Recurrent Streptococcal Pharyngotonsillitis Studies on etiology and treatment

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Recurrent Streptococcal Pharyngotonsillitis

Studies on Etiology and Treatment

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Lund 2006

To my three sons

Albert, Henrik and Filip

and to

Gunilla my love

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS

AP-PCR	Arbitrarily Primed Polymerase Chain Reaction
CFU	Colony Forming Unit
GAS	β-haemolytic Group A Streptococci
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
NF	Necrotizing Fasciitis
N.S.	Non Significant
STSS	Streptococcal Toxic Shock Syndrome
TSF	Tonsillar Surface Fluid
PcV	Phenoxymethyl penicillin

Bacterial treatment failure	presence of GAS of the same T-type as that of the pre-treatment strain, within two weeks after completing therapy.
Clinical and bacterial failure	as above in combination with clinical symptoms and signs of pharyngotonsillitis.
Reinfection	occurrence of a new GAS strain, irrespective of T-type, after successfull eradication of the primary GAS.
Penicillin tolerance	MBC/MIC ≥32 and survival rate ≥1% in "Time killing test".

LIST OF PUBLICATIONS

This thesis is based on studies reported in the following papers, referred to in the text by their respective Roman numerals (I –V).

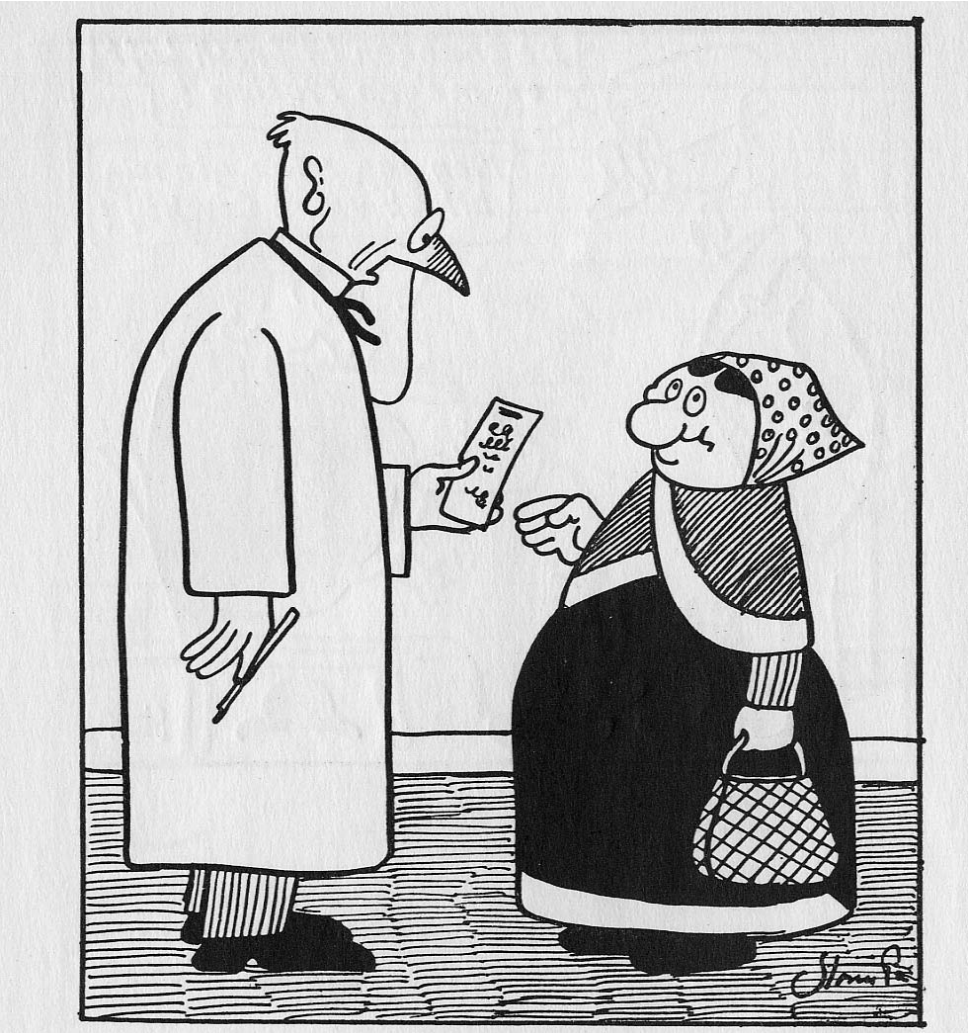
I Orrling A, Stjernquist-Desatnik A, Schalén C, Kamme C. Clindamycin in persisting streptococcal pharyngotonsillitis after penicillin treatment. Scand J Inf Dis 26:535-41, 1994.

II Orrling A, Stjernquist-Desatnik A, Schalén C, Kamme C. Clindamycin in recurrent group A streptococcal pharyngotonsillitis - An alternative to tonsillectomy. Acta Otolaryngol (Stockh) 117:618-22, 1997.

III Orrling A, Stjernquist-Desatnik A, Schalén C, Kamme C: Treatment failure in streptococcal pharyngotonsillitis. An attempt to identify penicillin tolerant streptococcus pyogenes. Scand J Infect Dis 28:143-7, 1996.

IV Orrling A, Karlsson E, Melhus Å, Stjernquist-Desatnik A. Penicillin treatment failure in group A streptococcal tonsillopharyngitis: No genetic difference found between strains isolated from failures and nonfailures. Ann Otology Rhinol Laryngol 110:690-5, 2001.

V Orrling A, Kamme C, Stjernquist-Desatnik A. Penicillin V, loracarbef and clindamycin in tonsillar surface fluid during acute group A streptococcal pharyngotonsillitis. Scand J Inf Dis 37: 429-35, 2005.



Hvis dette ikke hjælper, kan De komme igen – så skal De få noget andet.
Kunne jeg så ikke ligesågodt få det andet med det samme!

The therapeutic dilemma

The physician: If this does not help, then please come back – and I will prescribe another medicine.
The little woman: Couldn´t I as well get that other medicine at once!

(Publ. with permission from the Storm P. Museum, Frederiksberg Denmark)

INTRODUCTION

Acute pharyngotonsillitis is a common infection with an annual incidence in Sweden of approximately 300.000 cases (Tierpsprojektet**)** and group A streptococci (GAS) is the etiologic agent in 30-50% of cases (Wannamaker 1972; Roos 1985; Stjernquist-Desatnik et al 1987). GAS pharyngotonsillitis results in a high degree of absence from day-care, school and work, and it is agreed that antibiotic treatment is indicated. Phenoxymethylpenicillin (pcV) is the drug of choice in Sweden. In spite of exposure to β-lactams for decades and in contrast to several other common pathogens, GAS has over the years retained unchanged high susceptibility to these drugs. However, failure rates in pcV treated GAS pharyngotonsillitis are as high as 5-25% (Schwartz et al 1981; Strömberg et al 1988). A second course of pcV treatment is followed by still higher failure rates (Kaplan & Johnson 1988), and repeated failure in some cases necessitates tonsillectomy, one of the most common surgical procedures in the western world, although the benefit of operation in recurrent pharyngotonsillitis, lasts for only two to three years (Paradise et al 1984).

The background of failure remains largely elusive. Several factors possibly contributing to the recurrences have been mentioned: low compliance, reinfection from the environment, eradication of α-streptococci with inhibitory effect on GAS, increase in β-lactamase producing bacteria inactivating the drug, penicillin tolerant streptococci, low antibiotic concentration at site of infection and finally intracellular GAS surviving therapy.

PHARYNGOTONSILLITIS

Microbial etiology

Pharyngotonsillitis can be caused by a wide variety of pathogens. When symptoms are mainly restricted to the throat, however, a majority are of bacterial origin. GAS is the causative agent in about 50 % of cases. Group C and G streptococci cause 5-10% whereas other bacteria such as Arcanobacterium hemolyticum, Chlamydia pneumoniae, Mycoplasma pneumonie, Borrelia vincenti, Corynebacterium diphteriae and Neisseria gonorrhoeae are more seldomly seen (Hill et al 1969 ; Benjamin & Perriello 1976; Woodruff 1980; Telian 1986; Banck & Nyman 1986). Viruses account for 20-30 % of cases (Glezen et al 1967; Moffet et al 1968), and in the remaining 10-20% the causative agent is unknown (Ross et al 1971; Nordenfelt 1981).

Diagnosis

Clinical diagnosis

Signs and symptoms of acute pharyngotonsillitis include fever, throat angina, redness of tonsils and pharynx, tonsillar exudate, enlarged and tender cervical lymph nodes and dysphagia. Concurrent symptoms from the respiratory tract, e.g. cough or rhinorrhea, indicate viral origin. Established GAS throat and skin infection in the close surroundings like family, school or day care increases the probability of GAS origin. Certain symptoms could be more pronounced in pharyngotonsillitis of GAS origin than of other etiology. Thus the degree of redness in the throat (Roos 1985), fever (Hansen et al 1983), and a shorter duration of symptoms before seeking medical care (Stjernquist-Desatnik et al 1987) were reported to correlate significantly to recovery of GAS. The findings are, however, inconsistent and although the clinical picture could be of some guidance it is seldom sufficient for a reliable etiological diagnosis.

Microbiological diagnosis of GAS pharyngotonsillitis

Since there is no pathognomonic sign or combination of symptoms and signs in this condition, the definite diagnosis of GAS pharyngotonsillitis depends on identification of the bacteria. This might be done by a rapid antigen detection test or by a throat culture. A good view of the pharynx and correct sampling technique is essential to achieve a representative sample. The specimen should be obtained from the tonsillar surface, since in GAS pharyngotonsillitis the streptococci are predominantly localized on the tonsils and on the posterior oropharyngeal wall (Lilja et al 1997). A certain amount of bacteria, larger than for culture, is needed for a positive rapid antigen test. Quality of sampling may therefore influence on sensitivity and specificity of the rapid tests, currently reported as 74 % - 97 % and 89 % -95 % respectively (Nerbrant 2002; Lindbaek et al 2004).

Laboratory findings

A correlation between leucocytosis (Roos 1985; Hjortdahl & Melbye 1994) as well as increased levels of CRP and GAS pharyngotonsillitis has been reported (Kaplan & Wannamaker 1977) while other investigators failed to verify this (Putto et al 1986; Sun et al 2002).

β HAEMOLYTIC GROUP A STREPTOCOCCI

Grouping of streptococci

A basic tool for epidemiological investigations, as well as studies on treatment failure vs. reinfection of pharyngotonsillitis is the accurate identification of bacterial strains . The streptococci are classified into Lancefield´s serological groups A –U according to carbohydrate antigen in the cell wall (Lancefield 1933). Based on the presence of T-antigen, GAS are divided into approximately 30 T-types (Lancefield 1928).
Further subdivision is made on basis of the M-protein (Lancefield 1928). Advances in DNA-sequencing technology in the late twentieth century resulted in the development of methods for determining the M type of GAS from the sequence of the corresponding gene *emm*, and up to now more than 120 *emm*-types are identified (Facklam et al 2002).

Virulence factors

GAS exhibit a multitude of extracellular and cell-bound virulence factors with probable impact on different disease manifestations, and at various stages of the invasive process.
The cell wall *M-protein*, an extended α-helical protein with anticomplementary and antiphagocytic properties, is considered as a main factor determining virulence of GAS. Many M-proteins, by interacting with plasma proteins, such as IgG, fibrinogen and C4-binding protein, exhibit mechanisms specifically blocking the innate or acquired immune systems (Carlsson et al 2005). As established long ago, only type-specific antibodies directed to the N-terminal part of M-protein will be opsonic, and protect against GAS disease (Fischetti 1989)
The *hyaluronic capsule*, though poorly expressed *in vitro*, is a second, antiphagocytic part of GAS (Wessels et al 1991). The *T-protein*, previously not implicated as biologically important, was recently shown to mediate formation of fimbriae-like structures in GAS, of possible role for tissue adhesion (Mora et al 2005). *Pyrogenic exotoxins* (*erythrogenic toxins)*, are now established as so-called superantigens, viz highly active toxins triggering T cells to massive cytokine and interleukin release, thereby generating severe symptoms, such as fever, the scarlatiniform rash, tissue necrosis, hypotension and organ failure (Bisno et al 2003). The *cysteine protease* (identical to *exotoxin SpeB*) according to experimental work may be essential for severe clinical manifestations, such as circulatory shock and lung damage (Herwald et al 1998; Herwald et al 2004). This enzyme, and a second cysteine protease of GAS, may also cleave IgG, thereby interfering with immune opsonization of GAS (von

Pawel-Rammingen et al 2003). *Streptolysins S and O* are capable of lysing erythrocytes as well as leucocytes and platelets (Sierig 2003, Fontaine 2003). *Streptokinase*, which converts plasminogen to plasmin, may significantly contribute to rapid spread of GAS in infected tissue, i.a. by lysing blood clots (Lottenberg 1994).

