MATTI KARPPELIN

Acute and Recurrent Cellulitis

ACADEMIC DISSERTATION
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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following five original studies, which are referred to in the text by their Roman numerals I-V.

I Karppelin M, Siljander T, Vuopio-Varkila J, Kere J, Huhtala H, Vuento R, Jussila T, Syrjänen J. Factors predisposing to acute and recurrent bacterial non-necrotizing cellulitis in hospitalized patients: a prospective case-control study. Clin Microbiol Infect. 2010; 16:729-34.

II Siljander T, Karppelin M, Vähäkuopus S, Syrjänen J, Toropainen M, Kere J, Vuento R, Jussila T, Vuopio-Varkila J. Acute bacterial non-necrotizing cellulitis in Finland, microbiological findings. Clin Infect Dis. 2008; 46:855-61.

III Karppelin M, Siljander T, Haapala A-M, Huttunen R, Kere J, Vuopio J, Syrjänen J. Evidence of Streptococcal Origin of Acute Non-necrotising cellulitis, a serological study. Eur J Clin Microbiol Infect Dis. 2015; 34:669-72.

IV Karppelin M, Siljander T, Aittoniemi J, Hurme M, Huttunen R, Huhtala H, Kere J, Vuopio J, Syrjänen J. Predictors of recurrent cellulitis in a five year follow-up study. Clinical risk factors and the role of pentraxin 3 (PTX3) and C-reactive protein. J Infect (in press).

V Karppelin M, Siljander T, Huhtala H, Aromaa A, Vuopio J, Hannula-Jouppi K, Kere J, Syrjänen J. Recurrent cellulitis with benzathine penicillin prophylaxis is associated with diabetes and psoriasis. Eur J Clin Microbiol Infect Dis 2013; 32:369-72.

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ABBREVIATIONS

ADN	Anti-DNase B
ASO	Anti-streptolysin O
ASTA	Antistaphylolysin
BHS	β-haemolytic streptococcus
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
GAS	Group A β-haemolytic streptococcus
GBS	Group B β-haemolytic streptococcus
GCS	Group C β-haemolytic streptococcus
GFS	Group F β-haemolytic streptococcus
GGS	Group G β-haemolytic streptococcus
IU	International unit
LOS	Length of stay in hospital
NH	Negative history of cellulitis
OR	Odds ratio
PAR	Population attributable risk
PH	Positive history of cellulitis
PTX3	Pentraxin-3
SDSE	*Streptococcus dysgalactiae* subsp. e*quisimilis*
THL	National Institute for Health and Welfare (formerly KTL)

ABSTRACT

Acute bacterial non-necrotising cellulitis, or erysipelas, is an acute infection of the dermis and subcutaneous tissue with a tendency to recur. Erysipelas is mentioned already in ancient medical writings. There is considerable variation in the terminology regarding erysipelas and cellulitis. In the present study, cellulitis denotes acute, non-suppurative, superficial skin infection of presumed bacterial origin. This definition excludes abscesses, suppurative wound infections, and necrotising infections.

Cellulitis most typically occurs in in the leg, and less often in the upper extremity, in the face, or other parts of the body. β-haemolytic streptococci (BHS) are considered the main causative bacteria associated with cellulitis. Penicillin is the treatment of choice in most cases. The majority of cellulitis patients are probably treated as outpatients. The aim of the present study was to assess clinical risk factors for acute and recurrent cellulitis, to study the bacterial aetiology of cellulitis and characterize BHS associated with cellulitis. Also, the value of clinical features and inflammatory markers in predicting further recurrence was investigated.

A case control study was conducted comprising 90 patients hospitalized due to cellulitis and 90 population controls, matched by age and sex. Demographical data and data concerning the suspected clinical risk factors were collected. Bacterial cultures for isolation of BHS were obtained from the affected sites of the skin or skin breaks in the ipsilateral site. Also, pharyngeal swabs and blood cultures were collected on admission to hospital. In addition, sera were collected from patients in acute phase and in convalescent phase 1 month after the admission for subsequent analyses.

The median age of the patients was 58 years, 64% were male. The median body mass index (BMI) for patients and controls was 29.1 and 26.5, respectively. Cellulitis was located in the leg in 84%, in the upper extremity in 8%, and in the face in 8% of the cases. In the statistical analysis, chronic oedema, disruption of the cutaneous barriers (toe web maceration, ulcer, wound, or chronic dermatosis), and obesity were independently associated with cellulitis. BHS were isolated from skin swabs or blood cultures in 29% of the cases and group G BHS (GGS) was the most common streptococcal skin isolate. GGS were also isolated from throat swabs in 7% and 13% of the patients and their household members, respectively. No GGS was found in pharyngeal swabs in control subjects. Molecular typing revealed no distinct BHS strain associated with cellulitis. On the basis of the bacteriological and serological findings, BHS were associated with cellulitis in 73% of the cases.

Patients were contacted and interviewed by telephone five years after the initial recruitment and patient charts were reviewed. Eleven patients had died during the follow-up. On the basis of telephone interview and review of medical records 87 patients could be evaluated and a recurrence was verified in 36 (41%) and reliably excluded in 51 cases. The mean follow-up time was 4.7 years. The risk for recurrence

in five years was 26% after the primary cellulitis episode, and 57% in those who had a recurrent attack at the baseline study. A history of previous cellulitis at baseline was the only risk factor associated with recurrence in five years. At the baseline study, patients with a history of previous cellulitis showed a stronger inflammatory response, reflected by higher c-reactive protein (CRP) level and leukocyte counts, and longer hospital stay, than those with a primary episode. Based on this finding, it was hypothesized, that acute phase reactants CRP and pentraxin-3 (PTX3) could predict recurrence of cellulitis. However, the hypothesis could not be proved in the five year follow-up study.

