transesophageal echocardiography and better blood culturing techniques became accessible during the study period (see Figure 2). The use of echocardiography on wide indications may add to a still high proportion of CNE among infective endocarditis patients even though the sensitivity and specificity of the method is high (7, 26). Treatment and suspicion of infective endocarditis on wide indications probably increase the proportion of CNE in western countries, but early case detection is important to attain a low mortality from infective endocarditis (3, 20).

The male/female ratio in the CNE episodes (1.0) differs from the French CNE study (18) by a ratio of 3 (which may be due to selection bias of the reporting system in the study) and from other general clinical and population-based studies of infective endocarditis by a ratio of 1.3–2.5 (3, 6, 8, 16, 25, 35, 38,40). The even sex distribution in our study corresponds with the series described by von Reyn (39) and the Duke Endocarditis Service (11).

The median age of all patients in the present study (67 yr) was higher than that in the French CNE study (18) (53 yr). The median age in women was even higher (70 yr). These age and gender differences may be due to the prospective character of our study, with early diagnostic procedures in suspected cases of infective endocarditis. An aged population with a majority of elderly women with good access to hospital care, as in Sweden during the studied period, probably contributes to the group of infective endocarditis patients with an increase of the median age and a decrease of the male/female ratio

Classification

The modified Beth Israel criteria and the Duke criteria were compared (see Table 5) (11, 39). Only 20 episodes fulfilled the criteria for Duke definite, and 16 for Duke reject. The small number of Duke definite episodes, although more than the episodes classified as Beth Israel definite, may partly depend on strict use of the Duke criteria in patients who have undergone surgery or died. Valvular culture and/or PAD were not performed in 4 patients who underwent surgery, showed macroscopic signs of infective endocarditis, and were classified as possible infective endocarditis. Thus the Duke criteria may be insensitive when

used strictly, not accepting macroscopic evidence of infective endocarditis.

The Duke possible group is the largest, accounting for 80 (69%) of the 116 CNE episodes. It is heterogeneous, including patients with a high likelihood of infective endocarditis together with patients with a very low likelihood but with infective endocarditis treatment >4 days and no other obvious diagnosis. In our study, 39 of the episodes in the Duke possible group fulfilled 1 major and 2 minor criteria. Authors of a Swiss study (33) estimated the power of every Duke criterion. The major microbiologic criterion had the greatest impact (53%), followed by the major endocardial criterion (34%), when Duke definite and possible episodes were distinguished. In CNE the major endocardial criterion is very important in the absence of positive blood cultures. Only 38 of the CNE episodes in our study were investigated with transesophageal echocardiography. This more sensitive method may have been able to detect more episodes with the major endocardial criterion. The interobservational variation in echocardiographic investigations also stresses the risk of misclassification (15). Investigation of immunologic phenomena such as rheumatoid factor was not done, according to the study protocol. A positive rheumatoid factor test could have had an impact, classifying more episodes as Duke definite (33). Neither *Coxiella burnetii* nor *Bartonella* serology was done routinely as the incidences of Q-fever and *Bartonella* endocarditis were presumed to be low in the studied population.

In 43% (17/39) of the Duke possible episodes with 1 major and 2 minor criteria, ongoing or previous antibiotic treatment was found at the first blood culture. There was no significant difference in antibiotic pretreatment in this group compared with the other studied groups or subgroups. Our study did not add support to the suggested need for a new criterion of antibiotic pretreatment at blood culture in CNE patients with 1 major and 2 minor criteria to classify these episodes as Duke definite. It is necessary to have a larger study group with CNE patients with either surgical valve replacement or autopsy where PAD and valvular culture is performed consistently, to study the influence of antibiotic pretreatment in CNE.

In 27 of the 80 possible episodes, only 2 minor criteria were present (fever >38.0 °C and a predisposing heart condition). Valvular prosthesis, an earlier episode of infective endocarditis, atherosclerotic valves or congenital heart disorder, and fever were noted. Transesophageal echocardiography was done (since it became available in 1991) in 5 of 13 patients in the 2 minor criteria group compared with 19 of 24 in the 1 major 2 minor group during this period (p = 0.03). It is too simple to classify patients with a valvular prosthesis and fever as Duke possible. We agree with the proposed modifications of the Duke criteria to increase the specificity of the possible class and that at

least 3 minor criteria or 1 major and 1 minor criterion are necessary to classify an episode as possible infective endocarditis (24). Transesophageal echocardiography should be used when diagnosing suspected CNE to distinguish patients in the possible and reject groups.

Earlier evaluations of the Duke criteria have studied the sensitivity and the specificity in different patient groups (9, 11, 17, 34, 36). In our study the Duke criteria were more sensitive compared with the modified Beth Israel criteria in identifying the definite episodes (20 versus 13 episodes, respectively). However, it is more useful to compare the modified Beth Israel criteria definite and probable group (the latter including patients who did not undergo surgery or die) with the Duke definite group. This comparison, on the other hand, shows an increased sensitivity in the Beth Israel criteria (28 versus 20 episodes, respectively).

In the present study, the modified Beth Israel criteria distinguish 62 episodes as reject, indicating a better specificity compared with 16 reject episodes identified with the Duke criteria. This better discriminating ability is due to the fact that the Beth Israel criteria disregard the time of infective endocarditis treatment (more or less than 4 d) and do not require a firm alternative diagnosis to reject infective endocarditis, which sometimes is impossible in a clinical situation. Studies of the positive predictive value of the Duke criteria in different study populations are wanted.

We have not calculated the sensitivity and the specificity of the Duke criteria, as different methods were used to collect the patients. The alternative diagnosis in 16 reject episodes is presented (see Table 6) just to stress the importance of trying to rule out other important diagnoses in patients with suspected infective endocarditis (13).

Presentation

The mean duration from the first symptom to admission was 23 days, which is shorter than in earlier reports studying CNE (18, 30) (see Table 7). The median time to admission was considerably shorter, 10 days. A more important variable is the median time from first symptoms to infective endocarditis treatment, 14 days in this study. This is comparable to data from modern studies of infective endocarditis in general, with a delay of 11–13 days (3, 20).

Cardiac murmurs and embolism

In 89% of episodes a cardiac murmur was present (see Table 9). Murmurs have a great influence in prompting for diagnostic procedures, especially in episodes with a new murmur, 37% of the cases in our study. A new cardiac murmur is a major endocardial criterion that is of great importance when classifying infective endocarditis episodes with the Duke criteria.

In 16% of episodes, embolism preceded attendance to hospital. More than 50% of these emboli went to the central nervous system (CNS). CNS emboli often have serious symptoms and may have increased the suspicion of infective endocarditis in those patients.

Predisposing factors and risk factors

Data concerning earlier prosthetic surgery, earlier infective endocarditis, and location (see Table 8) correspond with previous studies (18). Alcohol abuse has been proposed as a risk factor in infective endocarditis (1). In the present study, 8% involved habitual drinkers, a higher proportion than earlier described in the Göteborg study (19). Homeless alcoholics with suspected or verified body lice infestation have been associated with *Bartonella quintana* infective endocarditis (5). No patient in this study was homeless or was noted to have body lice. The antibiotic effect of neuroleptic drugs has been shown in in vitro and in vivo experiments (22). The influence of neuroleptic drug treatment in CNE is probably low, as only 2% of the CNE patients used this medication.

Antibiotic treatment and blood culture

In the present study, antibiotic treatment preceded blood culture in 45% (95% CI 36–55) of all episodes of CNE, which corresponds with other studies that report previous antibiotic treatment in 48%-50% of episodes (18, 23). It differs from a study from Iowa (30), where antibiotic pretreatment was present at blood culture in 62% (52 episodes) of CNE episodes. We analyzed the episodes with ongoing and completed antibiotic pretreatment together in relation to blood culture because the influence of antibiotic pretreatment has been found to be important (29). The median time from withdrawal of antibiotic treatment to blood culture was 7 days in the patients with antibiotic pretreatment, who accounted for 16% of the episodes. The length of antibiotic pretreatment has been proposed to have a great influence on inhibition of bacterial growth on blood culture (37). The median duration of antibiotic therapy was 10 days in our study.