Internalization

The ability of GAS, especially in the stationary phase, to invade respiratory epithelial cells has been demonstrated in recent years (LaPenta et al 1994; Österlund & Engstrand 1995). GAS are mainly found extracellulary, but by specifically binding fibronectin, a protein that exists in human blood plasma and in the extracellular matrix GAS may be efficiently internalized into human mucosal cells. The fibronectin bound to the bacterial surface thereby acts like a bridging molecule towards host cell integrins, which in turn initiate the uptake process that leads to internalization (Kreikemeyer et al 2004).
Sela and Barziali (1999) found that GAS strains were able to survive for 4–7 days inside cultured epithelial cells, and also that GAS strains from patients with eradication failure harboured an internalization-associated gene in higher prevalence than strains recovered from patients with successful eradication. Internalized GAS have been found in asymptomatic carriers as well as in patients with pharyngotonsillitis (Österlund et al 1997**)** and various strains of streptococci have different capacity to internalize (LaPenta et al 1994; Österlund & Engstrand 1995). Interestingly strains from cases of eradication failure showed significantly increased intracellular survival compared to strains from non failures (Marouni et al 2004) Whether internalization into host cells may influence on the severity of GAS infections is not known; however, in an animal model, a GAS strain able to internalize was less prone to cause serious disease than GAS without that capacity (Nyberg et al 2004).

Disease manifestations

GAS are strict human pathogens giving rise to a wide range of infections. Impetigo, pharyngotonsillitis and erysipelas may be comparatively mild and are effectively treated with antibiotics. However, since late eighties a rising number of life threatening, invasive GAS infections such as necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) have been encountered (Cone et al 1987; Hoge et al 1993). Streptococci in these cases are often restricted to certain M-types, in particular M1, and produce powerful superantigens, such as pyrogenic exotoxin A – SpeA. Surgical intervention is often needed in the case of NF. However, in spite of antibiotics and intensive care the mortality in both NF and STSS is high.

(Davies et al 1996; Eriksson et al 1998). Acute rheumatic fever, the most serious non-suppurative complication to GAS pharyngotonsillitis, is the leading cause of acquired heart disease among children in developing countries (Bisno 1991). Although no longer a significant health problem in most socioeconomically advanced countries, limited outbreaks of acute rheumatic fever have occurred in the US in the eighties (Veasy et al 1987).
Acute post-streptococcal glomerulonephritis, a major cause of child renal failure occurs after throat as well as skin infections with GAS (Wannamaker 1970). Large epidemics are still noted in the developing countries, as compared to sporadic cases in our part of the world.

ANTIBIOTICS IN TREATMENT OF GAS PHARYNGOTONSILLITIS

β-lactam antibiotics

PcV and *cephalosporins* act on bacteria by inhibiting synthesis of the cell wall and are thus only active against growing bacteria, while organisms in lag phase and stationary phase, since they are not replicating, are not susceptible to these substances**.** The β-lactam antibiotics have low or no intracellular accessibility. Although GAS have been exposed to β-lactams for decades, there has been no development of resistance to these drugs. A possible explanation may be, that penicillin resistance in this species is not compatible with a virulent phenotype (Gutman & Tomasz 1982).
Penicillin is inactivated by β-lactamase produced by Staphylococci, Bacteroides and Fusobacteria spp in the throat.
In pcV treatment of GAS pharyngotonsillitis the importance of no less than 10 days treatment to achieve acceptably low recurrence rate, has been well documented (Schwartz et al 1981; Gerber et al 1987; Strömberg et al 1988; Zwart et al 2000). In a meta-analysis Lan and colleagues (2000) found the current recommended dosing frequency of 2 times daily for 10 days to be as efficacious as more frequent dosing regimens in treatment of GAS pharyngotonsillitis.
In primary GAS pharyngotonsillitis cephalosporins have been shown to be more effective than pcV (Pichichero et al 1987; Holm et al 1991; Milatovic & Knauer 1989). Cephalosporins may enable shorter treatment regimens than pcV in GAS pharyngotonsillitis, and some may be dosed once daily (Pichichero et al 1994).
Cephalosporins are less susceptible to the β-lactamases produced by the oral bacterial flora and probably have lesser impact on the bacteriocin producing α-haemolytic streptococci in the throat. Theoretically, lack of effect of cephalosporins on α-streptococci not disturbing the

bacterial interference could explain the better eradication of GAS by cephalosporins than by pcV (Holm et al. 1991; Roos et al. 1993).

Macrolides

Macrolides act by interfering with the protein synthesis and are mainly bacteriostatic. Stjernquist-Desatnik and colleagues (1993) investigated erythromycin in TSF and found detectable levels in half of the healthy persons investigated. Even though macrolides act intracellularly, and therefore may reach internalized GAS, the rate of failure in GAS pharyngotonsillitis is almost the same as by pcV treatment (Brook & Hirokawa 1985; Söderström et al 1991; Watkins et al 1997; Cohen et al 2002). In GAS pharyngotonsillitis, however, macrolides are less suitable because of tendency to induce resistance in GAS. Outbreaks of erythromycin resistant GAS are known from Japan (Maruyama et al 1979), Finland (Seppälä et al 1992) and many other countries. Although only 2 % of Swedish GAS strains are currently resistant to erythromycin, higher figures have been reported in Sweden in the eighties (Stjernquist-Desatnik et al 1994).

Clindamycin

Clindamycin also blocks protein synthesis and acts intracellularly. Log phase as well as stationary phase GAS are susceptible to the drug. Low recurrence rates have been achieved in treatment of pharyngotonsillitis by clindamycin (Brook & Hirokawa 1985; Jensen & Larsen 1991). The rate of GAS isolates resistant to clindamycin is generally low e.g. < 1% in Sweden in 2005. However clindamycin resistance in GAS may be linked to macrolide resistance and in areas with a high consumption of macrolides the proportion of GAS strains resistant to both antibiotics rapidly may reach alarming levels. For example, in the Olomouc region in the Czech Republic the proportion of clindamycin resistant GAS strains rose from 4% to 28% between 1999 and 2001 (Urbanek et al 2005).

THE CARRIER STATE

Asymptomatic carriage of GAS in the throat is more frequent in children than in adults. The frequency found in Scandinavian investigations was 2-11% in children <4 years of age, 5-21% in age group 4-15 years and 1-4% in adults (Hoffmann 1985; Strömberg et al 1988; Gunnarsson et al 1997). However, in outbreaks of GAS pharyngotonsillitis in for example day-care or school the carrier rate could be as high as 60 % (Falk & Kjellander 1992).

In a four year longitudinal study of school children, 5 -15 years old, the mean time for a period of carriage, during which the child harboured GAS of the same *emm* type, was 10.8 weeks (range: 3-34 weeks). Many children, however, experienced several periods of carriage during the study and frequently exhibited switches in *emm*-type (Martin et al 2004). The risk of becoming a carrier or contract disease, is related to the time spent in close contact with a patient during the week preceding onset of illness (Engelgau et al 1994; Weiss et al 1999). The background why some individuals become carriers is not known, but the carriership appears to be a harmless condition, as it probably does not result in clinical infection (Kaplan et al 1981). In addition, the streptococci are present in low numbers (Roos 1985) and the carrier probably does not transmit infection (Falk & Kjellander 1992). However, problems arise when a carrier acquires viral pharyngitis, as positive test for GAS will raise the issue of antibiotic treatment. This highlights the importance of careful evaluation of symptoms in order to avoid unnecessary antibiotic treatment.

REASONS TO TREAT GAS PHARYNGOTONSILLITIS

GAS pharyngotonsillitis is a self-limiting disease and the routine of pcV treatment has therefore been questioned (Flottorp et al 2000). However, GAS is one of the most virulent human pathogens, and in pharyngotonsillitis the patient can be seriously affected with high fever, dysphagia and severe pain. Irrespective of treatment, a majority of patients are free of symptoms within a week, but antibiotic treatment of GAS pharyngotonsillitis was shown to shorten the duration of symptoms (De-Meyere et al 1992; Dagnelie et al 1996). Treatment also in some degree reduces the risk of purulent complications, such as peritonsillitis, otitis and sinusitis (Del Mar et al 2000; Dagnelie et al 1996; Zwart et al 2000). In acute rheumatic fever, it is claimed that a majority of the patients have a history of pharyngotonsillitis. The decline of acute rheumatic fever in the western world might be the result of consequent antibiotic use in GAS pharyngotonsillitis, in strong support of the present principles of treatment. In NF and STSS, however, the port of entry is seldom reported to be the pharynx (Davies 1996; Eriksson et al 1998).

Thus the reasons for antibiotic treatment of GAS pharyngotonsillitis are: 1) Faster alleviation of symptoms; 2) Reducing the spread of GAS; 3) Reducing the risk for suppurative and non suppurative complications. Hence it is mostly agreed that benefits of antibiotic treatment outweigh disadvantages (Hoffman & Kolmos 2000; Roos et al 2000; Workshop 2001).

POSSIBLE REASONS FOR FAILURE IN PENICILLIN TREATMENT OF GAS PHARYNGOTONSILLITIS

Low compliance

In GAS pharyngotonsillitis treatment with pcV results in fast recovery (De Meyere et al 1992; Zwart et al 2000). Since the patient is often free of symptoms already after 2-3 days of treatment, further medication may appear unnecessary and discontinuation of treatment probably accounts for failure in many cases.

Reinfection from the environment

Since family members and other close contacts of patients with GAS pharyngotonsillitis are often infected by the same strain many supposed failures may in fact be due to reinfection (Falck et al 1997).

Eradication of α-streptococci with inhibitory effect on GAS

Some α-streptococci produce bacteriocins with inhibitory activity against GAS. Eradication of α-streptococci by pcV will theoretically reduce bacterial interference which could increase the risk of treatment failure (Sanders et al 1976). However, other investigations failed to show that lack of bacterial interference was related to bacterial treatment failure in GAS pharyngotonsillitis (Gerber et al 1999). Interestingly, administration of α-streptococci into the throat following β-lactam treatment of GAS pharyngotonsillitis has been shown to reduce the recurrence rate (Roos et al 1993; Falck et al 1999).

Increase in β-lactamase producing bacteria inactivating the drug

Treatment with pcV will promote selection of bacterial species producing β-lactamase conceivably accounting for inactivation of pcV (Brook 1985; Tuner & Nord 1986).
The benefit of β- lactamas inhibitors as supplements of penicillin is unclear. (Kaplan & Johnsson 1988; Tanz et al 1990) Gerber and colleagues 1999 comparing cefadroxil (stable to β- lactamas) and pcV in treatment of primary GAS pharyngotonsillitis found no evidence that β-lactamases produced by normal pharyngeal flora was related to bacterial treatment failure. The role of β-lactamases in treatment failure, thus remains unclear.

Penicillin tolerance

Tolerance to β-lactam antibiotics is a known phenomenon in some medically important species, such as Enterococcus faecalis, Streptococcus pneumoniae and various α- haemolytic streptococci (Tuomanen et al 1986) and it appears to account for failure of penicillin therapy of Arcanobacter haemolyticum infections (Nyman et al 1990). Penicillin tolerance in GAS has been suggested to promote failure in pcV treatment of GAS pharyngotonsillitis, but reports have been contradictory, conceivably due to the variability in the definition of "tolerance" as well as technical pitfalls of methods used (Kim & Kaplan 1985; Krasinski et al 1986; Grahn et al 1987; Smith et al 1987; Stjernquist-Desatnik et al 1992). The existence of penicillin tolerance in GAS has also been questioned (Woolfrey 1988).

Low antibiotic concentration at site of infection

In acute GAS pharyngotonsillitis the causative bacteria are mainly present in the secretion on surface and in crypts, rather than in the tonsillar parenchyma (Ebenfelt et al 1998; Lilja et al 1997). PcV was detected in the TSF in a majority of patients on the first day of treatment of acute GAS pharyngotonsillitis, but despite high concentrations in serum, rarely on the tenth day or in healthy treated subjects (Stjernquist-Desatnik et al 1993). Insufficient concentrations of antibiotics in TSF might contribute to treatment failure in GAS pharyngotonsillitis.