Risk factors for recurrent cellulitis were assessed in another clinical material comprising 398 patients with prophylactic benzathine penicillin treatment for recurrent cellulitis and 8005 controls derived from a national population-based health survey (Health 2000). The median age of the patients was 65 years. The mean BMI was 31.5 for male and 32.5 for female patients. In multivariable analysis psoriasis, other chronic dermatoses, diabetes, increasing BMI, increasing age, and a history of previous tonsillectomy were independently associated with recurrent cellulitis.

In conclusion, BHS were associated in the majority of the cellulitis cases. GGS was the most common streptococcal isolate in patients and their household members, but it was not found in the control population. Oedema, skin breaks, and obesity are risk factors for acute cellulitis. Same clinical risk factors probably predispose to acute and recurrent cellulitis, but the risk for further recurrence is higher after a recurrence than after the primary attack. Also, diabetes, psoriasis, and increasing age are risk factors for recurrent cellulitis with benzathine penicillin prophylaxis. High CRP or PTX3 do not predict recurrence of cellulitis.

The findings of the present study contribute to the understanding of factors behind the individual risk for cellulitis and especially the recurrence of cellulitis, and may influence the clinical use of antibiotics and non-pharmacological measures in treatment and prevention of cellulitis.

TIIVISTELMÄ

Ruusutulehdus on akuutti ihon ja ihonalaiskudosten bakteeri-infektio. Siitä käytetään myös nimityksiä selluliitti tai erysipelas. Kansainvälisessä kirjallisuudessa ruusutulehduksen nimitykset vaihtelevat, mikä voi vaikeuttaa tutkimustulosten tulkintaa. Tässä tutkimuksessa ruusutulehduksesta käytetään englanninkielistä termiä "cellulitis", jolla tarkoitetaan äkillistä, oletettavasti bakteeriperäistä ihon infektiota, johon ei liity märkäeritystä. Tämä määritelmä sulkee pois märkäiset haavainfektiot, paiseet ja kuolioivat tulehdukset.

Ruusutulehdus sijaitsee tyypillisesti alaraajassa, yleensä säären alueella, mutta se voi tulla myös yläraajaan, kasvoihin tai muulle ihoalueelle. β-hemolyyttisiä streptokokkeja (BHS) on pidetty pääasiallisina taudinaiheuttajina ja penisilliiniä käypänä hoitona ruusutulehduksessa. Stafylokokkien osuus ruusutulehduksessa on epäselvä, mutta ilmeisesti *Staphylococcus aureus* voi joskus aiheuttaa ruusutulehduksen, jota ei voi kliinisten merkkien perusteella erottaa streptokokin aiheuttamasta taudista.

Ruusutulehdukselle on tyypillistä sen uusiutuminen. Aiemmissa tutkimuksissa uusiutumisriski on ollut noin 10 % vuodessa. Uusiutumisriskiin vaikuttavia tekijöitä ei tunneta tarkasti, mutta pidetään todennäköisenä, että samat tekijät, jotka altistavat akuutille ruusutulehdukselle, altistavat myös sen uusiutumiselle.

Tämän tutkimuksen tarkoituksena oli selvittää ruusutulehduksen kliinisiä riskitekijöitä sekä akuutin ruusutulehduksen bakteerietiologiaa. Lisäksi tutkittiin kliinisten riskitekijöiden ja tulehdusmerkkiaineiden merkitystä ruusutulehduksen uusiutumisriskin arvioimisessa.

Akuutin ruusutulehduksen kliinisiä riskitekijöitä tutkittiin tapaus-verrokkitutkimuksessa, johon rekrytoitiin 90 potilasta ja 90 verrokkia. Potilaat olivat akuutin ruusutulehduksen vuoksi sairaalahoitoon otettuja aikuisia ja verrokit iän ja sukupuolen suhteen kaltaistettuja väestörekisteristä satunnaisesti valittuja henkilöitä. Kliinisten tietojen lisäksi kerättiin bakteeriviljelynäytteet 66 potilaan iholta ja veriviljely 89 potilaalta sairaalaan tullessa. Nieluviljely otettiin kaikilta potilailta ja verrokeilta. Potilailta otettiin seeruminäyte sekä sairaalaan tullessa että noin kuukauden kuluttua. Seeruminäytteistä tutkittiin tulehduksen välittäjäaineita ja bakteerivasta-aineita.

Potilaat olivat keskimäärin 58-vuotiaita ja 64 % oli miehiä. Potilaiden painoindeksi (BMI) oli keskimäärin 29.1 ja verrokkien 26.5. Ruusutulehdus oli alaraajassa 84 %:lla, yläraajassa 8 %:lla ja kasvoissa 8 %:lla potilaista. Tilastoanalyysin perusteella ruusutulehduksen itsenäisiä riskitekijöitä olivat krooninen raajaturvotus, ihorikkoumat ja ylipaino (BMI yli 30). Ihon bakteeriviljelyssä eristettiin BHS 24/66 (36 %) potilaalta. Eristetyistä 25 BHS-kannasta 18 (72 %) kuului ryhmään G (GGS), kuusi (24 %) ryhmään A (GAS) ja yksi ryhmään B. GGS eristettiin myös verestä kahdelta

(2 %) ja nielusta kuudelta (7 %) potilaalta sekä viideltä (13 %) potilaiden ruokakunnan jäseneltä, mutta ei yhdeltäkään verrokilta. GAS eristettiin kahden potilaan ja kahden verrokin nielunäytteestä, mutta ei yhdeltäkään ruokakunnan jäseneltä. Eristettyjen GAS ja GGS kantojen *emm*-geenin sekvenoinnin ja pulssikenttäelektroforeesin perusteella ei löytynyt yhtään erityisesti ruusutulehdukseen liittyvä tyyppiä.