The observation of no difference in the proportions of antibiotic pretreatment at blood culture between the Duke definite, possible, and reject groups underlines that antibiotic pretreatment is not the sole explanation for blood culture negativity in infective endocarditis. The thesis of continuous bacteremia in infective endocarditis as stated by Beeson and colleagues (2) in the classical work from the 1940s, where 6 patients with terminal infective endocarditis disease (5 patients with symptoms for 4–6 months and 1 patient with symptoms for 4 days) were investigated, needs to be reconsidered when patients with infective endocarditis are diagnosed earlier.

In the beginning of the present study, the blood volume used for blood culture was rather small (5 mL per puncture), but it has increased steadily and is now always

20 mL per culture. In spite of this, there was no significant change in the proportion of CNE among infective endocarditis patients in Göteborg during the years 1989–1996 (blood volume change from 10 to 20 mL). Problems in blood culturing, such as time from venipuncture to start of incubation, have not been analyzed but could have had an impact on the number of CNE patients.

The blood cultures in this study were analyzed in routine practice and were not always incubated more than 1 week. Fastidious bacteria such as the HACEK (*Hemophilus aphrophilus, Actinobacillus acinetocomitans, Cardiobacterium hominis, Eikenella, Kingella kingae*) group and *Granulicatella* may have been undetected. α-Streptococci may be inhibited from growing in blood culture by antibiotic pretreatment (20, 29, 43). The probability of α-streptococci as etiologic agents in some of the episodes of CNE is rather high, as the patients were on antibiotic treatment in more than 40% of the CNE episodes.

The FAN media have an antimicrobial neutralizing effect (41). The observation time in the present study was too short to evaluate a decrease in the proportion of CNE episodes after the introduction in 1995. To improve the diagnostic accuracy it is important that excised valves in all cases of suspected infective endocarditis are subjected to PAD investigation and that the necessary staining is performed to detect, for example, Whipple disease, *Bartonella* infection, and Q-fever (31). *Tropheryma whippelii* and *Bartonella* have been described as etiologic agents in infective endocarditis (10, 14, 32). The excised valves in the present study were not investigated for Whipple disease. New techniques such as the polymerase chain reaction (PCR) may improve our understanding of CNE and have been helpful in diagnosing agents such as *Tropheryma whippelii* and *Bartonella* in valves (12, 21, 42).

Surgery

The rate of early valvular surgery up to 1 month after the end of antibiotic infective endocarditis treatment was rather low in the CNE group (14%) and in the Duke definite group (15%) compared with the previously mentioned French study (18). Our study is a prospective study including all suspected cases, and thus differs from the French study which is based on reporting from physicians which may include an increased reporting of surgically proven cases. The low rate of surgery perhaps reflects the fact that patients with suspected infective endocarditis were treated following a scheme which earlier had yielded good results (20, 28).

Mortality

The mortality during treatment was low (7%) in our study as a whole compared with earlier series (14%-32%) (18, 30). The mortality was 20% in the Duke definite

episodes. There was an overrepresentation of Duke definite episodes among those who died (4/8). More episodes may have been defined as Duke definite if autopsies had been done regularly, as only 3 of 8 (38%) patients who died had autopsies.

Conclusions

The proportion of CNE is about 20% in a large group of prospectively investigated episodes of infective endocarditis in Scandinavia. Fewer than 50% of all episodes of CNE can be explained by antibiotic pretreatment. Among 116 episodes of CNE studied, 20 were definite, 80 possible, and 16 reject by the Duke criteria versus 13 definite, 15 probable, 27 possible, and 61 reject by the modified Beth Israel criteria. The Duke criteria probably both underestimate the real frequency of definite CNE episodes and overestimate the frequency of possible CNE episodes. This study supports the importance of performing PAD and valvular culture in every surgically treated episode or autopsy of suspected infective endocarditis, especially in episodes that are blood culture negative, to refine the diagnosis and treatment of infective endocarditis.

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II

Bartonella and Coxiella Antibodies in 334 Prospectively Studied Episodes of Infective Endocarditis in Sweden

MARIA WERNER[1], PIERRE-EDOUARD FOURNIER[2], RUNE ANDERSSON[1], HARRIET HOGEVIK[1] and DIDIER RAOULT[2]

From the [1]Department of Infectious Diseases, Göteborg University, Sweden, and [2]Unité de Rickettsies, Faculté de Médicine, Marseille, France

Bartonella spp. have been identified as aetiological agents in culture-negative infective endocarditis (IE). Coxiella burnetii may cause chronic Q-fever with endocarditis. 334 blood samples collected from 329 patients (334 episodes) with IE diagnosed between 1984 and 1996 in Göteborg, Sweden, were investigated for antibodies to Bartonella spp. and C. burnetii. 71 of the episodes (21%) were blood culture negative. A microimmunofluorescence assay revealed immunoglobulin G (IgG) antibodies to Bartonella in 13 of the culture verified episodes and in 2 of the culture-negative episodes. Three of the patients had IgG antibodies to ≥ 200 in the blood culture-verified group, but none had a titre ≥ 800, the cut-off level for Bartonella endocarditis. One patient had elevated antibodies to C. burnetii, diagnosing chronic Q-fever endocarditis. In conclusion, serologically verified Bartonella endocarditis is not prevalent in western Sweden and Q-fever endocarditis is rare.

M. Werner, Infektionskliniken, SÄS, Borås, SE-50182 Borås, Sweden (E-mail. maria.werner@vgregion.se)

INTRODUCTION

Negative blood cultures in suspected infective endocarditis (IE) are still a problem, although diagnostic techniques such as blood culture and echocardiography have improved. The prevalence of blood culture-negative infective endocarditis (CNE) is estimated to be 20% in series with IE from northern Europe (1–3). Coxiella burnetii and Bartonella species have been diagnosed as causative agents by valvular culture, blood culture, polymerase chain reaction (PCR), histological testing and/or serological tests in patients with CNE (4).

Bartonella quintana causes trench fever. Rats have been proposed as the reservoir (5), but humans are the only proven hosts. The body louse is the vector of the disease. The last Swedish case was described in 1945 (6). Recent investigations have reported the infection with B. quintana mainly in homeless patients with CNE (7–9). Antibodies to B. quintana have been found among homeless people in France and the USA, and in refugee camps in Burundi (10–12). Bartonella quintana has also been described causing bacillary angiomatosis in immunocompromised hosts.

Bartonella henselae is supposed to cause cat-scratch disease (CSD), which is characterized by a skin lesion after a cat scratch or cat bite and regional lymphadenopathy. Bartonella henselae has been detected in patients with CNE, bacillary angiomatosis or peliosis hepatitis in immunocompromised hosts (13). The reservoir of the disease is probably the cat. The infection may be spread directly from cat to human or indirectly via a vector (14). The cat flea (Ctenocephalides felis) may be such a vector (15). Genotypic variation of B. henselae has been proven; serotype 'Marseille' has been isolated from patients with endocarditis and

CSD. Antibodies to this serotype have been shown in CSD patients seronegative to B. henselae (16, 17).

Q-fever, a rickettsiosis caused by C. burnetii, is a zoonosis diagnosed worldwide. Coxiella burnetii is usually transmitted as an inhaled aerosol of parturient fluid from infected animals. The bacteria may survive for a long time in areas where animals have been present and the wind may transmit the bacteria to humans. (18). IE is the most severe form of chronic Q-fever sometimes diagnosed in CNE patients. In Sweden sporadic cases of domestic Q-fever have been reported (19, 20). Seroepidemiological studies in humans have revealed antibodies to C. burnetii from all over Sweden (21, 22).