Intracellular GAS surviving therapy

As shown *in vitro*, internalized GAS in human respiratory epithelial cells, in the absence of extracellular antibiotics, were mobilized and established infection. (Österlund & Engstrand 1995). In analogy to these findings the respiratory epithelial cells may act as a reservoir where internalized GAS with potential to cause infection can survive pcV treatment and account for recurrent pharyngotonsillitis after pcV treatment.

AIMS OF THE PRESENT STUDY

1 To investigate the short- and long-term effect of pcV versus clindamycin in patients with GAS pharyngotonsillitis who failed on pcV treatment.

2 To examine failure and non-failure GAS strains for possible penicillin tolerance.

3 To compare the DNA-profiles of failure and non-failure GAS strains.

4 To evaluate the kinetics of pcV, loracarbef and clindamycin in the tonsillar surface fluid during acute GAS pharyngotonsillitis, and to evaluate a possible correlation to their clinical efficacy.

THE PRESENT INVESTIGATION

Comparison of Penicillin and Clindamycin in Bacterial Failure after PcV Treated GAS Pharyngotonsillitis. A One-Year Follow up Study (I, II)

Patients and Methods

Patients

278 patients with acute GAS pharyngotonsillitis attending a private ENT clinic (Dr. Orrling) were treated with pcV for ten days. They all had a positive rapid test and a positive throat culture for GAS. 239 patients fulfilled the inclusion criteria by taking the drug as prescribed and showing up for scheduled control 4-6 days after completing therapy. At that time 53 patients manifested bacterial treatment failure. Their age range was 2-62 years (median 9, mean 15.2).

It was declared that the objective of the study was to eradicate the bacteria from the throat in case of bacterial treatment failure, and thus antibiotics could be given even if the patient was free from symptoms.

Bacterial failure was defined as presence of GAS of the same T-type as that of the pre-treatment strain within two weeks after completing therapy. Clinical and bacterial failure was defined as above in combination with clinical symptoms and signs of pharyngotonsillitis. Reinfection was defined as occurrence of another T-type within two weeks after completing therapy.

The study was approved by the Medical Ethics Committee of the University of Lund.

Treatment

The 53 patients with bacterial treatment failure were openly randomized to treatment with either pcV (n=25) or clindamycin (n=28) for ten days. The patients were followed for one year with examination and throat culture every third month. They were also told to return for examination, including a throat culture, in the event of sore throat.

For the rest of the follow up period the patients were treated with pcV in case of a positive throat culture or a primary GAS pharyngotonsillitis, and failures were treated with the drug to which the patient was randomized. Treatment was repeated until a negative throat culture was obtained.

However, owing to the poor effect of repeated pcV treatment of bacterial failures, and the superiority of clindamycin in this situation, 12 patients in the pcV group were crossed over to clindamycin in case of bacterial failure.

Bacteriological investigation

At inclusion a rapid test for GAS was performed. Culture specimens were obtained from the throat by rotating a sterile cotton swab along both tonsils. The GAS strains were T-typed and the growth was classified semi-quantitatively as sparse, moderate or abundant. All clinical examinations were performed, and all cultures were taken, by the same physician.

Statistical analysis

The χ^2-test with Yate´s correction was used for statistical analysis of the data *p* values below 0,05 being considered significant.

Results

Information in detail regarding culture and treatment, from initial treatment and throughout the 12 month observation period, is given in the flowchart – *Figure 1*.

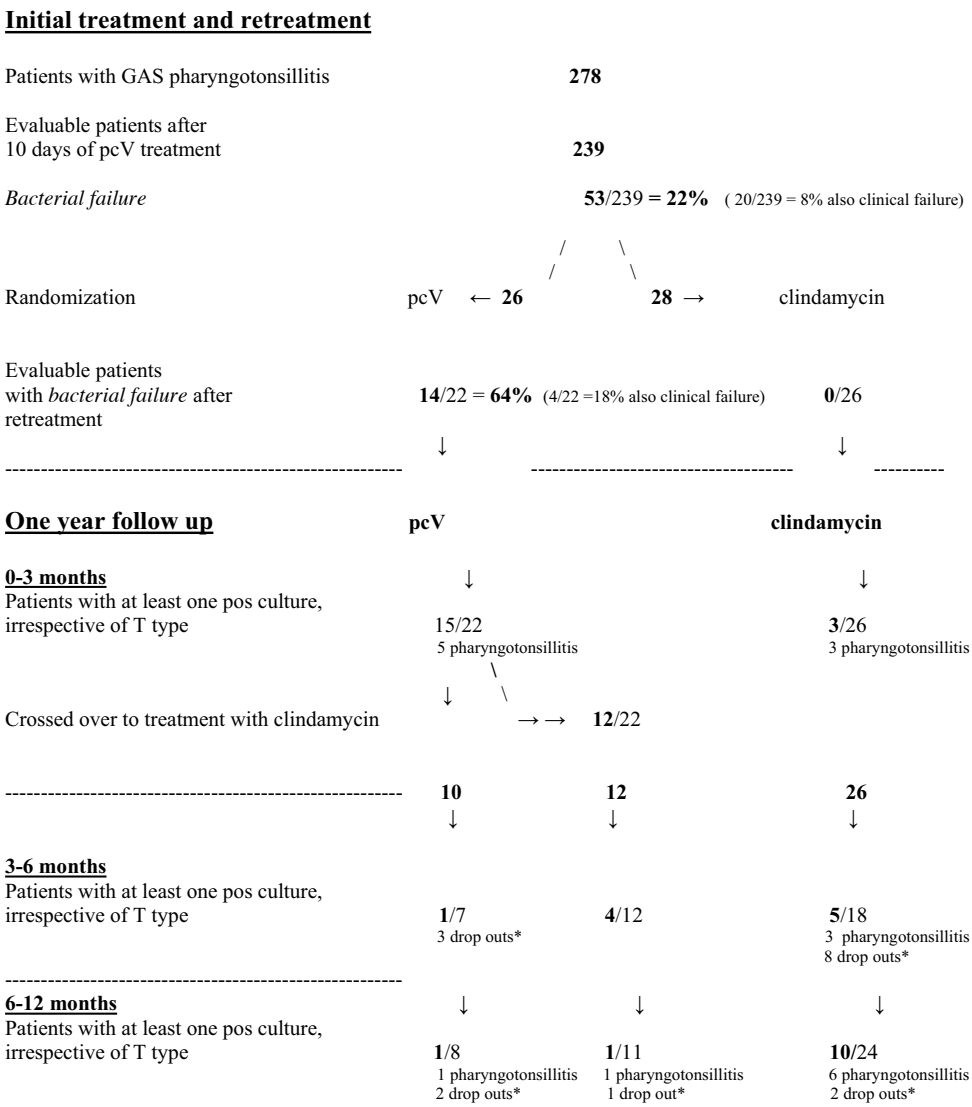
At examination within two weeks following the initial pcV treatment. 53 patients (22%) still harboured GAS of the same T-type as in the pre-treatment culture, 20 patients (8%) also had signs and symptoms of pharyngotonsillitis, while remaining 14 % were free of symptoms.

After randomization and treatment with either pcV or clindamycin, 48 patients (median 6.0, mean 9.5) were evaluable 22 in the pcV group and 26 in the clindamycin group. After the second treatment 14 patients (64%) in the pcV group showed bacterial failure, compared to 0% in the clindamycin group ($p< 0,001$). Of the 14 bacterial failures in the pcV group four patients also had signs and symptoms of pharyngotonsillitis (N.S.).

In the first 3-month period after the second treatment, one or more positive throat cultures of the same T-type were obtained from 15/22 (68%) patients in the pcV group, of which five also had clinical failure. In the same period there were no bacterial failures (0%) in the clindamycin group. However in this group three patients had pharyngotonsillitis and yielded a positive culture but with another T-type and were thus reinfections.

Within the first three months 12/22 patients in the pcV group were switched to treatment with clindamycin in case of bacterial failure. They were then separately registered as the “switched to clindamycin group”.

For the rest of the 12 month observation period the differences between the groups were diminished, and without statistical significance. All positive cultures, except two with sparse growth, were classified as abundant or moderate.



* Drop out during the period

Figure 1: One year follow up after treatment failure in pcV treated GAS pharyngotonsillitis – a flowchart

An Attempt to Identify Penicillin Tolerant GAS (III)

Patients and Methods

Patients and bacterial isolates

GAS strains were selected from the previous study on patients with pharyngotonsillitis (Orrling et al 1994). Samples were obtained before pcV therapy from patients who healed on pcV therapy as well as from patients with subsequent failure. The distribution of the isolates is shown in shown below.

GAS isolates tested for penicillin tolerance

	n
Before treatment: patients who healed	33
Before treatment: patients with bacterial failure:	25
After treatment *: patients with bacterial failure:	25
After second treatment **:patients with bacterial failure:	7
	90

* 13 patients with clinical failure
** 2 patients with clinical failure.

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Three control strains, one group A representing non-tolerance, and one group A and one group G, representing different levels of tolerance, were primarily selected from a total of approximately 150 clinical isolates examined by the disc diffusion test (Slater & Greenwood 1983).

Four streptococcal strains, reported by others as belonging to group A and penicillin tolerant (van Asselt & Mouton 1993; van Asselt et al 1995), and 16 own isolates from throat specimens – 12 group G and 4 group C – were also investigated.

Bacteriological investigation

The MBC/MIC ratios were determined by a modified plate dilution method (Kamme & Petersson 1993), and by broth dilution (Taylor et al. 1983). Survival rates were determined by the plate screening method and by the time killing kinetic test.

Log phase as well as stationary phase cultures from our previous study were investigated with the plate screening method. Log phase strains with a survival rate of 0.2-0.5% were subjected to time killing test. All strains were T-typed. The person performing the in vitro tests of clinical isolates was not informed of whether the various strains originated from cases of failure or not.

The four streptococcal strains mentioned above and the sixteen group G and group C streptococci were all investigated with the plate screening method and some of them by the time killing kinetic test.

Screening method for penicillin tolerance*:* Before screening all strains were subcultured five times on plates containing pcV in subinhibitory concentration. Log phase cultures were diluted to approximately 10^7 CFU/ml and two microliter of the suspension (approximately 10^4 CFU/ml) was applied onto horse blood agar plates containing pcV in a concentration of 4 times the MIC. This level was chosen based on the survival rate of the control strains in order to select as many isolates with delayed killing as possible. The plates were incubated for 6 h at 37°C, after which the antibiotic was inactivated with β-lactamase. The plates were reincubated for 4 h in room temperature and then at 37°C for 24 h after which the number of colonies for each inoculum was counted. The interassay variation was investigated with the control strains.

Time killing kinetic test*:* Log phase cultures in Todd–Hewitt broth were diluted to a density of approximately 10^5 CFU/ml, and pcV was added to a concentration of 12 times the MIC. The broth was then dispensed in aliquots 4.5 ml portions and incubated at 37°C. After 0, 2, 4 and 6 h of incubation respectively the antibiotic was inactivated with β-lactamase, and a viable count was performed.

Results

Penicillin tolerance was defined by normal MIC but elevated MBC (MBC/MIC ratio ≥ 32) and a survival rate of ≥1% in the screening test as well as in the time killing kinetic test.

The screening method for *log phase cultures* of the strains from our previous study showed killing rates of 99.8% or more, except for one strain with a survival rate of 0.2-0.5%. There was no difference between isolates from patients who healed and patients with one or two treatment failures.

Six isolates with survival rates in the screening test of 0.1-0.5% were obtained from five patients before or after one or two failures. On five occasions the patient also had a clinical pharyngotonsillitis. In the time killing kinetic test all isolates showed a survival rate of 0.01-0.03%.

With the plate screening method the *stationary phase cultures* showed survival rates of >0.5% in a majority of cases. As with log phase cultures there was no difference between isolates from patients who healed and patients with one or two treatment failures.

The 16 group C and G isolates all yielded a survival rate of >1% by the plate screening method. Two group C isolates that were subjected to the time killing kinetic test yielded survival rates of 2 and 5% respectively.

The four allegedly tolerant group A strains all turned out to be group G streptococci. They all yielded >1% survival rates in the plate screening test. One of them, when examined in the time killing kinetic test, showed a survival rate of approximately 5%.

Genetic Profiles in GAS Isolates from Failures and Nonfailures. An Investigation Using AP-PCR Technique (IV)

Patients and Methods

Patients

Isolates from four patients, selected from the previous study (Orrling et al 1997) with one or more bacterial treatment failures, were analyzed. They were compared with strains of the same T-type isolated during the same time period from patients who healed on a single course of pcV for pharyngotonsillitis and who lived in the same geographical area. All cultures showed abundant growth.