Bakteeriviljelyjen lisäksi ruusutulehduksen aiheuttaja pyrittiin osoittamaan streptokokkivasta-ainetutkimuksilla. Nämä viittasivat äskettäiseen GAS- tai GGS-infektioon 53:lla (69 %) 77 potilaasta, joilta oli saatu kuukauden välein pariseeruminäytteet. Yhdistettynä vasta-ainetutkimukset ja bakteeriviljelyt viittasivat siihen, että GGS tai GAS oli taudinaiheuttajana 56 (73 %) tapauksessa. Lisäksi niistä 21 potilaasta, joiden kohdalla vasta-ainetutkimukset tai bakteeriviljelyt eivät viitanneet BHS-infektioon, 9 potilasta hoidettiin penisilliinillä. Stafylokokit ovat nykyisin lähes aina resistenttejä penisilliinille, joten hyvää vastetta penisilliinihoidolle voidaan pitää epäsuorana viitteenä BHS:n osuudesta taudinaiheuttajana näissäkin tapauksissa. Näin ollen 65 (84 %) potilaan kohdalla BHS oli todennäköisin taudinaiheuttaja ja penisilliini olisi käypä hoito.

Tutkimuspotilaisiin otettiin yhteyttä viiden vuoden kuluttua tutkimukseen tulosta ja hankittiin potilaiden sairauskertomukset. Yksitoista potilasta oli kuollut seuranta-aikana ja kolmea muuta ei tavoitettu. Puhelinhaastattelun ja sairauskertomusten perusteella voitiin osoittaa ruusutulehduksen uusiutuneen 36 (41 %) potilaalla ja poissulkea uusiutuminen 51 potilaan kohdalla. Keskimääräinen seuranta-aika oli 4.7 vuotta. Jos potilaan alun perin tutkimukseen johtanut ruusutulehdusepisodi oli hänen elämänsä ensimmäinen, uusiutumisriski seuranta-aikana oli 26 %. Jos taas potilas oli sairastanut ainakin yhden ruusutulehduksen jo aiemmin, uusiutumisriski oli 57 %. Mikään muu kliininen riskitekijä ei ennustanut ruusutulehduksen uusiutumista. Alkuvaiheen tutkimuksessa niillä, joilla tutkimukseen tullessa oli jo uusiutunut ruusutulehdus, oli voimakkaampi tulehdusvaste kuin niillä joilla ruusutulehdus oli ensimmäinen. Tulehdusreaktiota arvioitiin mittaamalla C-reaktiivisen proteiinin ja pentraksiini-3:n pitoisuudet potilaiden tullessa hoitoon sekä kuumeen ja sairaalahoidon keston perusteella. Mikään näistä neljästä ei kuitenkaan ennustanut ruusutulehduksen uusiutumista seuranta-aikana.

Uusiutuvan ruusutulehduksen riskitekijöitä tutkittiin myös toisessa tapaus-verrokkitutkimuksessa, johon rekrytoitiin 398 potilasta, jotka olivat vuonna 2000 saaneet bentsatiinipenisilliniä uusiutuvan ruusutulehduksen ehkäisemiseksi. Verrokkeina oli Kansanterveyslaitoksen Terveys 2000 – tutkimukseen osallistuneet 8005 yli 30-vuotiasta henkilöä. Potilaat olivat iältään keskimäärin 65-vuotiaita. Monimuuttujamallissa itsenäisiä riskitekijöitä olivat krooniset ihosairaudet ja erityisesti psoriasis, diabetes, iän karttuminen ja painoindeksin kohoaminen sekä nielurisojen poisto.

Yhteenvetona voidaan todeta, että BHS ja erityisesti GGS on todennäköinen taudinaiheuttaja valtaosassa ruusutulehduksista. Krooninen turvotus, ihorikkoumat ja ylipaino ovat akuutin ruusutulehduksen riskitekijöitä. On todennäköistä, että nämä riskitekijät altistavat myös ruusutulehduksen uusiutumiselle samoin kuin diabetes, psoriasis ja iän karttuminen. Uusiutumisriski on kuitenkin yli kaksinkertainen jo uusiutuneen ruusutulehduksen jälkeen verrattuna ensimmäiseen episodiin. Tulehdusreaktion voimakkuus akuutin ruusutulehduksen yhteydessä ei ennusta ruusutulehduksen uusiutumista.

1. INTRODUCTION

Acute bacterial non-necrotising cellulitis, or erysipelas (most probably from Greek "erythros", red, and "pella", skin), is a skin infection affecting the dermis and subcutaneous tissue (Bisno and Stevens 1996). Until the recent decades, the most typical location of erysipelas was the face. At present, erysipelas is most commonly located in the leg (Ronnen et al. 1985).

There is some confusion in the terminology concerning cellulitis and erysipelas. Erysipelas is sometimes considered as a distinct disease, separate to cellulitis by means of the appearance of the skin lesion associated. Cellulitis, in turn may include abscesses and wound infections in addition to diffuse non-suppurative infection of the dermis and subcutaneous tissue. Variation in terminology and case definitions hampers interpretation of different studies (Chambers 2013). In the present study, cellulitis is defined as acute bacterial non-necrotising cellulitis, which corresponds to erysipelas or "rose" in Finnish clinical practice. Thus, suppurative conditions are excluded, as well as necrotising infections. For practical reasons, the term "cellulitis" is used in the text to denote acute non-necrotising cellulitis, which is the subject of the present study. Term "erysipelas" is used when citing previous studies using that definition.