There are no reported cases of Bartonella or domestic Q-fever endocarditis from Sweden and no earlier reports about serological testing for antibodies to Bartonella and C. burnetii in a prospectively collected series of IE. The aim of this study was to find undiagnosed cases of Bartonella or Q-fever endocarditis in a prospectively collected group of patients with IE.

MATERIALS AND METHODS

IE has been prospectively studied since 1984 at the Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden (23, 24), in a non-randomized descriptive study of consecutive patients with suspected IE. A study protocol with uniform antibiotic treatment regimens, clinical evaluation procedures and collection of specimens was used. The clinic is a tertiary referral centre in western Sweden, which also serves as a secondary referral centre for the population in Göteborg, a city with about 600,000 inhabitants situated on the west coast of Sweden. 750 patients were included in the study during 1984–1996. Blood samples were collected by venepuncture on inclusion of the patient in the study. Then, 1–2 ml of serum was separated and stored refrigerated ($-70°C$). Sera from patients included in the study between 1984 and

1988 classified as reject IE according to the modified Beth Israel criteria (23) were discarded in 1996. In addition, some of the samples were destroyed during storage. The episodes with treated (IE) were further studied and classified according to the Duke criteria (25).

One serum sample from each IE patient was analysed to find antibodies to B. quintana, B. henselae and C. burnetii. Bartonella quintana, Oklahoma strain, B. quintana, Marseille strain, isolated from a French patient (ATCC 49882T); B. henselae, strain Marseille, B. henselae, Houston strain, were used as antigens. The bacteria between the fourth and seventh passage in a human endothelial cell line (ECV) were harvested, pelleted and used as crude antigen in a microimmunofluorescence (MIF) assay (26, 27). The current cut-off levels for diagnosis of Bartonella endocarditis have been estimated to be ≥ 800 immunoglobulin G (IgG) antibodies with a positive predictive value (PPV) of 0.955 in patients with endocarditis (28). Patients with titres between 50 and 200 may have been in contact with cats and have a residual positive serology (27). IgA and IgM antibodies were not determined as they are not exhibited in patients with Bartonella endocarditis (28).

Q-fever serology was performed with an MIF assay to identify antibodies to C. burnetii antigen (29). The cut-off level for a diagnosis of chronic Q-fever was phase I IgG ≥ 800 with a PPV of 0.98.

RESULTS

Sera from 71 episodes of CNE (12 definite, 52 possible, 7 reject; Duke criteria) in 70 patients and from 263 episodes with culture-verified IE (171 definite, 69 possible, 23 reject) in 259 patients were examined (Table I). In total, sera from 334 episodes in 329 patients were examined.

Q-fever

Coxiella burnetii serology consistent with Q-fever endocarditis with phase I antigen IgG 1000 was detected in 1 patient.

Bartonella

Bartonella antibodies were found in 15/334 (4.5%) of the episodes (Table I). Cross-reacting antibodies between B. quintana and B. henselae were detected in 8 of these 15 seropositive episodes. Three patients with B. henselae antibody levels IgG ≥ 200 were from the blood-culture positive group and were recruited to the study during the 1980s. Case number 1, a previously healthy 53-y-old man who had a Streptococcus millerii IE (Duke definite), fully recovered. The B. henselae and serotype 'Marseille' IgG antibodies were 200. Case number 2, an 82-y-old man who lived in a sheltered accommodation, suffered from prostatic cancer and had had a myocardial infarction 3 times. He acquired an Enterococcus IE (Duke definite) and recovered completely after antibiotic treatment. Bartonella henselae antibodies were 200. Case number 3, a 67-y-old man with alcohol abuse, acquired an Enterococcus faecalis IE (Duke definite). He underwent acute valvular surgery and recovered. The B. henselae and serotype 'Marseille' IgG antibody titre was 400.

Case number 4, the only patient with CNE, had slightly increased levels of B. henselae antibodies (IgG 100). He was a 74-y-old man with chronic renal disease. He had no previous antibiotic treatment on blood culture. Echocardiography demonstrated a little firm vegetation on the aortic valve. He had fever and a new systolic murmur on heart auscultation. The episode was classified as possible by the Duke criteria. He recovered completely after antibiotic treatment.

No data was available about contact with cats or signs of cat-scratch disease in any of these patients.

DISCUSSION

This screening of sera from 334 episodes of IE did not find any undiagnosed cases of Q-fever or Bartonella infection. The only patient with increased levels of phase I antibodies to C. burnetii had an already diagnosed and treated Q-fever endocarditis, probably acquired in Crete, Greece (30, 31). Among the studied episodes 183 were classified as definite by using the Duke criteria. 12 of these episodes were CNE (7%) (Table I).

The sera with antibodies to Bartonella between 200 and 400 were to be found in the blood culture-positive episodes of IE. However, the antibody levels did not reach the cut-off for IE, which is 800 (28). There were no data in their records about cat contact or body lice. One of these 3 patients was a habitual drinker, but he lived in tidy conditions. The population of homeless people in Göteborg is quite small (32, 33) and none of the investigated patients was homeless. Body lice are seldom found in Swedish patients. It is not probable that the patients were infected with B. quintana, although it is not necessary to have an antibody response despite growth of B. quintana on blood culture (34). In another Swedish study PCR identified B. quintana in a liver biopsy from 1 patient. In another patient B. quintana was detected in the myocardium but the antibody response was directed to B. elizabethae. The presence of cross-reacting antibodies between different Bartonella species raises the hypothesis of the presence of antigenic variants of B. quintana and B. henselae (35). Two different genotypes of B. henselae, which may affect the antigenic expression, have been isolated in German cats and humans (36, 37).

The elevated antibody response to Bartonella in the group studied, 4.5% (95% confidence interval 2.3–6.7%) (15/334), indicates an earlier contact with cats infected with B.

Table I. *Distribution of Bartonella antibodies among 334 episodes of infective endocarditis evaluated according to the Duke criteria*

	Definite	Possible	Reject
Blood-culture negative	$n = 12$	$n = 52$	$n = 7$
Bartonella > 800	0	0	0
200–400	0	0	0
50–100	0	2	0
Blood-culture positive	$n = 171$	$n = 69$	$n = 23$
Bartonella > 800	0	0	0
200–400	3	0	0
50–100	9	1	0

henselae or the presence of cross-reacting antibodies to B. elizabethae. Antibodies to B. elizabethae have been detected in Swedish cats, with the highest prevalence in southern Sweden (38). Bartonella (including B. elizabethae) antibodies have previously been found in 4% of Swedish blood donors and in 8.3% of patients with suspected Bartonella infection from Sweden (35). It may be concluded that Q-fever rarely causes IE in western Sweden and that no cases of serologically undiagnosed Bartonella IE were found. Q-fever serology should be done in all patients with CNE, since domestic Q-fever has been diagnosed and travelling abroad is common. CNE patients with a history of homelessness, contact with body lice or owners of cats should be tested for Bartonella antibodies. If valvular surgery is performed, culture, PCR and histology should be done of the excised valves.

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III

CHLAMYDOPHILA (CHLAMYDIA) PNEUMONIAE INFECTION-A RISK FACTOR FOR INFECTIVE ENDOCARDITIS?

MARIA WERNER [1], JUDY GNARPE [2], ANDERS ODÉN [3], RUNE ANDERSSON [4] & HARRIET HOGEVIK [5].