Case 1: A 6-year –old boy was treated with a total of four pcV courses for growth of GAS of T-type 4 (*Figure 1 – paper IV*). On three of the occasions, he displayed signs and symptoms of pharyngotonsillitis. .

Case 2: A 2-year –old boy with pharyngotonsillitis and growth of GAS of T-type 12 was treated with four pcV courses (*Figure 1 – paper IV*). The boy displayed no symptoms or signs of pharyngotonsillitis at any of the five follow-up visits despite positive cultures.

Cases 3 and 4*:* A 28-year old mother and her 2-year-old son displayed clinical pharyngotonsillitis and growth of GAS of T-type R28. They were both treated with pcV for ten days (*Figure 1 – paper IV*). At the follow up visit the boy showed bacterial failure, but at the following controls the cultures were negative. In contrast the mother was prescribed three additional pcV courses because of repeated growth of the same T-type (twice) followed by a positive culture for group G streptococci. At this point she was treated with trimethoprim-sulfamethoxazole for urinary tract infection. After that the culture was still positive for group G streptococci, but the patient was asymptomatic, and no further treatment was given.

The DNA profiles of the isolates from case ***1 -4*** were compared with profiles of isolates of the same T-type respectively from nonfailures.

Bacteriological investigation

A total of 23 primers were initially tested, some of which produced no bands at all. Three primers giving 2-6 strong bands and 3-8 weak bands were chosen. *Figure2*.

Arbitrarily primed polymerase chain reaction (AP-PCR) was carried out (Jackson &Cook 1985), and the product was electrophoresed in a 1.5% agarose gel. The DNA bands were

visualized under UV-light and photographed. The size of the PCR product was compared using a DNA ladder. To check the reproducibility the samples were run twice.

Results

The reproducibility of the AP PCR technique was high. The method also demonstrated a high discriminatory capacity. Among the three types recognized by the conventional T-typing, 11 different DNA profiles could be detected by AP-PCR. The strains of T-type 4, 12 and R28 segregated into 5, 3 and 3 AP-PCR profiles respectively.

Case 1 showed the same DNA profile in 5 of 6 isolates, which were all of T-type 4. The aberrant isolate was obtained after the fourth pcV course. After the fifth pcV treatment the original DNA profile reappeared. The same profile was found in 4 of the 7 T-type 4 strains from non failures. The remaining three nonfailure strains were assigned to two different clones.

The genetic profiles of all the isolates from case 2 were identical, and this clone was also represented in 2 of the 7 nonfailure strains. The other five nonfailure strains of T-type 12 exhibited band patterns corresponding to four different clones.

Cases 3 and 4, mother and son, exhibited the same genetic profile in all five isolates, classified as T-type R28. This profile was also found in 4 of the 6 nonfailure strains, whereas 2 of the 6 had different genetic profiles.

Name	Sequence	Strong bands	Weak bands
A70-1*	CAT CCC GAA C	0	0
A70-2*	CAG CGT CGA C	0	0
A70-3*	ACG GTG CCT G	0-2	0-3
A70-4*	CGC ATT CGC C	0	0-2
A70-5*	GAG ATC CGC G	0	0
A70-6 *	GGA CTC CAC G	0-2	0-2
AT0-7*	ATCTCC CGG G	0	0
A70-8*	CTG TAC CCC C	0	0
1) A70-9*	TGC AGC ACC G	2-4	3-5
1) A70-10*	CAG ACA CGC C	2-6	4-8
A60-1*	CGC AGT ACT C	0	0
A60-2*	GTC CTA CTC G	0	0
A60-3*	CTA CAC AGG C	0-2	0-3
A60-4*	GTC CTT AGC G	0-1	0
A60-5*	GTC CTC AAC G	0	0
A60-6*	CTA CTA CCG C	0	0
A60-7*	GAG TCA CTC G	0	0
A60-8*	GTC CTC AGT G	0	0
A60-9*	CGT CGT TAC C	0	0
A60-10*	GCA GAC TGA G	0-1	0
1) H2LM**	CCT CCC GCC ACC	2-5	3-5
GAS 1LM**	GAT CAA GTC C	0-1	0
GAS 2LM**	GAT CTG ACA C	0-2	0-4

* Genosys Biotechnologies Inc. Texas
** DNA technology, Denmark
1) Primer used in the study

Figure 2 AP-PCR tested primers and number of bands on agarose gel after electrophoresis

Penicillin V, Loracarbef and Clindamycin in TSF During and After Treatment of GAS Pharyngotonsillitis (V)

Patients and Methods

Patients

35 consecutive patients attending a private ENT clinic with a history of no more than two days of acute pharyngotonsillitis (11 males, 24 females) were included. Their age range was 20 – 77 years (median: 35, mean 38.0), and they all had a normal serum creatinin. All patients manifested a positive rapid antigen test for GAS as well as a positive culture.

The study was approved by the Medical Ethics Committee of the University of Lund.

Treatment

Patients were randomly assigned to either pcV 12.5 mg/kg bodyweight b.i.d. (n=13), clindamycin 300 mg t.i.d. (n=11) or loracarbef 200 mg b.i.d. (n=11), all for ten days, which are the currently recommended dosages for GAS pharyngotonsillitis in Sweden.

Sampling

Sampling of serum, saliva and TSF was performed on four occasions: 1) On the day of inclusion before start of therapy, in order to exclude any unspecific antibacterial activity. 2) 1.5 h after intake of drug at two randomized days during treatment. 3) On one occasion within four days after end of therapy. No anaesthesia was used. Blood was drawn for C-reactive protein and orosomucoid on the same occasions. Three sterile filter paper disks were placed on the surface of the tonsils and three under the tongue. The disks were left in place for one minute and were then immediately sealed in plastic tubes and kept at -80° until assayed. The procedure was similar to that previously described (Strömberg et al. 1987; Stjernquist et al. 1993). A venous blood sample was obtained at each visit.

Antibiotic assays

The concentration of the drugs in serum was determined by agar-well diffusion. For saliva and TSF disk diffusion was used. Sterile powder of each drug with known potencies were used for the preparation of 2-fold standard solutions, of which 10 µl was added to sterile paper disks.

Sera, standard solutions and impregnated disks were assayed in duplicate. Zones of inhibition were measured after overnight incubation at 37°C. For the disks with TSF and saliva respectively the mean value of zones of inhibition for the three disks was given.

The detection limit for pcV was 0.03 mg/L, for loracarbef 0.12 mg/L, and for clindamycin 0.12 mg/L. MICs for susceptible GAS were 0.004-0.002, 0.06-0.25 and 0.03-0.12 mg/L respectively (Kamme & Petersson 1993, Kataja et al 1999).

Results

The results were given in five periods: I = day 1-3, II = day 4-7, III = day 8-10, IV = day 11-12, V = day 13 -14.

All included patients were examined three times within the first ten days, i.e. at inclusion and in two periods during treatment. Three patients in the pcV group and two patients in the loracarbef and clindamycin group respectively were examined twice in period I or II. The results were shown as the mean value of the two measurements in respective period.

One patient was excluded from further examination owing to zones of inhibition around the pre-treatment disks with TSF. Two patients in the pcV group, and one in the loracarbef group did not show up following treatment.

In order to find out the optimal time for sampling, two patients, one in the pcV and one in the loracarbef group, were repeatedly examined at 30 min intervals after taking the drug. The pcV concentration in the TSF showed a fast increase with a maximum after 90 min, followed by a fast decrease. The concentration of loracarbef in TSF showed a similar pattern, although the maximum concentration was reached 40 min later. *(Figure 3)*

***Antibiotic in serum*:** Detectable serum levels of all drugs were obtained throughout the treatment period. After end of therapy pcV and loracarbef did not reach detectable serum levels. In period IV clindamycin reached measurable concentrations in each of four tested sera at two days after end of therapy. On the following two days detectable levels of clindamycin were not obtained in any of six tested serum samples.

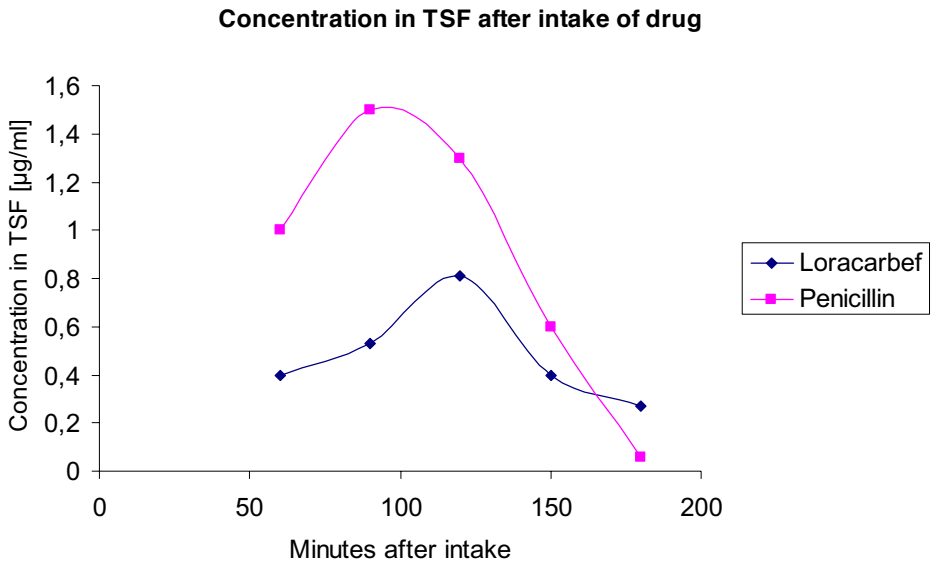

Figure 3

Antibiotic in TSF and saliva

Percentage of patients with measurable concentration of each drug in TSF in period I, II, III and IV+V is given in *Figure 4*.

***PenicillinV*:** The concentration in TSF in period I was in the range 0.05-1.5 followed by a rapid decline. In period III only 1 of 6 TSF samples yielded a measurable value, 0.05 mg/L. Detectable levels of pcV were not obtained in TSF after the end of therapy.

In the saliva pcV showed concentrations well above MIC even in period III. Detectable levels of pcV were not obtained in the saliva after the end of therapy.

Loracarbef: In TSF loracarbef showed a similar picture in period I as did pcV, range 0.1-1.7 mg/L but with no tendency to decrease in period II. Measurable concentrations were obtained both in period IV (2/2) and in period V (1/8).

In contrast to the other two drugs, loracarbef could not be detected in the saliva in any patient.

Clindamycin*:* The concentrations of clindamycin in TSF in periods I-III were practically all above 0.5 mg/L, and with no obvious tendency to decrease. Measurable concentrations in the TSF were obtained both in period IV (3/4) and in period V (1/7).

The salivary concentrations in periods I-IV were all above 0.5 mg/L.

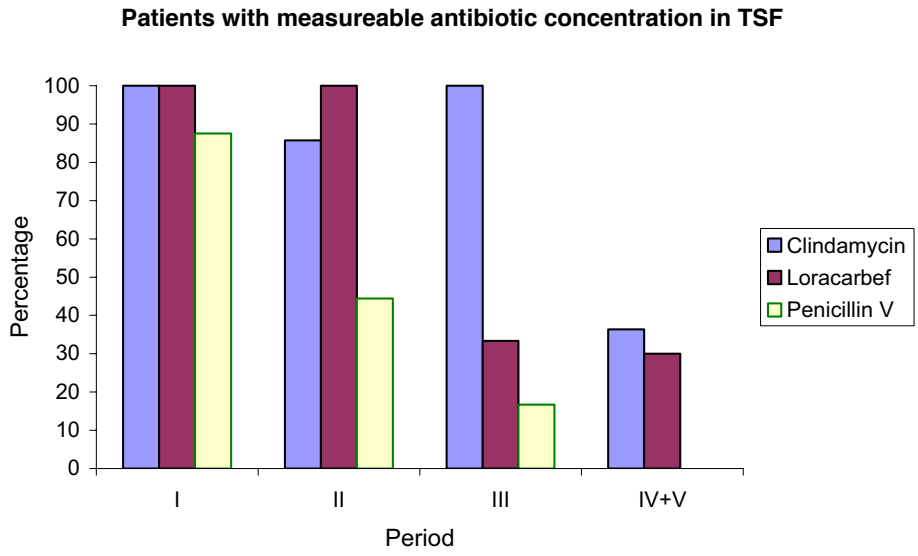

Figure 4

CRP and orosomucoid

At day 1 CRP was elevated >30 mg/L in 12/35 patients and >50 mg/L in 7/35 patients. After five days of treatment the median of CRP was below 10 mg/L.