Cellulitis is not uncommon. Incidence is estimated to be 200/100 000 persons/year (McNamara et al. 2007b). The incidence of cellulitis has likely been quite stable throughout the 20th century, but case fatality rate has declined close to zero after the introduction of penicillin (Madsen 1973). The infectious nature of cellulitis has been accepted after the early experiments of Friedrich Fehleisen in the end of the 19th century (Fehleisen 1883). However, the exact pathogenetic mechanisms behind the clinical manifestations of cellulitis are unknown. Although bacterial aetiology is not always possible to ascertain, BHS and especially group A BHS (GAS) is considered the main pathogen. The role of *Staphylococcus aureus* as a causative agent in diffuse non-suppurative cellulitis is controversial (Bisno and Stevens 1996, Swartz 2004, Gunderson 2011).

A typical clinical picture of cellulitis is an acute onset of erythematous skin lesion, with more or less distinct borders, accompanied with, often high, fever. The differential diagnosis includes a wide variety of infectious and non-infectious conditions (Falagas and Vergidis 2005, Gunderson 2011, Hirschmann and Raugi 2012b). Treatment of cellulitis consists of administration of antibiotics and supportive measures. The majority of cellulitis cases are probably treated as outpatients, but the exact proportion is not known (Ellis Simonsen et al. 2006).

A typical feature of cellulitis is recurrence. The rate of recurrence, according to the previous studies, has been roughly 10% per year (Jorup-Rönström and Britton 1987, Eriksson et al. 1996, McNamara et al. 2007a). Clinical risk factors for erysipelas and cellulitis have been investigated in previous studies. Skin breaks, chronic oedema and obesity have most consistently been found associated with acute and recurrent cellulitis (Dupuy et al. 1999, Roujeau et al. 2004, Björnsdottir et al. 2005, Mokni et al. 2006, Bartholomeeusen et al. 2007, Halpern et al. 2008, Eells et al. 2011). Bacterial aetiology has been studied by various methods (Leppard et al. 1985, Bernard et al. 1989, Jeng et al. 2010, Gunderson 2011). However, the interpretation of these studies is particularly difficult due to high variation in case definition and terminology in the studies (Gunderson 2011, Chambers 2013).

C-reactive protein (CRP) and pentraxin-3 (PTX3) are so called acute phase proteins, the production of which is increased in infections and other inflammatory conditions (Black et al. 2004, Mantovani et al. 2013). CRP measurement is widely used in current clinical practice as a diagnostic marker and in monitoring of treatment success in infectious and rheumatologic diseases. The role of PTX3 as a diagnostic and prognostic marker is recently studied in a variety of conditions (Peri et al. 2000, Mairuhu et al. 2005, Outinen et al. 2012, Uusitalo-Seppälä et al. 2013).

In the present study, clinical risk factors for acute cellulitis and recurrent cellulitis were assessed in two patient populations: (1) hospitalised patients with an acute cellulitis and (2) patients with a recurrent cellulitis. Both groups were compared to respective controls. The risk of cellulitis recurrence in five years and associated risk factors for recurrence were studied in patients hospitalised with acute cellulitis. The bacterial aetiology of acute cellulitis was investigated by culture and serology. BHS strains isolated in cases of acute cellulitis were characterised by molecular methods.

Furthermore, the value of CRP and PTX3 in predicting a recurrence of cellulitis was assessed.

2. REVIEW OF THE LITERATURE

2.1 Cellulitis and erysipelas

2.1.1 Definition of cellulitis

Cellulitis is an acute bacterial infection of the dermis and subcutaneous tissue (Bisno and Stevens 1996, Swartz 2004, Stevens et al. 2005). Bacterial aetiology distinguishes it from other inflammatory conditions affecting dermis and hypodermis, such as eosinophilic cellulitis or Well's syndrome (Wells and Smith 1979), and neutrophilic cellulitis or Sweet's syndrome (acute febrile neutrophilic dermatosis**)** (Cohen and Kurzrock 2003).

Erysipelas, or classic erysipelas, has sometimes been distinguished from cellulitis by its feature of a sharply demarcated, skin lesion which is slightly elevated from the surrounding normal skin. However, it is impossible to make a clear distinction between erysipelas and cellulitis in many cases. Erysipelas may be considered as a special form of cellulitis affecting the superficial part of dermis (Bisno and Stevens 1996, Swartz 2004).

The qualifier "acute" in the context of bacterial cellulitis refers to a single episode or an attack of cellulitis, whether the first for a given patient or a recurrent episode, and separates it from the phenomenon of recurrent cellulitis, i.e. two or more acute cellulitis episodes suffered by a patient. On the other hand it emphasises the fact that bacterial cellulitis is not a chronic condition. However, in very rare cases nontuberculous mycobacteria may cause skin infections resembling cellulitis with an insidious onset, and without fever or general malaise (Bartralot et al. 2000, Elston 2009). The term "chronic cellulitis", used by laypersons and occasionally by health care professionals, refers most often to recurrent cellulitis or is a misinterpretation of the chronic skin changes due to venous insufficiency or lymphoedema (Hirschmann and Raugi 2012b).