Department of Infectious Diseases, South Älvsborg Hospital, Borås, Sweden [1].
Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada [2]. Statistical consultant, Chalmers University of Technology, Sweden [3]. Research and Development Centre, Skaraborg Hospital, Skövde, Sweden [4]. Department of Infectious Diseases, Uddevalla Hospital, Sweden [5].

Abstract

The aims of this study were to investigate whether serologically documented *Chlamydophila pneumoniae* (*Cp*) infection is more prevalent in patients with blood culture negative endocarditis (CNE) than in those with blood culture positive endocarditis (CPE), and to investigate if increased levels of *Cp* antibodies may indicate a risk factor for infective endocarditis (IE), and if there might be any difference between men and women in this respect.

  Cp antibodies were analyzed by a microimmunofluorescence technique in 71 CNE episodes and 216 CPE episodes as well as in a randomly selected population control group of 70-year olds. The proportion of *Cp* IgG and IgA antibodies were compared in specimens from CPE and CNE episodes. No significant differences were found. *Cp* antibodies were compared in specimens from IE episodes and population controls. The results for <u>men</u> were *Cp* IgG≥512: 33% vs. 26 % of the controls, (aOR 1.37 [0.83-2.27]) and *Cp* IgA≥64: 45 % vs. 31% (aOR 1.80 [1.12-2.91]). The results for <u>women</u> were *Cp* IgG ≥512: 32% vs. 17%, (aOR 2.21 [1.32-3.70]) and Cp IgA ≥64: 32% vs. 10% (aOR 3.98 [2.26-7.01]).

  Increased levels of *Cp* IgA and IgG indicating infection with *Cp* were not found to be significantly different in CNE and CPE. High levels of *Cp* IgA were found more often in IE patients than in controls.

Key Words*:* Infective endocarditis, valvular disease, Chlamydophila pneumoniae

Introduction

Patients with valvular disease are prone to acquire infective endocarditis (IE) (1) and valve calcifications are prevalent as a cause of valvular disease (2). *Chlamydophila pneumoniae (C. pneumoniae)* has been suggested to be associated with arteriosclerosis and valvular disease, but its role in IE is unclear (3-10) Blood culture negative endocarditis (CNE) affects 10-20 % of patients with IE (11, 12). One of the microbes proposed as a cause of CNE is *C. pneumoniae* (13, 14).

The aims of this study were:

1. To investigate whether serologically documented *C. pneumoniae* infection is more prevalent in patients with CNE than in those with blood culture positive endocarditis (CPE).

2. To investigate if increased levels of *C. pneumoniae* antibodies may be a risk factor for IE and if there might be any difference between men and women in this respect.

Study groups and methods

Study groups

IE has been prospectively studied since 1984 at the Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden (1), a secondary and tertiary referral center in southwest Sweden. It is an ongoing non-randomized, descriptive study of consecutive patients with suspected endocarditis. The protocol includes clinical evaluation and collection of specimens. The protocol does not include questions about smoking, lung disease, hypertension and obesity. In all 750 patients were diagnosed with presumptive endocarditis between the years of 1984-1996. Blood samples were collected at inclusion and sera were stored at -70°C. Sera from patients included in the study between 1984 –1988, but later classified as Reject according to the modified Beth Israel criteria for IE, were discarded (1). Some samples were destroyed during storage.

In all, 314 episodes in 308 patients diagnosed as IE were analyzed with *Chlamydia* serology. The episodes were classified according to the Duke criteria; Duke Definite, Possible or Reject (15). Patients who did not qualify according to the Duke criteria were excluded from further analysis (27 episodes). Blood specimens for serological analysis were taken at the start of IE therapy in all but four patients. Data about sex and age distribution in CPE and CNE episodes are shown in Table I.

Table I. Age and the sex distribution in blood-culture positive endocarditis (CPE), blood-culture negative endocarditis (CNE) and in all studied cases of infective endocarditis (IE).

	All patients	Men	Women
CNE: mean age, range [] years. (number)	62.0 [26-84]. (71)	62.3 [41-83]. (38)	61.6 [26-84]. (33)
CPE mean age, range ([]) years. (number)	58.7 [20-89]. (216)	56.3 [20-89]. (122)	61.7 [18-87]. (94)
IE mean age, range []years, (number)	59.5 [20-89]. (287)	57.7 [20-89]. (160)	61.7 [18-87]. (127)

The Longitudinal Gerontological and Geriatric Studies in Göteborg, Sweden is a population study, which was initiated 1971/72 in Göteborg. From this study a sample of 1148 70-year old men and women were randomly selected in the Göteborg area (16), sera from a sample from this group (102 men and 142 women) were analyzed for *C. pneumoniae* antibodies. In 1992, another randomly selected sample of 753 70-year old people were included in the study and sera from a sample from this later group (76 men and 106 women) were also analyzed for *C. pneumoniae* antibodies (17).

Methods

Serology was analyzed using a modified microimmunofluorescence technique (18). Sera were diluted 1:16 (for IgM and IgA antibodies) and 1: 32 (for IgG antibodies) in phosphate buffered saline (PBS), pH 7.4, and tested for IgG, IgA and IgM antibodies on 21 well antigen slides containing elementary body preparations of *C. psittaci*, *C. pneumoniae* and *Chlamydia trachomatis* in each well (Lab Systems Oy, Finland), according to an earlier report (18). Sera positive in screening tests for IgG were rediluted and tested in doubling dilutions. Sera positive in screening tests for IgA and/or IgM were

mixed with Gullsorb (Gull Laboratories, USA) at a dilution of 1:16 to remove all IgG and then titrated in doubling dilutions with PBS (19). The same investigator blinded to case/control status read all slides. Control sera routinely used in the laboratory was included in every test run and tests were only accepted if the control sera titers were within one titer step of the mean earlier calculated. The last dilution step to give a specific fluorescent pattern was reported as the reciprocal titer (18). A *C. pneumoniae* titer of IgG antibodies ≥512 and a *C. pneumoniae* IgA ≥64 antibody titer were chosen as cut-offs for presumptive acute reinfection or persistent infection (19).

Statistics

The probability of *C. pneumoniae* IgG ≥512 and *C. pneumoniae* IgA ≥64 as a function of age, gender (men=0, women=1) and 0-1 variable CNE was estimated with help of the logistic regression analysis. The probability of *C. pneumoniae* IgG ≥512 and *C. pneumoniae* IgA ≥64 at the age of 70 years (the age of the individuals in the control group) can be calculated as $1/(1+exp (-S))$, where S is a linear combination of the following type. $ß0+ ß 1· 70 + ß 2· 1 + ß 3·0$. The number 70 represents age, 1 female and 0 presence of CNE. The last two variables may be changed depending upon the group of patients studied. The standard deviation (SD) of the linear combination was estimated by use of the matrix of covariance. In the pooled group of CPE (CNE=0) and CNE (CNE=1) was the logistic model used without the CNE variable. Sex differentiated analogous calculations were done on the control group. The variables age and sex were not included in this calculation. An adjusted odds ratio (aOR), taking age into account by use of the multiple logistic regression, was used comparing the presence of C. pneumoniae IgG ≥512 and IgA≥64 in the patient group CNE =0 and CNE=1 versus controls of the same sex and in the CNE=1 group versus CNE=0 group. Confidence limits (95% level) are given in brackets [CI 95%]. Values of quantitative variables were expressed as means and range (in brackets []).

Results

A comparison of *C. pneumoniae* antibodies in CNE and CPE patients

CPE and CNE episodes were compared taking age into account (Figure 1 and 2). The results for men were IgG ≥512 (CPE 30% vs. CNE 42% (aOR 1.69 [0.84-3.33])) and for IgA ≥64: (CPE 41% vs. CNE 53% (aOR 1.61 [0.83-3.13])).