Orosomucoid was elevated >1.17g/l in 13/35 patients and >1.5 g/l in 3/35 at inclusion. After five days of treatment the median orosomucoid level was below 1.1 g/l.

There was no difference in the decrease of either CRP or orosomucoid between the three groups.

GENERAL DISCUSSION

Treatment failure

The bacterial treatment failure rate in our study, 22% after the first and 64% after the second course of pcV, was in accordance with other studies (Kaplan & Johnson 1988; Schwartz et al. 1981). In the clindamycin group, on the other hand, none of the patients exhibited failure. Furthermore, in the following three month period, three patients in the clindamycin group had clinical pharyngotonsillitis, but in all cases the GAS strain isolated was of a new T-type thus representing reinfection. In the pcV group, however, five patients developed one or more clinical pharyngotonsillitis episodes, in each case due to GAS of the same T-type as in the primary episode. Thus clindamycin treatment apparently, by eradicating carriage of GAS, interrupted the vicious circle of recurrent GAS pharyngotonsillitis among patients originally treated with pcV. Accordingly none of the patients receiving clindamycin had to undergo tonsillectomy.

The difference between the two groups was reduced later in the follow up period, when positive cultures for GAS were seen in a few patients left in the pcV group. Owing to the study design, however, "tonsillitis-prone" patients in the pcV group were switched to clindamycin treatment in case of bacterial failure. The remaining pcV group, therefore was selected and consisted of individuals with comparatively high resistance to GAS.

During the 12 month follow up period some patients in the clindamycin group manifested failure after pcV treated primary GAS pharyngotonsillitis. Our findings partly disagree with those obtained by Brook & Hirokawa (1985) where only one of 15 patients treated with clindamycin manifested a single recurrence of pharyngotonsillitis during one year of follow up. Jensen and Larsen (1991) found the frequency of episodes of acute pharyngotonsillitis to be significantly reduced among patients treated with one course of clindamycin, as compared to untreated controls, in a 12 month follow up period. Unfortunately in their study, diagnosis of pharyngotonsillitis was based on anamnestic data rather than throat cultures.

Reinfection

Since close contacts, such as family members often harbour the same strain as the patient, a distinction between failure and reinfection is not always possible. Efforts were made to minimize the risk of including patients with reinfection from the environment in the study. This was done by obtaining the follow-up throat culture as soon as four days after cessation of

therapy. In addition, family members with symptoms and a positive culture for GAS were treated with pcV.
In contrast to the pcV group, there was no GAS pharyngotonsillitis caused by the original T-type in the clindamycin group in the first three month follow-up period. Therefore our findings strongly suggest that the recurrences after pcV treated GAS pharyngotonsillitis mainly were due to bacterial treatment failure rather than reinfection.

Genetic profiles

When exploring the isolates with the AP-PCR technique, we found that isolates from four patients with several failures exhibited the same genetic profile as the pre-treatment isolate from each patient. A minor exception was an extra band found in one of the isolates. Since the same profile as the original was found in the next isolate, this extra band was probably due to two different bacterial populations of GAS at that time. Our findings minimize the probability that the bacterial failures were due to infection with a new GAS strain and were in accordance with Österlund and Engstrand (1995) and Bingen and colleagues (1992) who also found most pre-treatment and post-treatment isolates to have the same genetic profile.
Similar genetic profiles were also found in pre-treatment isolates from patients in the same area who healed on their first pcV course, as in isolates from patients with multiple failures. Moreover, the isolates from a mother who had repeated clinical and bacterial failures showed the same profile as those from her son who healed after a second pcV course. We were thus unable to identify special strains accounting for treatment failures. Analogously Norgren and colleagues (1992) found the same genetic profile of GAS of T-type 1 in patients with bacteraemia as in asymptomatic carriers in the same family.
The findings suggest that host factors, such as local or systemic immunity to streptococci, or local production of peptides with antibacterial properties (Nizet et al 2001; Bessen & Fishetti 1988) may significantly influence the outcome of treatment of GAS.

Penicillin tolerance

In our study, using log phase bacteria, only a minor proportion of strains showed survival rates above 0.1 % after exposure to pcV, at four times the MIC. When these strains were subjected to time killing kinetic test, however, they did not show any delayed killing. There was no difference between isolates obtained before compared to after treatment, and results were similar for isolates from healed cases and isolates from one or two treatment failures, respectively.

Stationary phase cultures of GAS have been shown to yield a phenotypic response to β-lactam antibiotics affecting the killing rate rather than MIC (Kamme & Petersson 1993). This property of many GAS strains might be due to the fact that non-replicating bacteria are not sensitive to the inhibitory effect on cell wall synthesis accomplished by β-lactam drugs. As with log phase cultures however there was no difference in killing rate between isolates from patients who healed and from those with one or two treatment failures.

One single GAS strain, out of 150 tested clinical isolates, showed delayed killing, with a survival rate close to tolerance as defined above. This strain, however, showed a MBC/MIC ratio = 1 in the modified plate dilution test as well as in the broth dilution test. This shows the difficulties in investigating penicillin tolerance, and emphasizes the need for appropriate confirmation of positive findings.

Our failure to detect tolerance in GAS was in accordance with Ciftci and colleagues (2002) who could not identify penicillin tolerant strains among 263 isolates from children with GAS pharyngotonsillitis. Thus penicillin tolerance seems to be of little or no significance in accounting for failures after pcV treated GAS pharyngotonsillitis. Furthermore, penicillin tolerance or resistance, in this species may not be compatible with a virulent phenotype (Gutman & Tomasz 1982).

Somewhat unexpectedly, our results suggested that penicillin tolerance may be rather common in group C and G streptococci. However, clinical experience does not indicate, that penicillin tolerance in these species should represent a therapeutic problem in the context of pharyngotonsillitis.

Antibiotic concentrations in TSF

The high initial concentrations of pcV in TSF, followed by a rapid decline and measurable level in only one late sample, was in contrast to results for clindamycin and loracarbef which showed detectable concentrations in TSF even after end of therapy. Hence early, high concentrations of the three investigated drugs in TSF, as here found, may account for prompt clinical recovery. All patients had satisfactory serum levels of antibiotic during treatment, so the rapid decline of pcV in TSF was not due to low compliance or low resorption of the drug. The post treatment persistence in TSF of loracarbef and clindamycin may conceivably be explained by mobilized tissue depots. Levels of all three antibiotics in TSF and saliva were essentially unrelated, and loracarbef did not reach measurable levels in saliva, thus in contrast to TSF.

The finding of high initial concentrations of pcV in TSF, followed by a rapid decline, was in agreement with a previous study by Stjernquist – Desatnik and colleagues (1993). These authors found measurable concentration of pcV in TSF in all patients on the first day of treatment of GAS pharyngotonsillitis, but in only one of nine patients at the end of treatment. Furthermore, pcV could not be detected in TSF in healthy individuals. In contrast, Strömberg and co-workers (1987) found the concentrations in TSF of both pcV and cefadroxil to be higher than those in tonsillar tissue from patients tonsillectomized because of recurrent tonsillitis. However, these patients manifested chronic inflammation in the tonsils implying plasma leakage and exudation of fluid through the epithelium. Furthermore, sampling was made under general anaesthesia which might have affected the tonsillar blood flow. Our study comprised patients with ongoing acute GAS pharyngotonsillitis and no local anaesthesia was used during sampling.

In acute pharyngotonsillitis, GAS are predominantly localized in the secretion on the tonsillar surface and in the crypts (Ebenfelt et al 1998). The concentration of antibiotics in TSF thus would most probably be crucial in treatment of GAS pharyngotonsillitis.

CRP and orosomucoid

We also evaluated CRP and orosomucoid as indicators on the degree of inflammation in the throat and a possible linkage between serum levels of these markers and drug concentrations in TSF. However, no such correlation was found. Only in a minor proportion of the patients CRP and orosomucoid showed serum levels indicating a bacterial infection, and there were no differences between the three groups receiving different antibiotics. Our findings were in accordance with other studies (Stjernquist et al 1987; Putto et al 1986; Sun et al 2002) and provided no support for the use of CRP or orosomucoid as tools to separate bacterial from viral pharyngotonsillitis.

Penicillin for ten days

In pcV treated acute otitis media Ingvarsson and colleagues (1980) recorded a rapid decline in pcV concentration in the middle ear as the inflammation abated. This resulted in a change in Swedish treatment praxis from 10 to 5 days of pcV in primary acute otitis media. However, in pcV treated GAS pharyngotonsillitis, despite fast clinical recovery, the importance of no less than 10 days therapy, in order to keep the failure rate on acceptable level, has been reported (Schwartz et al 1981; Strömberg et al 1988; Zwart et al 2000).

The need for a 10 days course of pcV in the treatment of GAS pharyngotonsillitis then seems paradoxical, since the drug concentration in TSF later in therapy often does not reach detectable levels. A possible reason might be that although the log phase bacteria are killed early in treatment, GAS in lag phase, as well as intracellulary GAS, are not affected by pcV. However, the gradual passage to log phase, as well as the externalization and start of replication of intracellulary GAS, will successively render additional bacteria susceptible to the drug. PcV is probably transferred to the tonsillar surface by inflammatory exudation, hence later in therapy at lower levels. The continued, local inflammation caused by the replicating bacteria may still result in exudation of pcV in sufficient amount to kill the bacteria. A course long enough to cover the shift from lag phase to log phase for most bacterial cells, as well as the start of growth of most internalized bacteria, thus might be important for efficacy of pcV in GAS pharyngotonsillitis. It seems likely that an extended course, i.e. for more than the arbitrarily chosen 10 days, although it would result in compliance problems, could result in further reduced failure rate in GAS pharyngotonsillitis.

Loracarbef

In a study of loracarbef versus pcV in recurrent GAS pharyngotonsillitis Roos and Larsson (1997) found significantly higher bacterial eradication rate among the loracarbef treated patients (90% versus 66%). The better eradication rates by the cephalosporins, as compared to pcV, in primary and recurrent GAS pharyngotonsillitis is usually ascribed to a higher stability of cephalosporins to β-lactamases as well as lesser impact on the interfering resident α-streptococci. It seems reasonable that maintained high concentrations of loracarbef, and perhaps also other cephalosporins, in TSF is another important factor for their lower failure rates as compared to pcV.

Clindamycin

Since α-streptococci are susceptible to clindamycin, bacterial interference does not seem to contribute to the low failure rate after clindamycin treated GAS pharyngotonsillitis. However, clindamycin enters and accumulates in human cells. The longstanding concentrations in TSF, the insusceptibility to β-lactamase and the effect on extra- as well as intracellular GAS, whether resting or actively growing, are all properties that might be of crucial importance for the superior capacity of clindamycin to eradicate GAS in recurrent pharyngotonsillitis.

Conclusion

In primary GAS pharyngotonsillitis there is still support for use of pcV, due to narrow spectrum and absence of penicillin resistance. However, in treatment failure use of clindamycin, due to proven ability to eradicate GAS from the throat, can be justified. This drug may also interrupt the vicious circle of repeated recurrences of GAS pharyngotonsillitis often leading to tonsillectomy. However, since clindamycin also is of high value in the treatment of life threatening streptococcal diseases such as streptococcal toxic chock syndrome and necrotizing fasciitis, it is imperative, in order to avoid development of resistance, that the drug is used on strict indications.

CONCLUSIONS

1 In patients with GAS pharyngotonsillitis who failed on pcV treatment, clindamycin could prevent further failures for at least the following three months.

2 Reinfection is of less importance than bacterial treatment failure as an explanation for recurrent GAS pharyngotonsillitis.

3 Penicillin tolerant GAS could not be identified, and we conclude that penicillin tolerance seems to be of no significance in failures of pcV treated GAS pharyngotonsillitis.

4 Failure and nonfailure strains exhibited similar DNA profiles, indicating that failures are associated with host rather than bacterial factors.

5 The longstanding concentrations of both loracarbef and clindamycin in TSF may contribute to their capacity to eradicate GAS in patients who failed on pcV treatment of GAS pharyngotonsillitis.

6 CRP is of no diagnostic value in GAS pharyngotonsillitis.

SUMMARY

In acute pharyngotonsillitis group A streptococci (GAS) is the etiological agent in 30-50% of cases. GAS are virulent human pathogens, and may cause both suppurative and nonsuppurative complications, and sometimes life threatening diseases such as "streptococcal toxic shock syndrome" and necrotising fasciitis. GAS pharyngotonsillitis results in a high degree of absence from day care, school and work, and it is agreed that antibiotic treatment is indicated in these cases. Phenoxymethylpenicillin (pcV) is the drug of choice in Sweden. Although penicillin resistance is not recorded in GAS, the failure rate is as high as 5-25%. A second course of pcV treatment is followed by still higher failure rates, in some cases necessitating tonsillectomy.