Erysipelas and cellulitis together make up a clinical entity with the same risk factors and clinical features and mostly the same aetiology (Bernard et al. 1989, Björnsdottir et al. 2005, Chambers 2013). However, some authors emphasise that erysipelas is a specific type of cellulitis and should be studied as a separate disease (Bonnetblanc and Bedane 2003). French dermatologists have proposed the terms bacterial dermo-hypodermitis or acute bacterial dermo-hypodermatitis to replace erysipelas or non-necrotising cellulitis as they more clearly define the anatomical location of the inflammation (Dupuy et al. 1999, Bonnetblanc and Bedane 2003). The two extremes of acute bacterial cellulitis can be clinically defined but clear distinction between them is not always possible (Bisno and Stevens 1996, Swartz 2004). The histological findings in cellulitis and erysipelas are dermal neutrophilic infiltration, dermal fibrin-rich oedema and dilated lymphatic vessels (Bonnetblanc and Bedane 2003, McAdam and Sharpe 2010). Bacteria may, or may not, be seen in Gram staining of the histological sample. When present, there is no difference in the localisation of bacteria between erysipelas and cellulitis (Bernard et al. 1989). Thus, there is no clear histological definition distinguishing erysipelas from cellulitis, which is also reflected by the frequent imprecise statement, that erysipelas is more superficially or more dermally situated than cellulitis (Bonnetblanc and Bedane 2003, Falagas and Vergidis 2005, Lazzarini et al. 2005, Gunderson 2011). In studies on risk factors, bacteriology and serology of cellulitis only clinical case definitions have been used. The U.S. Food and Drug Administration has proposed the following composite clinical definition of cellulitis and erysipelas for drug development purposes: " A diffuse skin infection characterized by spreading areas of redness, oedema, and/or induration … accompanied by lymph node enlargement or systemic symptoms such as fever greater than or equal to 38 degrees Celsius." (http://www.fda.gov/downloads/Drugs/%E2%80%A6/Guidances/ucm071185.pdf).

Necrotising infections, caused by GAS or other BHS and a variety of other micro-organisms cover a clearly different clinical entity from non-necrotising cellulitis in respect of epidemiology, risk factors, pathogenesis, treatment and prognosis (Humar et al. 2002, Hasham et al. 2005, Anaya and Dellinger 2007).

In clinical studies on cellulitis, various case definitions have been used (Chambers 2013). Common feature in these studies is the acute onset of the disease, and signs of localised inflammation of the skin, and usually fever or chills or general malaise

(Bernard et al. 1989, Eriksson et al. 1996, Dupuy et al. 1999, Roujeau et al. 2004, Björnsdottir et al. 2005, Mokni et al. 2006). In some studies, however, general symptoms have not been a prerequisite (Leppard et al. 1985, Lazzarini et al. 2005, Jeng et al. 2010). Erysipelas and cellulitis have occasionally been clearly distinguished (Leppard et al. 1985, Bernard et al. 1989), but most often only the patients with a clearly demarcated skin lesion have been included (Jorup-Rönström 1986, Dupuy et al. 1999, Björnsdottir et al. 2005, Mokni et al. 2006). In some studies no clear description of the skin lesion is provided (Semel and Goldin 1996). There is also variation in the exclusion criteria concerning abscesses, osteomyelitis and necrotic infections (Jorup-Rönström 1986, McNamara et al. 2007b).

In the present study acute bacterial non-necrotising cellulitis is defined as follows: an acute onset of fever or chills and a localized erythema of the skin in one extremity or in the face. In the case of facial cellulitis fever was not a prerequisite. Cellulitis of other localities (trunk, breast, genitals) were excluded because they are rare (Lazzarini et al. 2005). Abscess, bursitis, septic arthritis, osteomyelitis and necrotising infections were excluded. Also, orbital, periorbital, buccal, and perianal cellulitis are excluded from the present study, because they represent different clinical entities, although partly share the same bacterial aetiology (Swartz 2004). Henceforth, for practical reasons, acute bacterial non-necrotising cellulitis is referred to as cellulitis. However, when referring to other studies, the definition chosen by the authors of the corresponding study is used, when appropriate.

2.1.2 Clinical characteristics of cellulitis

2.1.2.1 Diagnosis and differential diagnosis of cellulitis

The diagnosis of cellulitis is clinical. No exclusive pathological description exists for acute bacterial cellulitis (Swartz 2004). Constitutional symptoms are present in most cases, i.e. fever, chills, general malaise, and not infrequently these precede the appearance of local manifestations of inflammation (Eriksson et al. 1996). Fever may be absent in elderly patients and in diabetic patients, but its absence should raise suspicion of an alternative diagnosis to cellulitis (Chartier and Grosshans 1990,

Bonnetblanc and Bedane 2003). However, current clinical experience implicates that cellulitis of the face is more often afebrile than cellulitis in other locations, even though there is no scientific literature found on this subject. Regional adenopathy may be present, but not in the majority of the patients (Bisno and Stevens 1996, Lipsky et al. 2012a). The typical skin lesion in cellulitis fulfils the cardinal signs of inflammation: tumor, rubor, calor, dolor, i.e swelling, redness, warmth and pain. The fifth classic sign (Rather 1971), functio laesa, disturbance of function, may not be obvious in this context. In a typical case of classic erysipelas the inflamed area of the skin is bright red, clearly demarcated and elevated from the surrounding normal skin, and is indurated giving the skin a typical appearance of orange peel, "peau d' orange" (Figure 1a). Often, however, the lesion has a sharp border, but is not elevated or indurated (Figure 1b). The other end of the continuum of local manifestation of cellulitis is a localised but diffuse reddish swelling of the skin, without any clear margin between inflamed and healthy skin (Hirschmann and Raugi 2012a) (Figure 1c). Bullae containing yellowish fluid are occasionally seen in cellulitis cases (Figure 1d), more frequently in female patients (Hollmen et al. 1980, Krasagakis et al. 2006).

Sometimes only a tingling or itching sensation is the first local symptom of cellulitis. The pain in the site of the skin lesion in cellulitis varies from nearly painless to very intense. (Hook et al. 1986, Bisno and Stevens 1996). However, very severe pain, especially when it seems to be disproportionate to the skin lesion in a severely ill patient, should raise a suspicion of a necrotising infection and needs prompt investigation (Anaya and Dellinger 2007).