The results for the women were: IgG ≥512 (CPE 29% vs. CNE 40% (aOR 1.69 [0.83-3.45])) and IgA ≥64 (CNE 29% vs. CPE 40% (aOR 1.61 [0.79-3.23])).

A comparison of *C. pneumoniae* antibodies in IE patients and population controls

IE episodes with an estimated prevalence of *C. pneumoniae* antibodies at the age of 70 years were compared with controls (Figure 1 and 2). The results for men were *C. pneumoniae* IgG≥512: (33% vs. 26 % of the controls, (aOR 1.37 [0.83-2.27])) and *Cp* IgA≥64: (45 % vs. 31% (aOR 1.80 [1.12-2.91])). The results for women were *C. pneumoniae* IgG ≥512: (32% vs. 17%, (aOR 2.21 [1.32-3.70]) and *Cp* IgA ≥64: 32% vs. 10% ((aOR 3.98 [2.26-7.01])).
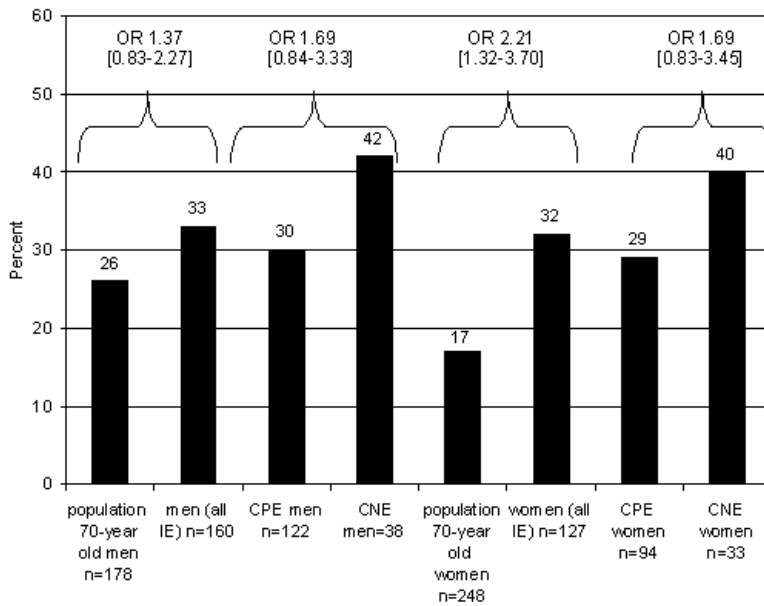
Figure 1

Proportions of C. pneumoniae IgG $\geq$ 512 in the 70-year old control group compared with the estimated probability (percent) at the age of 70 years in infective endocarditis patients (IE). Blood culture positive endocarditis (CPE) and blood culture negative endocarditis (CNE) episodes were compared and the odds ratios OR were age adjusted

Figure 2

Proportions of *C. pneumoniae* IgA $\geq$64) in the 70-year old control group compared with the estimated probability (percent) at the age of 70 years in infective endocarditis patients (IE). Blood culture positive endocarditis (CPE) and blood culture negative endocarditis (CNE) episodes were compared and the odds ratios (OR) were age adjusted
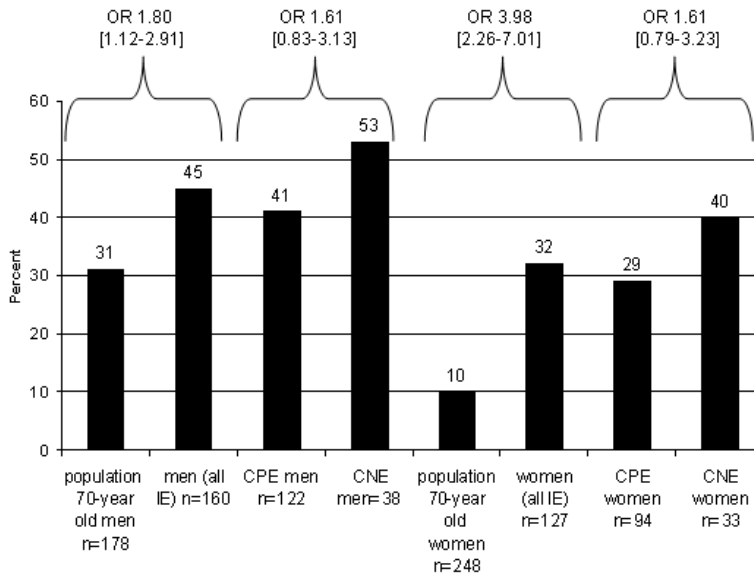
Discussion

No significant differences were seen in the proportions of episodes with high levels of *C. pneumoniae* antibodies when comparing CNE and CPE episodes and consequently, the study does not support the hypothesis of *C. pneumoniae* as a cause of blood culture negative endocarditis.

The *C. pneumoniae* antibody levels in IE patients were compared with the results from the antibody determination from the control population. We found a significant difference with increased *C. pneumoniae* IgG and IgA antibody levels in the female IE cases compared to the female controls. There were also increased *C. pneumoniae* IgA antibody levels in IE men compared to control men. The results should however be judged with caution. Although adjusted for age and sex the results were not adjusted to smoking, hypertension and obesity, proposed confounding factors in studies of aortic valve sclerosis (20, 21)

To our knowledge this is the first seroepidemiological study of *C. pneumoniae* in IE and in a control population. Antibodies to Bartonella were looked for but not found in the IE episodes and it is unlikely that cross-reacting antibodies could have caused the increased proportions of *C. pneumoniae* antibodies (22)

The specimens from the IE cases were collected during 13 years. About half of the specimens from the control group were collected 10 years earlier than the first specimens from an endocarditis patient. However, an ongoing epidemic that could have affected the controls seems not to have negated the significant differences found.

There is no validated reference test universally agreed upon for the diagnosis of persistent *C. pneumoniae* infection. The microimmunofluorescence test, considered to be the gold standard, is technically complex and diagnostic criteria can differ from laboratory to laboratory (23). IgG antibodies can be expected to be detected in serum for many years after an infection and serum IgG of ≥512 may demonstrate a possible chronic infection, but may of course also indicate recent infection with the bacterium. It might have been possible to distinguish between these two types of infection with analysis of sequential sera. IgA antibodies have a short half-life (about one week) and therefore it is thought that high levels of this immunoglobulin should be more indicative of an acute reinfection or a sustained infection. We used humoral IgA as a marker of persistent infection, although it is not a universally accepted criterion, and chose IgA ≥ 64 as a cut-off level because this level of antibody is not usually found in healthy, uninfected individuals (23, 24).

The association between *C. pneumoniae* antibodies and aortic valve sclerosis is not proven (6). *C. pneumoniae* has been detected in calcified aortic valves with immunohistochemistry and / or polymerase chain reaction (4, 5, 7, 25). In other such studies, *C. pneumoniae* have not been found(6, 8, 10). The relevance of raised *C. pneumoniae* IgG and IgA antibodies in serum and the finding of the bacteria in heart valves is not fully understood (6, 7, 26). Also it is uncertain whether the results of antibody tests in our patients mirror true *Chlamydophila* infections in heart valves, since we did not have the opportunity to study tissues from our patients. .

The men in all groups had higher proportions of IgG and IgA antibodies than women, a finding that is consistent with other serological studies (27-29). The reason for this is unknown.

Increased levels of *C. pneumoniae* IgA and IgG antibodies may be risk factors in women with infective endocarditis; men demonstrate a significant increase only of IgA. *C. pneumoniae* has been found in atherosclerotic lesions and it is possible that either this bacterium could be an innocent bystander or a co-pathogen in the pathogenesis of endocarditis. The study does not support the hypothesis of *C. pneumoniae* as a cause of blood culture

negative endocarditis. Future studies are necessary to establish a gold standard for the diagnosis of suspected *C. pneumoniae* infection in valves.