Several factors possibly contributing to the recurrences have been mentioned: low compliance, reinfection from the environment, eradication of α-streptococci with inhibitory effect on GAS, increase in β-lactamase-producing bacteria inactivating the drug, penicillin tolerant streptococci, low antibiotic concentration at site of infection and finally intracellular GAS surviving therapy.

Object

The aim of the present studies was:

1 To investigate the *short- and long-term efficacy of pcV versus clindamycin* in patients with GAS pharyngotonsillitis who failed on pcV treatment.

2 To examine failure and non-failure strains considering so called *penicillin-tolerance*.

3 To compare the *DNA-profiles* of failure and non-failure strains.

4 To evaluate *the kinetics of pcV, loracarbef and clindamycin in tonsillar surface fluid* in order to find a possible correlate to their clinical efficacy.

5 To investigate the diagnostic value of CRP and orosomucoid in GAS pharyngotonsillitis.

Material and methods

We defined bacterial failure as presence of GAS of the same T-type as in pre treatment samples within two weeks after completing therapy.

239 patients with GAS pharyngotonsillitis were treated with pcV for ten days. At examination 4-6 days after therapy, 53 patients still harboured GAS of the same T-type as in the pre-treatment culture. These 53 patients were randomized to treatment with either pcV or clindamycin and were then followed for one year with throat culture every third month and in case of pharyngotonsillitis. To investigate the role of penicillin tolerance, failure and non-failure strains were screened for tolerance. Isolates with a high survival rate were subjected to time killing tests. Using arbitrarily primed polymerase chain reaction (AP-PCR), the DNA-profiles of failure and non-failure strains were compared. Three different antibiotics - pcV, loracarbef and clindamycin - were investigated regarding concentration in tonsillar surface fluid (TSF) during and after ten days treatment of GAS pharyngotonsillitis and CRP and orosomucoid were analyzed throughout the investigation period.

Results

In the pcV group 64% yielded GAS in the throat culture, compared to 0% in the clindamycin group. In the first three months 68% in the pcV group yielded one or more positive cultures for GAS of the same T-type compared to 0% in the clindamycin group. However, for the remaining investigation period the difference between the groups was reduced.

No penicillin tolerant strains could be identified. The strains were of three different T-types, and using AP-PCR technique eleven different clones were identified. The same clones were found in both failures and non-failures.

PcV was found in TSF during the first three day period of treatment, after which the concentrations declined rapidly. This was in contrast to loracarbef and clindamycin, both of which showed longstanding concentration in TSF, with measurable values throughout, and even after, therapy.

Neither CRP nor orosomucoid was of any significance as indicators of GAS as a cause of pharyngotonsillitis.

Conclusions

1 Treatment with clindamycin could prevent further treatment failures for at least the following three months in patients with GAS pharyngotonsillitis who failed on pcV treatment.

2 Penicillin tolerance seems to be of no significance in failures of pcV treated GAS pharyngotonsillitis.

3 Failure and nonfailure strains exhibited similar DNA profiles, indicating that failures are associated with host rather than bacterial factors.

4 The longstanding concentration of both loracarbef and clindamycin in TSF may contribute to their capacity to eradicate GAS in patients who failed on pcV treatment of GAS pharyngotonsillitis.

5 CRP and orosomucoid is of no diagnostic value in GAS pharyngotonsillitis.

SVENSK SAMMANFATTNING

BAKGRUND

Akut faryngotonsillit orsakas till ca 40 % av betahaemolytiska grupp A streptokocker (GAS) och till ca 10% av andra bakterier. Ca 30% av fallen är virusutlösta och i ca 20% är genesen okänd. GAS är mycket virulenta humanpatogener som förutom svalg- och hudinfektioner även kan förorsaka livshotande tillstånd som ”streptococcal toxic shock syndrome” och nekrotiserande fasciit. Tidigare fruktade, men i Sverige numera sällsynta komplikationer är reumatisk feber och glomerulonefrit.

GAS faryngotonsillit medför hög arbets- och skolfrånvaro. Trots att GAS alltid är känsliga för penicillinV (pcV), så återfinns GAS i svalget hos 15-25% av patienterna ett par veckor efter avslutad behandling. Återinsjuknande i GAS faryngotonsillit sker i 5-10% av fallen, och efter ytterligare pcV kurer stiger återfallsfrekvensen markant. Upprepade recidiv leder ibland till tonsillektomi.

Som orsak till den höga recidivfrekvensen har bland annat följande faktorer föreslagits:

1. Bristande compliance, dvs. icke fullföljd behandling.
2. Reinfektion från omgivningen,
3. Utradering av normalt skyddande α-streptokocker under pcV behandlingen,
4. Tillväxt av penicillinresistenta β-laktamasproducerande bakterier med åtföljande inaktivering av pcV.
5. Penicillintolerans, dvs. fördröjd avdödning vid exponering för pcV.
6. Dålig penetration av penicillin till infektionsfocus, dvs. tonsillyta och kryptor.
7. Intracellulärt belägna streptokocker som inte nås av penicillinet**.**

MÅLSÄTTNING

Målsättningen med studierna har varit

1 Att på såväl kort som lång sikt jämföra effekten av pcV respektive klindamycin vid behandling av patienter med bakteriell terapisvikt efter pcV behandling av GAS faryngotonsillit.

2 Att undersöka GAS stammar från såväl patienter som blev bakteriefria efter pcV behandling som från patienter med bakteriell terapisvikt avseende förekomst av penicillintolerans och eventuell skillnad i DNA-profil.

3 Att undersöka huruvida koncentrationen av pcV, loracarbef och klindamycin i sekret på tonsillytan (TSF) under och efter behandling, kan korreleras till deras kliniska effekt på GAS faryngotonsillit.

4 Att utvärdera den kliniska signifikansen av CRP och orosomucoid vid GAS-faryngotonsillit.

MATERIAL och METODER

I studierna är bakteriell terapisvikt definierad som förekomst i svalget av GAS av den ursprungliga T-typen inom två veckor efter avslutad behandling.

Arbete I och II

239 patienter med faryngotonsillit och positiv snabbtest för GAS behandlades med pcV i tio dagar. Positiv svalgodling verifierade snabbtestfyndet. Kontrollodling utfördes 4-6 dagar efter avslutad behandling Patienter med bakteriell terapisvikt randomiserades därefter till behandling antingen med penicillin eller klindamycin i tio dagar. Kontroll med snabbtest och svalgodling utfördes åter ca fyra dagar efter avslutad behandling.

Patienterna följdes därefter under ett år. Svalgodling togs var tredje månad och dessutom vid faryngotonsillit.

T-typning och kvantitativ odling utfördes genomgående. Fynd av GAS i svalgodling föranledde behandling med pcV, och vid bakteriell svikt gavs behandling enligt tidigare randomisering.

Arbete III

GAS stammar *före* behandling från patienter som sedan blev streptokockfria (n=33) och från patienter med kommande terapisvikt (n=25), samt stammar från odling *efter* första (n=25) och andra (n=7) terapisvikten undersöktes avseende förekomsten av penicillintolerans. Kulturer i såväl stationär som i log-fas undersöktes.

Lägsta inhiberande (MIC) och lägsta baktericida (MBC) koncentrationen av pcV bestämdes. Tolerans definierades som en MBC/MIC kvot ≥ 32 och en överlevnad på ≥ 1 % i screening test och i ”time killing kinetic test”. Stammarna screenades med en modifierad

plattspädningsmetod där de under 6 timmar exponerades för pcV i en koncentration av 4 x MIC. Isolat med hög överlevnad undersöktes med s.k. ”time killing test”.

Arbete IV

Med arbitrarily primed polymerase chain reaction (AP-PCR) undersöktes DNA-profilen hos GAS stammar från fyra patienter med upprepade recidiv och jämfördes med profilen hos stammar från patienter som blev streptokockfria efter en pcV kur. Odlingarna togs under samma tidsperiod och i samma geografiska område.

Arbete V

Trettiofem konsekutiva patienter med faryngotonsillit och positivt snabbtest för GAS randomiserades till behandling med antingen pcV, klindamycin eller loracarbef. Positiv svalgodling verifierade snabbtestfyndet. Prov på antibiotikakoncentrationen i serum, tonsillsekret och saliv togs vid två randomiserade tillfällen under pågående behandling samt 1-3 dagar efter avslutad behandling. Prov på tonsillsekret och saliv togs med filterpapperslappar som placerades på tonsillytan och under tungan. CRP/s och orosomucoid/s kontrollerades vid varje besök.

RESULTAT

Arbete I o II

Totalt 53, dvs 22 % av de 239 patienterna hade positiv svalgodling för GAS vid kontroll fyra dagar efter avslutad behandling. 8% hade dessutom faryngotonsillit, medan 14% var symptomfria . 43 patienter hade riklig bakterieväxt i odlingen. 25 patienter randomiserades till pcV - och 28 till klindamycinbehandling. .

Bland patienter med terapisvikt kvarstod 48 evaluerbara fall. Av dessa uppvisade 14/22 (64 %) i penicillingruppen ånyo terapisvikt jämfört med 0/26 (0 %) i klindamycingruppen ($P<0.001$).

Under den följande tremånadersperioden förekom en eller flera positiva odlingar med samma T-typ hos 15 patienter i pcV gruppen, fem av dessa hade klinisk faryngotonsillit.

I klindamycingruppen erhölls under första tremånadsperioden ingen positiv odling med samma T-typ, men tre patienter hade faryngotonsillit med GAS av annan T-typ.

Upprepade recidiv under den första tremånadersperioden föranledde att 12 patienter i penicillingruppen korsades över till behandling med klindamycin vid terapisvikt, och i den gruppen förekom i fortsättningen fem positiva odlingar.
Under den resterande uppföljningstiden (3-12 månader) förekom positiva odlingar och klinisk faryngotonsillit både i den starkt decimerade pcV-gruppen och i klindamycingruppen - men utan signifikant skillnad. Samtliga odlingar utom en visade riklig eller måttlig växt.

Arbete III

Ingen penicillintolerans kunde konstateras hos log-fas bakterier, medan stammar i stationär fas uppvisade ett fördröjt avdödande. Ingen skillnad i detta avseende förelåg mellan isolat från de patienter som läkte på sin första pcV behandling och de som sviktade en eller två gånger.

Arbete IV

Stammarna tillhörde tre T-typer. Med AP-PCR teknik kunde elva olika genetiska profiler identifieras. Samma kloner fanns hos patienter som läkte på sin första pcV behandling och hos dem som sviktade en eller flera gånger. Vid bakteriell svikt sågs genomgående samma genetiska profil hos isolaten från respektive patient.

Arbete V

Under behandlingsdag 1-3 hade alla testade patienter i klindamycin och loracarbefgruppen samt 7 av 8 patienter i pcV-gruppen antibiotikakoncentrationer över MIC i TSF.
Under resterande behandlingstid sjönk koncentrationen av pcV snabbt, i kontrast till de båda andra preparaten.
Efter avslutad behandling påvisades mätbara antibiotikakoncentrationer i TSF hos 4 av 11 patienter i klindamycingruppen och hos 3 av10 patienter i loracarbefgruppen, men inte hos någon patient i pcV-gruppen. Såväl pcV som klindamycin påvisades i saliven i koncentrationer över MIC under hela, och vad gäller klindamycin även efter, behandlingen. Detta i kontrast till loracarbef som ej kunde påvisas i saliv.
Initialt sågs CRP värden >50 hos 7/35 (20%) och orosomucoid >1.17 g/l hos 13/35 (37%) av patienterna.

SLUTSATSER

1 Hos patienter med terapisvikt efter pcV behandling av GAS faryngotonsillit kunde behandling med klindamycin bryta den onda cirkeln med upprepade insjuknanden och ge ett skydd mot förnyad terapisvikt under minst tre månader.

2 Penicillintolerans var inte en faktor av betydelse vid terapisvikt efter pcV behandlad GAS faryngotonsillit.

3 Värdrelaterade faktorer var troligen viktigare vid terapisvikt än bakterierelaterade, eftersom samma DNA profil sågs hos GAS stammar från patienter som läkte som från patienter med bakteriell terapisvikt efter pcV behandling.