At present, cellulitis is most often located in the leg (Hollmen et al. 1980, Ronnen et al. 1985, Jorup-Rönström 1986, Chartier and Grosshans 1990, Bisno and Stevens 1996, Eriksson et al. 1996), which was not the case in the pre-antibiotic era when facial cellulitis was the most common manifestation (Boston and Blackburn 1907, Erdman 1913, Hoyne 1935, Sulamaa 1938). The reason for this shift is unclear, but is thought to be associated with the introduction of penicillin and early antibiotic treatment of streptococcal sore throat. Furthermore, improved hygiene made possible by running water has been proposed (Ronnen et al. 1985, Chartier and Grosshans 1990).

The differential diagnosis of cellulitis comprises a variety of infectious and non-infectious conditions. The most common, and various less common but important

conditions are outlined in Table 1. In addition, there are numerous other conditions causing erythematous lesions on the skin that can be confused with cellulitis, for example lymphoma (Puolakka et al. 2013), "seal finger" (a mycoplasmal infection associated with seal handling) (Hartley and Pitcher 2002), necrobiosis lipoidica (Wake and Fang 2006), diabetic muscle infarction (Kermani and Baddour 2006), carcinoma erysipelatoides (Choi et al. 2011, Chow et al. 2012), and urticarial vasculitis (Suh et al. 2013). Lipodermatosclerosis is a consequence of chronic oedema, which is most often associated with venous insufficiency. In a typical case the leg resembles a bottle or a baseball bat (Walsh and Santa Cruz 2010). Cellulitic inflammation may be difficult to detect in a leg with chronic stasis dermatitis and especially in the most severe cases of chronic oedema, or elephantiasis. An acute form of lipodermatosclerosis has been suggested (Greenberg et al. 1996). However, it is debatable and uncommon (Bruce et al. 2002). Chronic venous insufficiency often leads to a hyperpigmentation due to extravasation of erythrocytes. This may be confused with inflammation as the leg with venous insufficiency is often painful and warm. Furthermore, a sudden exacerbation of chronic oedema may cause redness of the skin and warmth in the affected leg, thus leading to a misdiagnosis of cellulitis: the more so, as patients with chronic venous insufficiency are also prone to have true cellulitis (Westerman 2005). The differential diagnosis of cellulitis has been extensively reviewed recently (Falagas and Vergidis 2005, Gunderson 2011, Hirschmann and Raugi 2012b, Hirschmann and Raugi 2012a, Keller et al. 2012).

Figure 1. Different types of skin lesions in cellulitis. a) Classic erysipelas. The skin lesion is clearly demarcated with slightly elevated borders and a typical “peau d’orange” appearance. b) Cellulitis lesion with sharp borders but with no elevation. Cellulitis in the upper extremity is most often associated with mastectomy and axillary lymph node evacuation. c) Acute diffuse cellulitis with no clear demarcation of the skin lesion in the right leg. Chronic hyperpigmentation in the right leg. d) Bullous cellulitis. Figure 1a kindly provided by a study patient and all figures by permission of the patients.