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Ethics

The Research Ethic Committee Göteborg University (Ö500-99) approved this study.

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IV

A ten-year survey of blood culture negative endocarditis in Sweden - aminoglycoside therapy is important for survival

MARIA WERNER [1], RUNE ANDERSSON [2], LARS OLAISON [3], HARRIET HOGEVIK [4]
 & *THE SWEDISH SOCIETY OF INFECTIOUS DISEASES QUALITY ASSURANCE STUDY GROUP FOR ENDOCARDITIS*

Department of Infectious Diseases, South Älvsborg Hospital, Borås, Sweden [1]. Research and Development Centre, Skaraborg Hospital, Skövde, Sweden [2]. Department of Infectious Diseases, Sahlgrenska University Hospital, Göteborg, Sweden [3]. Department of Infectious Diseases, Uddevalla Hospital, Sweden [4].

Abstract

We analyzed data from patients with infective endocarditis (IE) reported to the Swedish endocarditis registry during the 10-year period 1995-2004. The patients were cared for at all 29 Departments of Infectious Diseases in Sweden. We estimated the prevalence of blood culture negative endocarditis (CNE) and described and analyzed data with special attention to antibiotic treatment. During the 10-year period, 2509 IE episodes (78% Duke definite) were identified in 2410 patients.

   Three hundred and four (12%) CNE episodes were found, of which 42 % were women. The number of Duke Definite CNE episodes was 83. Fatal outcome occurred in 10.3 % of all IE episodes and in 5 % of the CNE episodes. The risk of dying was significantly increased in female (9%) compared to male CNE episodes (2%), OR 5.5. Two hundred and sixty-one episodes (88%) received beta–lactam antibiotics and 249 episodes (84%) received aminoglycoside therapy. The mortality was significantly decreased in episodes with aminoglycoside therapy (3%) vs. episodes without aminoglycoside therapy (13%), aOR 0.23.

Key Words: Blood culture negative infective endocarditis, antibiotic therapy, epidemiology, Sweden

Background

Diagnosis and treatment of patients with blood culture negative infective endocarditis (CNE) is a challenge for most clinicians. CNE is found in 8-54% (1-5) of infective endocarditis (IE) cases (IE). Diagnostic criteria, clinical settings and the health care organization are important factors for the estimated prevalence of CNE. To our knowledge there are no studies on antibiotic treatment in CNE. The objectives of this study, using data from a national registry for infective endocarditis, were:

   1. to estimate the proportion of patients with CNE among Swedish IE patients ,

   2. to describe and analyze data from CNE patients regarding demography, predisposing factors and clinical features,

   3. to describe the antibiotic treatments used in the CNE patients in relation to mortality and relapse.

Abbreviations

Blood culture negative endocarditis	CNE
Blood culture positive endocarditis	CPE
Infective endocarditis	IE
Intravenous drug use	IVDU
Native valve endocarditis	NVE
Odds ratio	OR
Prosthetic valve endocarditis	PVE
Transesophageal echocardiography	TEE
Transthoracal echocardiography	TTE

Correspondence: Maria Werner, Department of Infectious Diseases. South Älvsborg Hospital, S-50182 Borås.
E-mail: maria.werner@vgregion.se

Patients and methods

In 1995 a national registry collecting data about IE patients was established in Sweden (6), organized by the Swedish Society for Infectious Diseases. All 29 Departments of Infectious Diseases in Swedish hospitals participate in registering data from patients with diagnosed IE.

Study design

The patient's physician filled in a standardized questionnaire at the time of the patient's hospital discharge. The form includes questions about the patient's age, sex and predisposing factors such as history of heart valvular disease, intravenous drug use (IVDU) or prosthetic heart valve (PV). The form also includes data about the current episode of IE, length of hospital stay, patient's and doctor's delays, symptoms and signs, blood culture results, echocardiography, treatment and outcome. The form does not report *Bartonella* and /or *Coxiella burnetti* serology results. At the follow-up visit, the physician filled in a second form answering questions about morbidity and mortality during follow-up. Data from the questionnaires were entered into a computerized database and categorized according to the Duke criteria (7). The Swedish Society of Infectious Diseases in 2002 published the first Swedish guidelines for treatment in IE These guidelines were supplemented in 2004 by recommendations regarding therapy in cases with CNE (8).

Definitions

CNE is defined as an episode of IE with absence of growth in blood culture and classified as Duke definite or possible (7).

Mortality is defined as a death registered in form one.

Possible relapse of IE is defined as a new event of IE during the 6 months after completed IE treatment.

Surgery is defined as cardiac surgery during the period of parenteral antibiotic treatment.

The first day of an episode is defined as the first day of intravenous antibiotic treatment.

Blood cultures

The local hospital departments of microbiology analyzed the blood cultures. The automated blood culture systems most often used in Sweden during this period were the Bac-T Alert and BACTEC systems. The blood was incubated for 7-10 days before test results were answered as culture negative.

Statistics

Values for quantitative variables were expressed as means, medians and range (in brackets []). Odds ratio(s) (OR) were calculated with 95% confidence limits [95% CI] or exact confidence limits when needed (StatCalc). Adjusted odds ratios were calculated using the multiple logistic regression analysis (EpiInfo-6). A two-tailed test was calculated with a significance level of 0.05; Yates correction and Fishers exact test were used when appropriate.

Results

Proportion of CNE among Swedish IE patients

During the ten-year study, 2,546 IE episodes were registered. We excluded thirty-seven episodes because of duplicates, scarcity of data, blood cultures not done or classified as rejected by the Duke criteria. Data from the remaining 2,509 treated episodes (2,410 patients) were analyzed in the study. Three hundred and four episodes (301 patients) were CNE and 2,205 episodes (2,109 patients) were blood culture positive endocarditis (CPE). The proportion of CNE was calculated as 304/2,509 12% [95%CI 9-15%]. One thousand nine hundred and fifty-two IE episodes fulfilled Duke definite criteria and 83 of these episodes had CNE. The

proportion of Duke definite CNE episodes was 83/1,952, 4.2%[95%CI 3.6-5.0].

Clinical features and predisposing factors in CNE patients

Data are presented in Table 1 and 2.

Table 1. A comparison of demographic and clinical features in 2509 blood culture negative and blood culture positive episodes of infective endocarditis

	Blood culture negative (n=304)	Blood culture positive (n=2205)	OR [95%CI]
Females	127 (42%)	764 (35%)	1.35 [1.05-1.74]
Age in years, median and range[ ]	68 [17-89]	66 [14-100]	-
Valvular disease	111 (37%)	884 (40%)	0.86 [0.67-1,11]
Prosthetic valve	57 (19%)	394 (18%)	1.07 [0.77-1.46]
Previous endocarditis	32 (10%)	218 (10%)	-
Injection drug use	10 (3%)	267 (12%)	0.25 [0.12-0.48]
Aortic valve infection	151(50%)	837 (38%)	1.61 [1.26-2.07]
Mitral valve infection	89 (29%)	754 (34%)	0.80 0.61-1.04
Aortic and mitral valve infection	20 (7%)	167 (7%)	-
Tricuspid valve infection	15(5%)	246 (11%)	0.63 [0.35 -1.11]
Duke criteria (Definite / Possible)	83 (27 %) /221 (73 %)	1869 (85 %) /336 (15 %)	-
Vascular phenomena (emboli)	63 (21%)	770 (35%)	0.49 [0.36-0.66]

The male-female ratios were 1.39 (177:127) in CNE and 1.89 (1441:764) in CPE Acute surgery was performed in 46 (15 %) of the CNE patients (median day 12, range [0-75]), as compared to 453 (21%) of the blood culture positive IE (CPE) patients on (median day 11 range [7-68]).