4 Tillräcklig koncentration i TSF av såväl loracarbef som av klindamycin under, och även efter avslutad, behandling bidrog sannolikt till att preparaten har bättre förmåga än penicillin att utplåna GAS från svalget.

5 CRP och orosomucoid var inte användbara som diagnostiska hjälpmedel vid GAS tonsillit.

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REFERENCES

1. Banck G, Nyman M. Tonsillitis and rash associated with Corynebacterium haemolyticum. J Infect Dis 154:1037-40, 1986.
2. Benjamin JT, Perriello VA Jr. Pharyngitis due to group C hemolytic streptococci in children. J Pediatr 89:254-6, 1976.
3. Bessen D, Fischetti VA. Passive acquired mucosal immunity to group A streptococci by secretory immunoglobulin A. J Exp Med 167:1945-50, 1988.
4. Bingen E, Denamur E, Lambert-Zechovsky N, Braimi N, El Lakany M, Elion J. DNA restriction fragment length polymorphism differentiates recurrence from relapse in treatment failures of *Streptococcus pyogenes* pharyngitis. J Med Microbiol 37:162-4, 1992.
5. Bisno AL. Group A streptococcal infections and acute rheumatic fever. N Engl J Med 325:783-93, 1991.
6. Bisno AL, Brito MO, Collins CM. Molecular basis of group A streptococcal virulence. Lancet Infect Dis 3:191-200, 2003.
7. Brook I. The role of β-lactamase-producing bacteria in the persistence of streptococcal tonsillar infection. Rev Infect Dis 6:601-7, 1984a.
8. Brook I. β-lactamase-producing bacteria recovered after clinical failures with various penicillin therapy. Arch Otolaryngol 110:228-31, 1984b.
9. Brook I. Role of β-lactamase-producing bacteria in the failure of penicillin to eradicate group A streptococci. Pediatr Infect Dis 4:491-5, 1985a.
10. Brook I, Hirokawa R. Treatment of patients with a history of recurrent tonsillitis due to group A β-hemolytic streptococci. A prospective randomized study comparing penicillin, erythromycin, and clindamycin. Clin Pediatr (Phila) 24:331-6, 1985b.
11. Carlsson F, Sandin C, Lindahl G. Human fibrinogen bound to Streptococcus pyogenes M protein inhibits complement deposition via the classical pathway. Mol Microbiol 56:28-39, 2005.
12. Ciftci E, Dogru U, Guriz H, Aysev AD, Ince E. Penicillin tolerance in group A β-haemolytic streptococci isolated from throat cultures of children with tonsillopharyngitis. Mikrobiyol Bul 36:147-52, 2002.
13. Cohen R, Reinert P. DeLa Rocque F, Levy C, Boucherat M, Robert M et al. Comparison of 2 dosages of azithromycin for 3 d vs penicillin V for 10 d in acute group A streptococcal tonsillopharyngitis,. Pediatr Infect Dis J 21:297-303, 2002.

14. Cone LA, Woodard DR, Schlievert PM, Tomory GS. Clinical and bacteriologic observations of a toxic shock-like syndrome due to Streptococcus pyogenes. N Engl J Med 317:146-9, 1987.
15. Dagnelie CF, van der Graaf Y, De Melker RA. Do patients with sore throat benefit from penicillin? A randomized double-blind placebo-controlled clinical trial with penicillin V in general practice. Br J Gen Pract 46:589-93, 1996.
16. Davis HD, McGeer A, Schwartz B, Green K, Cann D, Simor AE, Low DE. Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A streptococcal Study Group. N Engl J Med 335:547-54, 1996.
17. Del Mar CB, Glasziou PP, Spinks AB. Antibiotics for sore throat. Cochrane Database Syst Rev (4):CD000023, 2000.
18. De Meyere M, Mervielde Y, Verschraegen G, Bogaert M. Effect of penicillin on the clinical course of streptococcal pharyngitis in general practice. Eur J Clin Pharmacol 43:581-5, 1992.
19. Ebenfelt A, Ericson LE, Lundberg C. Acute pharyngotonsillitis is an infection restricted to the crypt and surface secretion. Acta Otolaryngol (Stockh) 118:264-71, 1998.
20. Engelgau MM, Woernle CH, Schwartz B, Vance NJ, Horan JM. Invasive group A streptococcus carriage in a child care centre after a fatal case. Arch Dis Child 71:318-22, 1994.
21. Eriksson BK, Andersson J, Holm SE, Norgren M. Epidemiological and clinical aspects of invasive group A streptococcal infections and the streptococcal toxic shock syndrome. Clin Infect Dis 27:1428-36, 1998.
22. Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: emm103 to emm124. Clin Infect Dis 34:28-38, 2002.
23. Falck, G, Kjellander J. Outbreak of group A streptococcal infection in a day-care center. Pediatr Infect Dis J 11:914-9, 1992.
24. Falck G, Holm SE, Kjellander J, Norgren M, Schwan Å. The role of household contacs in the transmission of group A streptococci. Scand J Infect Dis 29:239-44, 1997.

25. Falck G, Grahn-Håkansson, E, Holm SE, Roos K, Lagergren L. Tolerance and efficacy of interfering α-streptococci in recurrence of streptococcal pharyngotonsillitis: a placebo-controlled study. Acta Otolaryngol (Stockh) 119:944-8, 1999.
26. Fischetti VA. Streptococcal M protein: molecular design and biological behaviour. Clin Microbiol Rev 2:285-314, 1989.
27. Flottorp S, Oxman AD, Cooper JG, Hjortdahl P, Sandberg S, Vorland LH. Guidelines for diagnosis and treatment of sore throat. Tidskr Nor Laegeforen 10:1754-60, 2000.
28. Fontaine MC, Lee JJ, Kehoe MA. Combined contributions of streptolysin O and streptolysin S to the virulence of serotype M5 Streptococcus pyogenes strain Manfredo. Infect Immun 71:3857-65, 2003.
29. Gerber MA, Randolph MF, Chanatry J, Wright LL, De Meo K, Kaplan EL. Five vs ten days of penicillin V therapy for streptococcal pharyngitis. Am J Dis Child 141:224-7, 1987.
30. Gerber MA, Tanz RR, Kabat W, Bell GL, Siddiqui B, Lerer TJ, Lepow ML, Kaplan EL, Shulman ST. Potential mechanisms for failure to eradicate group A streptococci from the pharynx. Pediatrics 104:911-7, 1999.
31. Glezen WP, Clyde WA Jr, Senior RJ, Sheaffer CI, Denny FW. Group A streptococci, mycoplasmas, and viruses associated with acute pharyngitis. JAMA 202:119-24, 1967.
32. Grahn E, Holm S-E, Roos K. Penicillin tolerance in betastreptococci isolated from patients with tonsillitis. Scand J Infect Dis 421-6, 1987.
33. Gunnarsson RK, Holm SE, Söderström M. The prevalence of β-haemolytic streptococci in throat specimens from healthy children and adults. Implications for the clinical value of throat cultures. Scand J Prim Health Care 149-55, 1997.
34. Gutman L, Tomasz A. Penicillin-resistant and penicillin-tolerant mutants of group A streptococci. Antimicrob Agents Chemother 22:128-36, 1982.
35. Hansen J, Schmidt H, Bitsch N. Sore throat. Principles of diagnosis and treatment. Practitioner 227:937-48, 1983.
36. Herwald H, Mörgelin M, Olsen A, Rhen M, Dahlbäck B, Muller-Esterl W, Björck L. Activation of the contact-phase system on bacterial surfaces – a clue to serious complications in infectious diseases. Nat Med 4:298-302, 1998.

37. Herwald H, Cramer H, Mörgelin M, Russel W, Sollenberg U, Norrby-Teglund A, Flodgaard H, Lindbom L, Björck L. M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. Cell 116:367-79, 2004.
38. Hill HR, Caldwell GG, Wilson E, Hager D, Zimmerman RA. Epidemic of pharyngitis due to streptococci of Lancefield group G. Lancet ii:371-4. 1969.
39. Hjortdahl P, Melbye H. Does near-to-patient testing contribute to the diagnosis of streptococcal pharyngitis in adults? Scand J Prim Health Care 12:70-6, 1994.
40. Hoge CW, Schwartz B, Talkington DF, Breiman RF, MacNeill EM, Englender SJ. The changing epidemiology of invasive group A streptococcal infections and the emergence of streptococcal toxic shock-like syndrome. A retrospective population-based study. JAMA 269:384-9, 1993.
41. Hoffmann S. The throat carrier rate of group A and other β hemolytic streptococci among patients in general practice. Acta Path Microbiol Immunol Scand sect B. 93:347-51, 1985.
42. Hoffman S, Kolmos HJ. Effect of antibiotics on symptoms and complications of sore throat. Comments on a meta-analysis from the Cochrane Collaboration. Läkartidn 97:2730-2, 2000.
43. Holm SE, Roos K, Strömberg A. A randomized study of treatment of streptococcal pharyngotonsillitis with cefadroxil or phenoxymethylpenicillin (penicillin V). Pediatr Infect Dis J 10:S68-71, 1991.
44. Ingvarsson L, Kamme C, Lundgren K. Concentration of penicillin V in serum and middle ear exudate during treatment of acute otitis media. Ann Otol Rhinol Laryngol Suppl May-Jun 89:275-7, 1980.
45. Jackson DA, Cook PR. A general method for preparing chromatin containing intact DNA.EMBO J 4:913-8, 1985.
46. Jensen JH, Larsen S: Treatment of recurrent acute tonsillitis with clindamycin. An alternative tonsillectomy? Clin Otolaryngol 16:498-500**,** 1991.
47. Kamme C, Petersson A-C. In vitro effect on group A streptococci of loracarbef versus cefadroxil, cefaclor and penicillin V. Scand J Infect Dis 25:37-42, 1993.
48. Kaplan EL, Wannamaker LW. C-reactive protein in streptococcal pharyngitis. Pediatrics 60:28-32, 1977.

49. Kaplan EL, Gastanaduy AS, Huwe BB. The role of carrier in treatment failures after antibiotic for group A streptococci in the upper respiratory tract. J Lab Clin Med 98:326-35, 1981.
50. Kaplan EL, Johnson DR. Eradication of group A streptococci from the upper respiratory tract by amoxicillin with clavulanate after oral penicillin V treatment failure. J Pediatr 113:400-3, 1988.
51. Kataja J, Huovinen P, Skurnik M. The Finnish study group for antimicrobial resistance, Seppälä H. Erythromycin resistance genes in group A streptococci in Finland. Antimicrob Agents Chemother 43:48-52, 1999.
52. Kim KS, Kaplan EL. Association of penicillin tolerance with failure to eradicate group A streptococci from patients with pharyngitis. J Pediatr 107:681-4, 1985.
53. Krasinski K, Hanna B, LaRussa P, Desiderio D. Penicillin tolerant group A streptococci. Diagn Microbiol Infect Dis 4:291-7, 1986.
54. Kreikemeyer B, Klenk M, Podbielski A. The intracellular status of Streptococcus pyogenes: role of extracellular matrix-binding proteins and their regulation. Int J Med Microbiol 294:177-88, 2004.
55. Lan AJ, Colford JM, Colford JM Jr. The impact of dosing frequency on the efficacy of 10-day penicillin or amoxicillin therapy for streptococcal tonsillopharyngitis: A meta-analysis. Pediatrics 105:E19, 2000.
56. Lancefield RC. The antigenic complex of Streptococcus haemolyticus. I. Demonstration of a type-specific substance in extracts of Streptococcus haemolyticus. J Exp Med 47:91-103, 1928.
57. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med 57:571-95, 1933.
58. La Penta D, Rubens XC, Chi E, Cleary P. Group A streptococci efficiently invade human respiratory epithelial cells. Proc Natl Acad Sci USA 91:12115-9, 1994.
59. Lilja M, Myklebust R, Raisanen S, Stenfors LE. Selective attachment of β-haemolytic streptococci group A to oropharyngeal epithelium in health and disease. Acta Otolaryngol (Stockh) 117:744-9, 1997.
60. Lindbaek M, Hoiby EA, Lermark G, Steinsholt IM, Hjortdahl P. Which is the best method to trace group A streptococci in sore throat patients: culture or GAS antigen test? Scand J Prim Health Care 22:233-8, 2004.
61. Lottenberg R, Minning-Wenz D, Boyle MD. Capturing host plasmin(ogen): a common mechanism for invasive pathogens. Trends Microbiol 2:20-4, 1994.