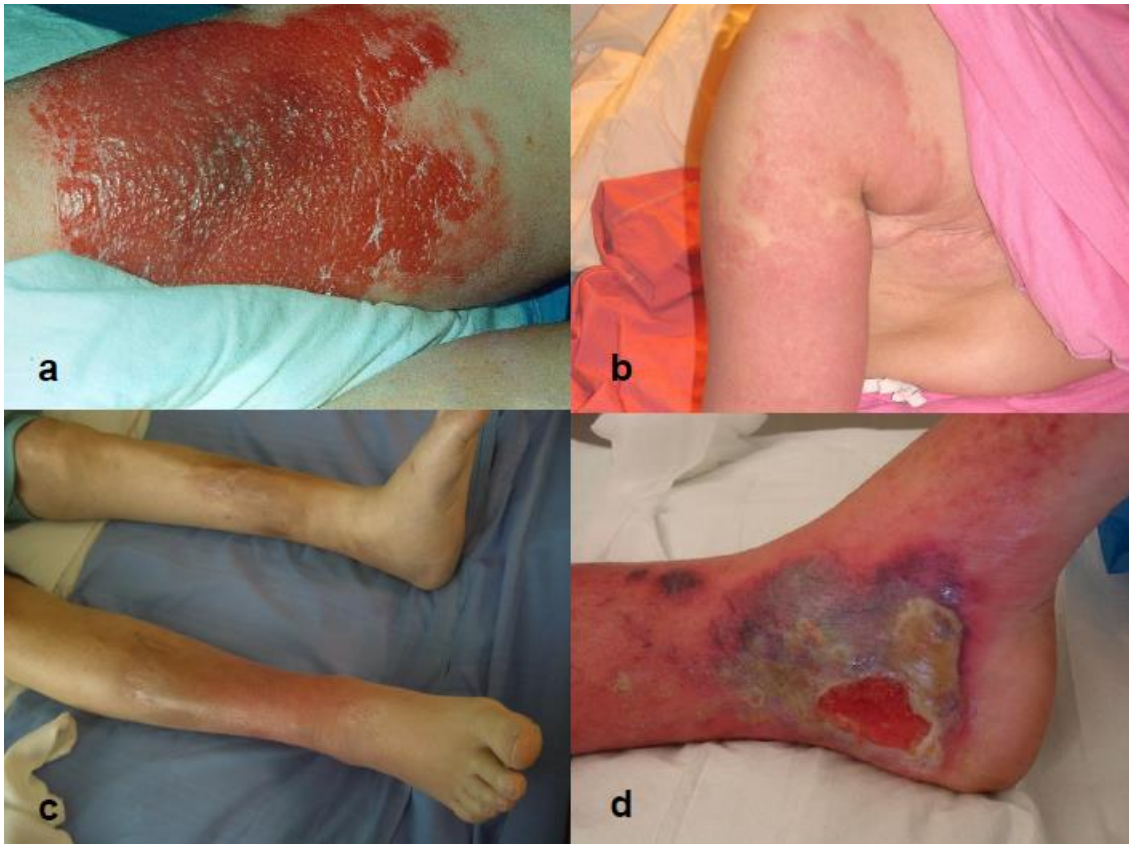


Table 1. Clinical features of conditions that may resemble bacterial cellulitis

Infectious diseases	Clinical features resembling cellulitis	Clinical features not typical of cellulitis
Erythema migrans	Demarcated erythema	Gradual spreading of the lesion in a few days or weeks, not oedematous, only mild fever occasionally (Hytönen et al. 2008)
Necrotising infections	Ecchymosis, blisters and bullae may occasionally accompany cellulitis (Guberman et al. 1999)	Local pain disproportionate to skin lesion, oedema outside the erythema, patient severely ill and deteriorating, , hypotension (Anaya and Dellinger 2007)
Septic arthritis	Fever, erythema, warmth, swelling	Joint effusion, painful movement restriction of the joint (Sharff et al. 2013)
Herpes zoster	Tingling sensation, pain, erythema	Typical clinical picture, when vesicles appear, no fever
Primary Herpes simplex infection	Erythema, local swelling, occasionally fever	Typical vesicles, usual location in genital area, finger, "herpes gladiatorum" (Belongia et al. 1991)
Erysipeloid	Skin erythema with distinct border, bullae	mild/no systemic symptoms, animal contact (Veraldi et al. 2009)
Non-infectious conditions		
Deep venous thrombosis	Diffuse erythema, warmth, swelling	Mild temperature rise, no fever or chills, no local adenopathy (Goodacre 2008)
Stasis dermatitis	Demarcated erythema, warmth, recurrent exacerbations	Chronic condition, often bilateral, no fever (Weingarten 2001)
Dependent rubor	Diffuse erythema of the leg, oedema	No systemic signs, disappears when leg elevated, severe peripheral arterial disease (Uzun and Mutluoglu 2011)
Gout	Diffuse erythema, pain, recurrent attacks	No fever, mild temperature rise possible, clinical picture often typical (Terkeltaub 2003)
Systemic lupus erythematosus (lupus panniculitis)	Demarcated skin lesion, recurrent	History of systemic lupus, no systemic signs of infection (Fabbri et al. 2003)
Charcot arthropathy	Erythema, warmth, swelling of the foot, occasionally pain	No systemic signs, CRP and leukocyte count may be normal (Pakarinen et al. 2003)

Non-infectious conditions (continued)	**Clinical features resembling cellulitis**	**Clinical features not typical of cellulitis**
Erythema nodosum	Raised erythematous lesions, painful, may be recurrent	Often multiple lesions, underlying infection or other cause (Psychos et al. 2000)
Contact dermatitis	Erythema, swelling, vesicles, demarcated lesion	Systemic signs absent, in chronic state eczematous (Saint-Mezard et al. 2004)
Insect bite	Acute onset, erythema, swelling, pain	Pruritus, systemic signs often absent, occasionally anaphylaxis (Reisman 1994)
Auricular relapsing polychondritis	Acute inflammation, redness, warmth, swelling, tenderness, often recurrent	Occurs in cartilaginous part of ears (not in earlobe), usually bilateral, no systemic signs of infection, rare (Mathew et al. 2012)
Erythema fixum	Clearly demarcated erythema, recurrent	Always associated with a drug, no systemic signs (Shiohara and Mizukawa 2007)
Eosinophilic cellulitis (Wells syndrome)	Indurated, annular lesion, or diffuse erythema	Often multiple lesions in different parts of the body, itching, usually no fever, very rare (Wells and Smith 1979)
Neutrophilic cellulitis (Sweet's syndrome)	Fever, systemic signs, erythematous skin lesions	Usually multiple lesions, most often in upper extremities, papular or nodular (Cohen and Kurzrock 2003)
Hereditary Mediterranean fever	Acute onset erythematous lesion, fever, recurrent	Hereditary (Mediterranean descent), sometimes bilateral, abdominal pain (Soriano and Manna 2012)
Erythromelalgia	Redness, swelling and pain in hands or feet, recurrent	Typical clinical picture, heat intolerance, cold reliefs symptoms (Norton et al. 1999)

Infections associated with foot ulceration in diabetic persons, i.e. diabetic foot infections, comprise a clinical entity distinct from cellulitis. Diabetic foot infections are usually considered to be polymicrobial, although *S. aureus* and other gram positive cocci are the most important pathogens in this context (Lipsky et al. 2004, Lipsky et al. 2012b).

2.1.2.2 Recurrent cellulitis

The recurrent nature of erysipelas has been recognised for long (Erdman 1913, Hosford 1938, Sulamaa 1938). Recurrences occur with highly variable intervals, ranging from weeks to years (Jorup-Rönström 1986, Eriksson et al. 1996, Baddour 2001). Recurrences most often occur in the ipsilateral site, but also in contralateral limb or other site (Björnsdottir et al. 2005).

Recurrence of cellulitis is common (Baddour and Bisno 1984, Eriksson et al. 1996, Dupuy et al. 1999, Eriksson 1999, Björnsdottir et al. 2005, Lazzarini et al. 2005), and even in multiple form (Cox 2006, Bartholomeeusen et al. 2007). Cohort studies on the risk of recurrence are outlined in Table 2. Also, the proportions of recurrent cases in case-control studies and some descriptive studies are included, if available. The large difference of the lowest recurrence rate observed (16% in 11 years) (Bartholomeeusen et al. 2007) as compared to other studies may be explained by differences in the database structure and different diagnostic criteria used.