Two of the CNE patients (4%) and 37 of the CPE patients (8%) who underwent surgery died.

Table 2.A comparison of echocardiographic findings, treatment and follow up in blood culture positive and negative patients

	Blood culture negative n=304	Blood culture positive n=2205	OR [95%CI]
Echocardio-graphy,	300 (99%)	2189 (99%)	-
TEE	247 (82%)	1717 (78%)	1.23 [0.90-1.69]
TTE	201 (66%)	1527 (69%)	0.87 [0.67-1.13]
New dehiscence of prosthetic valve	10 (3%)	89 (4%)	0.81 [0.39-1.62]
Vegetation	236 (78%)	1512 (69%)	1.59 [1.19-2-14]
Abscess	10 (3%)	155 (7%)	0,45 [0.22-0.89]
Mean in-hospital time (days)	32	34	-
Acute surgery	46 / 297[1] (15%)	453/ 2152[2] (21%)	0.69 [0.49-0.97]
Mortality	14 (5%),	246 (11%),	0.38 [0.21-0.68]
Relapse	3 / 152 (2 %)	23/ 1116 (2%)	-
Deceased during follow up	4 /152 (3 %)	39/1116 (3.5%)	-

[1]. Missing data about antibiotic treatment in seven episodes
[2]. Missing data about antibiotic treatment in 53 episodes

Mortality during treatment was 10.3 % in all IE-episodes (CNE and CPE included) over the ten-year study period (1995-2004). Fatal outcome occurred in 14 CNE patients (5% of the episodes) as compared to 246 CPE patients (11% of the episodes) 0.38 [95%CI 0.21-0.68]. The mortality in CNE male episodes was 3/177 (2 %) vs. CPE male episodes 149/1441 (10%), OR 0.15 [95%CI 0.03-0.45]. The mortality in CNE female episodes was 11/127 (9%) vs. 97/764 (13%) in CPE female episodes, OR 0.6 [95%CI 0.32-1.30]. Fatal outcome during treatment occurred in 11/127 (9%) of the female CNE episodes vs. 3/177 (2%) in male CNE episodes, aOR 5.59 [95%CI 1.40-31.2] (adjusted for age and aminoglycoside treatment).

In 152 (50.0%) of the CNE episodes and in 1,116 (51 %) of the CPE episodes a follow-up Case Report Form (CRF) was completed. Time to follow-up was mean

96 days in CPE episodes and mean 98 days in CNE episodes. A comparison between blood culture negative native valve endocarditis (NVE) and prosthetic valve (PVE) are presented in Table 3. Acute surgery was performed in 13% NVE episodes as compared with 21% in PVE episodes. Death during treatment occurred in 3% of the NVE episodes vs. 11% of the PVE episodes.

Antibiotic therapy in CNE

Data are presented in Table 4. In the two hundred and ninety-seven episodes the patients received a cell wall–active antimicrobial drug for a median of 28 days and an aminoglycoside was added for a median 14 days in 84% of these episodes. The antibiotics used included Penicillin G, ampicillin, oxacillin, cephalosporin and carbapenem antibiotics in 261 (88%) episodes, vancomycin in 81 (27%) episodes and sequential therapy with these antibiotics in 57 episodes. Rifampicin treatment was administered in twelve episodes: Eight NVE episodes and four PVE episodes. In 48 (16%) episodes no aminoglycoside therapy was given. Fatal outcome occurred in eight of the episodes (3%) that received amino-glycosides as therapy for IE versus six of the episodes (13%) who did not receive amino-glycosides, aOR 0.23 [0.08-0.85] p=0.03 (adjusted age and sex). Eight patients with blood culture negative NVE and six PVE patients, all who died are described in Tables 5 and 6. In NVE were 7/8 and in PVE were 4/6 of the dead patients females.

Relapses

Three CNE patients had a new IE during follow-up within the first 6 months of completed antibiotic therapy. The new episodes were deemed as possible relapses. The first patient was an 85-year-old woman with a pacemaker. Echocardiography detected a vegetation on her pacemaker cable. *Staphylococcus* a*ureus* was isolated from her pacemaker

pouch. She underwent treatment with oxacillin for 27 days combined with an aminoglycoside for 7 days. Relapse occurred 24 days after initial antibiotic treatment was stopped and *Staphylococcus*

Table 3. A comparison of clinical data in 297[1.] episodes of blood culture negative IE divided in native valve and prosthesis valve infective endocarditis

		Native valve blood culture negative IE	Pros-thesis valve blood culture negative IE	OR [95%CI]
Clinical and demo-graphic data	Number of episodes [1.]	240	57	-
	Age median [range] years	68 [17-89]	66 [25-85]	-
	Duke criteria (Definite/ Possible)	63 / 177	20 / 37	-
	Surgery, n (%) (treatment day median range [],	33 (13), 12 [0-75]	12, (21), 10 [0-49],	0.6 [0.27-1.33]
	Deaths during treatment n (%)	8 (3%)	6 (11%)	0.29 [0.09-1.08]

[1]Seven episodes excluded, no data about antibiotic therapy).

aureus was isolated from her blood. She underwent a new period of treatment with oxacillin (28 days) combined with an aminoglycoside (14 days), followed by oral flucloxacillin. Pacemaker extraction was impossible. The patient has been followed for three years and is still alive.

The second patient was a 57-year-old woman with a valvular prosthesis. She had fever and heart failure. Echocardiography diagnosed a vegetation and an abscess engaging the aortic and mitral valves. She underwent heart valve replacement the same day she arrived at the hospital. There are no data about valve culture or histological examination. Antibiotic treatment with vancomycin and rifampicin continued for 56 days combined with aminoglycoside for 7 days. She relapsed 18 days after the initial period of treatment was stopped. There are no data about blood culture findings or the antibiotic treatment during the relapse. She died 7 months later.

The third patient was a 50-year-old woman who had a mitral IE with vegetation and central nervous system embolism. She was treated with vancomycin and later teicoplanin for a total of 60 days. The possible relapse, occurred about 90 days after initial IE therapy was completed. She underwent new antibiotic treatment with vancomycin and teicoplanin for 60 days and cardiac surgery was performed with a valvular prosthesis insertion. The patient has been followed for five years without any further complications.

Table 4. Antibiotic therapy in blood culture negative IE

	Antibiotic therapy	β-lactam	Aminoglycoside	Aminoglycoside and β-lactam	Vancomycin	Rifampicin
ALL CNE N (%), treatment (days) median, range []	297 (100) 28 [1-101]	261 (88) 28 [2-77]	249 (84) 14 [2-63]	232 (78) 14 [1-49]	81 (27) 21 [2-83]	12 (4) 27 [6-56]
NATIVE VALVE CNE N (%), treatment (days) median, range []	240 (100), 28 [1-101]	216 (90), 28 [2-77]	201, (84), 14 [2-56]	192 (80), 14 [1-40]	57 (24), 15 [2-83]	8 (3), 21 [6-43]
PROSTHETIC VALVE CNE, N (%), treatment (days) median, range []	57, (100), 28 [1-89]	45 (79) 25 [2-80]	48 (84), 14 [3-63],	40 (70), 14 [1-27]	24, (42), 30 [2-56]	4 (7) 42 [21-56]

Abbreviations: β-lactam: Penicillin G, ampicillin, oxacillin, cephalosporin and carbapenem, blood culture negative IE (CNE).