62. Marouni MJ, Barzilai A, Keller N, Rubinstein E, Sela S. Intracellular survival of persistent group A streptococci in cultured epithelial cells. Int J Med Microbiol 294:27-33, 2004.
63. Martin DR. Rheumatogenic and nephritogenic group A streptococci. Myth or reality? An opening lecture. Adv Exp Med Biol 481:21-7, 1997.
64. Martin JM, Green M, Barbadora KA, Wald ER. Group A streptococci among school-aged children: clinical characteristics and the carrier state. Pediatrics 114:1212-9, 2004.
65. Maruyama S, Yoshioka H, Fujita K, Takimoto M, Satake Y. Sensitivity of group A streptococci to antibiotics. Prevalence of resistance to erythromycin in Japan. Am J Dis Child 133:1143-5, 1979.
66. Milatovic D, Knauer J. Cefadroxil versus penicillin in the treatment of streptococcal tonsillopharyngitis. Eur J Clin Microbiol Infect Dis 8:282-8, 1989.
67. Moffet HL, Siegel AC, Doyle HK. Nonstreptococcal pharyngitis. J Pediatr 73:51-60, 1968.
68. Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, Telford JL. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens**.** Proc Natl Acad Sci USA 102:15641-6, 2005.
69. Nerbrand C, Jasir A, Schalen C. Are current rapid detection tests for Group A Streptococci sensitive enough? Evaluation of 2 commercial kits. Scand J Infect Dis 34:797-9, 2002.
70. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, Gallo RL. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414:454-7, 2001.
71. Nordenfelt E. Virusetiologi vid svalginfektion. In: Ekedahl C, Holm SE, eds. Akuta svalginfektioner. Round table conferense 1980, AB Leo, Helsingborg, Sweden: 17-22, 1981.
72. Norgren M, Norrby A, Holm SE. Genetic diversity in T1M1 groupA streptococci in relation to clinical outcome of infection. J Infect Dis 166:1014-20, 1992.
73. Nyberg P, Sakai T, Cho HK, Caparon MG, Fässler R**,** Björck L. Interactions with fibronectin attenuate the virulence of Streptococcus pyogenes. EMBO J 23:2166-74, 2004.

74. Nyman M, Banck G, Thore M. Penicillin tolerance in Arcanobacterium haemolyticum. J Infect Dis 161:261-5, 1990.
75. Orrling A, Stjernquist-Desatnik A, Schalén C, Kamme C. Clindamycin in persisting streptococcal pharyngotonsillitis after penicillin treatment. Scand J Infect Dis 126:535-41, 1994.
76. Orrling A, Stjernquist-Desatnik A, Schalén C. Clindamycin in recurrent group A streptococcal pharyngotonsillitis – an alternative to tonsillectomy? Acta Otolaryngol (Stockh) 117:618-22, 1997.
77. Paradise JL, Bluestone CD, Ruth Z, Bachman RZ, Colborn DK, Bernard BS, Taylor FH, Rogers KD, Schwarzbach, RH, Stool SE, Friday GA, Smith IH, Saez CA. Efficacy of tonsillectomy for recurrent throat infection in severely affected children. Results of Parallel Randomized and Nonrandomized Clinical Trials. New Eng J Med 310:674-83, 1984.
78. Pichichero ME, Disney FA, Aronovitz GH, Talpey WB, Green JL, Francis AB. Randomized, single-blind evaluation of cefadroxil and phenoxymethyl penicillin in the treatment of streptococcal pharyngitis. Antimicrob Agents Chemother 31:903-6, 1987.
79. Pichichero ME, Gooch WM, Rodriguez W, Blumer JL, Aronof SC, Jacobs RF, Musser JM. Effective short-course treatment of acute group A β-haemolytic streptococcal tonsillopharyngitis. Ten days of penicillin V vs 5 days or 10 days of cefpodoxime therapy in children. Arch Pediatr Adolesc Med 148:1053-60, 1994.
80. Putto A, Meurman O, Ruuskanen O. C-reactive protein in the differentiation of adenoviral, Epstein-Barr viral and streptococcal tonsillitis in children. Eur J Pediatr 145:204-6, 1986.
81. Roos K. The diagnostic value of symptoms and signs in acute tonsillitis in children over the age of 10 and in adults. Scand J Infect Dis 17:259-67, 1985.
82. Roos K, Grahn E, Holm SE, Johansson H, Lind L. Interfering alpha-streptococci as a protection against recurrent streptococcal tonsillitis in children. Int J Pediatr Otorhinolaryngol 25:141-8, 1993.
83. Roos K, Larsson P. Loracarbef versus phenozxymethylpenicillin in the treatment of recurrent streptococcal pharyngotonsillitis. Scand J Infect Dis 29:141-5, 1997.
84. Roos K, Prellner K, Holm S, Larsson P, Stjernquist-Desatnik A, Strömberg A. Norwegian guidelines for “sore throats” nothing for Swedish throats! Läkartidn 97:5144-5, 2000.

85. Ross PW, Chisty SMK, Knox JDE. Sore throat in children: Its causation and incidence. Br Med J 624-6, 1971.
86. Sanders C, Sanders E, Harrow D. Bacterial interference: effects of oral antibiotics on the normal throat flora and its ability to interfere with group A streptococci. Infect Immun 13:808-12, 1976.
87. Schwartz RH, Wientzen Jr RL, Pedreira F, Feroli EJ, Mella GW, Guandolo VL. Penicillin V for group A streptococcal pharyngotonsillitis. A randomized trial of seven vs ten days' therapy. JAMA 246:1790-5, 1981.
88. Sela S, Barzilai, A. Who do we fail with penicillin in the treatment of group A streptococcus infections? Ann Med 31:303-7, 1999.
89. Seppälä H, Nissinen A, Jarvinen H, Huovinen S, Henriksson T, Herva E, Holm SE, Jahkola M, Katila ML, Klaukka T, et al. Resistance to erythromycin in group A streptococci. N Engl J Med 326:292-7, 1992.
90. Sierig G, Cywes C, Wessels MR, Asbaugh C. Cytotoxic effects of streptolysin O and streptolysin S enhance the virulence of poorly encapsulated group A streptococci. Infect Immun 71:446-55, 2003.
91. Slater GJ, Greenwood D. Detection of penicillin tolerance in streptococci. J Clin Pathol 36:1353-6, 1983.
92. Smith TD, Huskins WC, Kim KS, Kaplan EL. Efficacy of β-lactamase resistant penicillin and influence of penicillin tolerance in eradicating streptococci from the pharynx after failure of penicillin therapy for group A streptococcal pharyngitis. J Pediatr 110:777-82, 1987.
93. Stjernquist-Desatnik A, Prellner K, Christensen P. Clinical and laboratory findings in patients with acute tonsillitis. Acta Otolaryngol (Stockh) 104:351-9, 1987.
94. Stjernquist-Desatnik A, Orrling A, Schalen C, Kamme C. Penicillin tolerance in group A streptococci and treatment failure in streptococcal tonsillitis. Acta Otolaryngol (Stockh) Suppl 492:68-71, 1992.
95. Stjernquist-Desatnik A, Samuelsson P, Walder M. Penetration of penicillin V tonsillar surface fluid in healthy individuals and in patients with acute tonsillitis. Laryngol Otol 107:309-12, 1993.
96. Stjernquist-Desatnik A, Schalen C, Ekedahl A. Erythromycinresistenta grupp A streptokocker. Läkartidn 91:812-4, 1994.

97. Strömberg A, Friberg U, Cars O. Concentrations of phenoxymethylpenicillin and cefadroxil in tonsillar tissue and tonsillar surface fluid. Eur J Clin Microbiol 6:525-9, 1987.
98. Strömberg, A, Schwan, Å, Cars O. Five versus ten days treatment of group A streptococcal pharyngotonsillitis: a randomized controlled clinical trial with phenoxymethylpenicillin and cefradroxil. Scand J Infect Dis 20:37-46, 1988a.
99. Strömberg A, Schwan Å, Cars O. Throat carrier rates of β-haemolytic streptococci among healthy adults and children. Scand J Dis 20:411-7, 1988b.
100. Sun J, Keh-Gong W, Hwang B. Evaluation of the etiologic agents for acute suppurative tonsillitis in children. Zhonghua Yi Xue Za Zhi (Taipei) 65:212-7, 2002.
101. Söderström M, Blomberg J, Christensen P, Hovelius B. Erythromycin and phenoxymethylpenicillin (penicillin V) in the treatment of respiratory tract infections as related to microbiological findings and serum C-reactive protein. Scand J Infect Dis 23:347-54, 1991.
102. Tanz RR, Shulman ST, Sroka PA, Marubio S, Brook I, Yogev R. Lack of influence of β-lactamase-producing flora on recovery of group A streptococci after treatment of acute pharyngitis. J Pediatr 117:859-63, 1990.
103. Taylor PC, Schoenknecht FD, Sherries JC, Linner EC. Determination of minimum bactericidal concentrations of oxacillin for Staphylococcus aureus. Influence and significance of technical factors. Antimicrob Agents Chemother 23:142-50, 1983.
104. Telian SA. Sore throat and antibiotics. Otolaryngol Clin North Am 19:103-9, 1986.
105. Tierpsprojektet. Arbetsrapport Socialstyrelsen. Soc Med Inst, Uppsala. B Smedby.
106. Tunér K, Nord CE. Emergence of β-lactamase producing anaerobic bacteria in the tonsils during penicillin treatment. Eur J Clin Microbiol 5:399-404, 1986.
107. Tuomanen E, Durack DT, Tomasz A. Antibiotic tolerance among clinical isolates of bacteria. Antimicrob Agents Chemother 30:521-7, 1986.
108. Urbanék K, Kolár M, Cekanová L. Utilisation of macrolides and the development of Streptococcus pyogenes resistance to erythromycin. Pharm World Sci 27:104-7, 2005.
109. van Asselt GJ, Mouton RP. Detection of penicillin tolerance in Streptococcus pyogenes. J Med Microbiol 38:197-202, 1993.

110. van Asselt GJ, de Kort G, van de Klundert JAM. Differences in penicillinbinding protein patterns of penicillin tolerant and non-tolerant group A streptococci. J Antimicrob Chemother 35:67-74, 1995.
111. von Pawel-Rammingen U, Björck L. IdeS and SpeB: Immunoglobulin-degrading cysteine proteases of Streptococcus pyogenes. Curr Opin Microbiol 60:50-5, 2003.
112. Wannamaker LW. Differences between streptococcal infections of the throat and of the skin. N Eng J Med 8:78-85, 1970.
113. Wannamaker LW. Perplexity and precision in the diagnosis of streptococcal pharyngitis. Am J Dis Child 124:352-8, 1972.
114. Watkins VS, Smietana M, Conforti PM, Sides GD, Huck W. Comparison of dirithromycin and penicillin for treatment of streptococcal pharyngitis. Antimicrob Agents Chemother 41:72-5, 1997.
115. Veasy LG, Wiedmeier SE, Orsmond GS, Ruttenberg HD, Boucek MM, Roth SJ, Tait VF, Thompson JA, Daly JA, Kaplan et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. N Engl J Med 316:421-7, 1987.
116. Weiss K, Laverdiere M, Lovgren M, Delorme J, Poirier L, Beliveau C. Group A Streptococcus carriage among close contacts of patients with invasive infection. Am J Epidemiol 149:863-8, 1999.
117. Wessels MR, Moses AE, Goldberg JB, DiCesare TJ. Hyaluronic acid capsule is a virulence factor for mucoid group A streptococci. Proc Natl Acad Sci USA 88:8317-21, 1991
118. Woodruff C. Microbiology of infectious diseases of Waldeyer's ring. Ear Nose Throat J 59:454-6, 1980.
119. Woolfrey BJ. Penicillin tolerance in β-streptococci. Scand J Infect Dis 20:235-7, 1988.
120. Workshop arrangerad av Läkemedelsverket. Handläggning av pharyngotonsilliter. Information från Läkemedelsverket 12(7/8):44-75, 2001.
121. Zwart S, Sachs APE, Ruijs GJHM, Gubbels JW, Hoes AW, de Melker RA. Penicillin for acute sore throat: randomized double blind trial of seven days versus three days treatment of placebo in adults. BMJ 320:150-4, 2000.
122. Österlund A, Engstrand L. DNA fingerprinting of Streptococcus pyogenes from patient with recurrent pharyngotonsillitis by means of random amplified polymorphic DNA analysis. Scand J Infect Dis 27:119-21, 1995.

123. Österlund A. Popa R, Nikkilä T. Scheynius A, Engstrand L. Intracellular reservoir of Streptococcus pyogenes in vivo: a possible explanation for recurrent pharyngotonsillitis. Laryngoscope 107:640-7, 1997.