Table 5. Fatal outcome in eight episodes of blood culture negative <u>native valve endocarditis</u> with special attention to antibiotic therapy

	Death day	Age, years sex	Duke criteria	Echo-cardiography	Affected valve	Post-mortem examination, cause of death	ANTIBIOTIC TREATMENT (DAYS)				
							β-lactam	Amino-glycoside	β-lactam and amino-glycoside	Vanco-mycin	All antibiotics
1	21	75 F	D	TTE, TEE. Vegetation, pericardial effusion	AO	Not done, myocardial infarction suspected	21	16	16	0	21
2	21	81 F	D	TTE. Vegetation	MI	IE	21	10	10	0	21
3	17	78 F	D	Not done	AO	IE. Diagnosed as pneumonia ante mortem	17	0	0	0	17
4	15	52 F	D	TEE. Vegetation	AO	Yes, multi organ failure	Number of days unknown	Number of days unknown	Number of days unknown	0	0
5	5	79 F	D	TTE, TEE Difficult to visualize structures	AO	Yes, sepsis, hepatocellular cancer	5	0	0	0	5
6	20	46 F	D	TTE. Vegetation	MI	Yes. Heart rupture, IVDU	20	11	11	0	20
7	57	42 F	D	TEE. Vegetation, ventricular septum defect	MI	Yes, multi-organ failure (Streptococcus anginosus in pleural fluid	17	0	0	2	19
8	17	76 M	P	TTE, TEE Suspected pace maker vegetation	TRI	Yes. Result unknown	15	0	0	0	15

Abbreviations: Female (F), Male (M), Definite (D), Possible (P), Aortic valve (AO), mitral valve (MI), tricuspid valve (TRI).

Table 6. Fatal outcome in six episodes of blood culture negative prosthetic valve endocarditis with special attention to antibiotic therapy

No.	DEATH DAY	Age (YS) sex	Duke	ECHOCARDIO-GRAPHY	Affected valve	Postmortem examination, cause of death	Antibiotic treatment (days)				
							β-lactam	Amino-glycoside	β-lactam and amino-glycoside combined	Vanco-mycin	All antibiotics
1	24	75 F	P	TTE, TEE Vegetation valvular dehiscence	MI	Not done, heart failure	0	3	0	24	24
2	19	71 F	D	TEE Vegetation	AO and MI	Cerebral bleeding	19	16	16	1	20
3	23	81 F	P	TTE Vegetation, heart failure	AO	Not done, not described	24	4	4		24
4	21	58 M	D	TEE Vegetation	MI	Not done, renal failure	22	12	12	0	22
5	17	80 F	D	TTE, TEE Vegetation	MI	Not done, gastro-intestinal bleeding, pulmonary embolism	14	0	0	0	14
	11	81 M	P	TTE, TEE Vegetation, valvular stenosis	MI	No data	9	8	8	2	11

Abbreviations: Female (F), Male (M), Years (YS), Definite (D), Possible (P), Aortic valve (AO), mitral valve (MI), tricuspid valve (TRI).

Discussion

The proportion of CNE found (12%) is less than in an earlier prospective study of CNE from Western Sweden (5). The prevalence of CNE was approximately 20% in all episodes of IE in that study. This may be due to selection bias, since the present study is based on data from reporting doctors and CPE episodes may have been reported to a higher degree than CNE episodes.

The Duke possible group is heterogeneous; CNE patients who would be classified in the possible Duke criteria group in a prospective study may have been given other diagnoses such as heart failure or pneumonia. (7). Also there are opportunities for misclassification of CNE with the Duke criteria, diagnosing obvious definite cases as possible (9).

The absence of positive blood cultures may be one reason not to perform an echocardiography. Although IE is not a self-limiting disease, short treatment (four days or less) with intravenous antibiotics may cure it, which the Duke criteria consider when differentiating between the "Possible" and "Reject" groups. The low proportion of Duke definite CNE episodes (4 %) of all Duke definite IE episodes corresponds to our previous findings (5), and is probably attributable to underestimation of the "real" proportion of IE with the classification system (5).

Mortality in all IE-episodes during the study period was 10.3 %. The mortality is low as compared with recently published studies of IE; 16% France (1999), 24%, Argentina (2002), 14% Northern Italy (2000-1), 26% Minnesota US (1970-2000), (10-13)

Methicillin resistant *Staphylococcus aureus* still has a low prevalence in Sweden and nearly all patients with IE are treated at specialized Infectious Diseases Departments. These factors may contribute to the estimated low mortality.

Data from CNE and CPE episodes are in good agreement. The absence of positive blood cultures explains the low proportion

of Duke definite episodes in the CNE group (27%) as compared with the CPE group (85%) (3, 5, 14, 15) as blood culture findings are one of the cornerstones of the Duke criteria (7). There were fewer IVDU in the CNE group than in the CPE group (3% vs. 12%). Tricuspid IE was also less common (5% vs. 12%). Pacemaker endocarditis with right-sided IE has been associated with CNE but we have not been able to test this hypothesis since the CRF did not contain this information.

Vascular phenomena were less common in CNE patients than in CPE patients (21 vs. 35%). This finding corresponds to other studies (5, 14).

The aortic valves were more often affected in CNE patients than in CPE patients (50% vs. 38%). Also this is in accordance with findings by others (15, 16). We suggest that the hemodynamic influence of a diseased aortic valve makes the clinicians to order an echocardiography in spite the fact that the blood cultures are negative.

It is well known that patients who have received antibiotic treatment before blood cultures are often found to have CNE (3-5, 17). Data on antibiotic treatment before admission were not reliable enough to draw any conclusions about this.

False positive echocardiographic findings such as nodular thickening, non-oscillating tissue and uninfected thrombi may be difficult to differentiate from IE vegetation (18) and might to some extent contribute to increase the group of CNE patients.

Surgery was performed in 16% of the CNE episodes as compared to 21 % in the CPE episodes. This corresponds well to the findings in a previous study with surgery in 15% of the episodes (5). Mortality was lower in the CNE than the CPE group (5% vs. 11%). The relatively low mortality and low prevalence of acute valvular surgery in CNE patients may indicate that CNE in Sweden might be a milder variant of IE. Preparedness for early parenteral antibiotic treatment may have affected the positive outcome. It is also of importance to point out that there were no reported episodes of Q-fever or infection with *Bartonella,* which, if present could have contributed to a worse outcome.

There was a fatal outcome in 9% of the women with CNE episodes vs. 2% in males, aOR 5.5. The increased odds ratio indicates that treatment and diagnostic procedures for women with CNE need improvement.

The proportion of PVE was nearly the same in the CNE and the CPE groups (19 % vs. 18%). The data forms did not include questions about how long the PVE patients had had their heart valve prostheses, a factor that influences which microorganisms most frequently infect the valve. Seventy-eight percent of all patients got combination therapy with an aminoglycoside and a beta-lactam antibiotic, but fewer patients were treated with this combination in the PVE group (70%). Vancomycin treatment was predominant in the PVE group (42% vs. 24%). Only twelve patients received treatment with rifampicin four (7%) PVE and eight (3%) NVE patients. The impact of rifampicin treatment in CNE warrants further studies. The higher mortality in patients without aminoglycoside therapy (p=0.03) supports the inclusion of aminoglycoside in the treatment regimen of CNE, as suggested in recently published guidelines from the US (19) and also from Europe (8, 20). This is important, as the impact of aminoglycoside treatment in IE has attracted a great deal of attention in recent years (21, 22).

We used the term *possible relapse* as a new event of IE during the 6 months after completed IE therapy. If confirmational testing by molecular analysis is not performed, the terms relapse and reinfection are inadequate (23). It is obvious that the term reinfection is difficult to use in patients with CNE.

In conclusion, in a ten-year survey of IE in Sweden the proportion of CNE in the reported episodes treated as IE was 12%.

Mortality in IE during the studied ten-year period was low (10.3%) and it was even lower (5%) in CNE. There were no great differences in demographic or other predisposing factors between CNE and CPE. Patients with CNE who received aminoglycoside therapy survived more frequently than CNE patients who did not receive this therapy. The study indicates that aminoglycosides should be used in the treatment of CNE.

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