Table 2. Risk of recurrence and proportions of recurrent erysipelas or cellulitis cases in previous studies.

Prospective cohort studies	**Patient characteristics**	**Recurrent cases baseline**[1]	**Follow-up time**	**Recurrence rate**[2]
Jorup-Rönström 1984	≥15 years, hospitalised	n/a	6 months	12% (7/60)
Jorup-Rönström and Britton 1987	≥15 years, hospitalised, prophylactic ab in 9 pts	n/a	3 years[3]	29% (41/143)
Eriksson et al 1996	≥18 years, hospitalised	28% (63/229)	16-40 months	21% (48/229)
Retrospective cohort studies				
Lazzarini 2005	Hospitalised	17% (34/200)	1 year	11% (16/145)
Cox 2006	Hospitalised	n/a	3 years	47% (81/171)
Bartholomeeusen 2007	Hospitalised and outpatients	n/a	11 years	16% (211/1336)
McNamara et al 2007a	≥18 years, hospitalised and outpatients	0[4]	2 years	17% (35/209)
McNamara et al 2007b	≥18 years, hospitalised and outpatients	n/a	2 years	22% (38/176)
Other studies				
Dupuy et al 1999	≥15 years, hospitalised	23% (38/167)	n/a	n/a
Björnsdottir et al 2005	≥18 years, hospitalised	35% (35/100)	n/a	n/a
Halpern et al 2008	≥16 years, hospitalised	37% (56/150)	n/a	n/a
Jeng 2010	≥18 years, hospitalised	19% (34/179) [5]	n/a	n/a
Eells 2011	Hospitalised	22% (11/50)	n/a	n/a

[1]Proportion of patients with a positive history of previous cellulitis at the beginning of the study
[2]Proportion of recurrent cases during the follow-up
[3]data extracted from the earlier report (Jorup-Rönström et al. 1984) on the same patient population.
[4]Patients with a history of previous cellulitis at the ipsilateral site (n=45, 18%) were excluded from the analysis. There were 15 patients with a history of contralateral cellulitis included in the analysis. Thus, there were 24% (60/254) recurrent cases at baseline.
[5]Patient excluded from the study, if a previous episode within 1 year

2.1.2.3 Treatment of cellulitis

Before the antibiotic era various general and local measures and topical agents, such as oil from cyprus seeds, leaves of ivy (*Hedera helix*) (Celsus, trans. 1961), and incisions of the inflamed tissue were used for the treatment of cellulitis (Lawrence 1828, Hosford 1938). Also, many different symptomatic remedies such as systemic iron, quinine (Erdman 1913), lead, iodine, zinc, magnesium sulphate (Hosford 1938, Sulamaa 1938) have been used. Bed rest, immobilisation and warming (Hosford 1938) or cooling (Erdman 1913, Sulamaa 1938) the affected extremity have been considered essential. After the discovery of the bacterial origin of erysipelas various antisera products ("antistreptococcus serum", "erysipelas antitoxin", "human convalescent erysipelas serum") and streptococcal vaccine preparations, "streptococcal antivirus cream" (Hoyne 1935) and “Phylacogen" were tried. In general, however, the value of the many different remedies and treatments was considered low, bar relieving of symptoms, and before the antibiotic era erysipelas was perceived as a mild disease with a low mortality as compared to other infectious diseases (Erdman 1913, Hoyne 1935, Hosford 1938, Sulamaa 1938).

Sulphonamides were introduced for the treatment of bacterial infections in the 1930s. Three controlled studies on a synthetic dye Prontosil (which is in vivo metabolised to sulphanilamide) and sulphanilamide were conducted in 1936-7 (Snodgrass and Anderson 1937a, Snodgrass and Anderson 1937b, Snodgrass et al. 1938). These studies suggested the superiority of sulphanilamides over ultraviolet light treatment. Penicillin came to widespread use in the late 1940s and has since been the mainstay of treatment of streptococcal cellulitis (Bisno and Stevens 1996, Bishara et al. 2001, Bonnetblanc and Bedane 2003, Stevens et al. 2005).

Today, the appropriate treatment consists of antibiotics, usually targeted to gram positive cocci (Stevens et al. 2005, Morris 2008). A combination therapy with penicillin and antistaphylococcal penicillin has been a common practice in the United Kingdom, aiming at an assumed maximal efficacy against both streptococci and staphylococci (Cox 2002, Leman and Mukherjee 2005, Quirke et al. 2013).

Local treatment aiming at reducing oedema, and healing possible skin breaks, e.g. toe-web maceration and tinea pedis has also been strongly advocated (Dupuy et al. 1999, Roujeau et al. 2004, Stevens et al. 2005, Lewis et al. 2006, Mokni et al. 2006, Morris 2008). These measures are primarily based on clinical experience. Relieving swelling in acute cellulitis is thought to promote healing of the local inflammation. As skin breaks have been associated with acute cellulitis in case-control studies (see below), maintaining skin integrity has been considered to lower the risk of cellulitis recurrence. However, no studies have been published on the effectiveness of such measures. In case of an abscess draining is essential.

The few randomised controlled studies on antibiotic treatment of non-suppurative cellulitis in the penicillin era are outlined in Table 3. Of other studies, a prospective, non-controlled observational study on diffuse non-culturable cellulitis including 121 patients reported (Jeng et al. 2010) a 95% overall efficacy of ß-lactam antibiotics. The authors concluded that treatment with ß-lactams is effective despite of high prevalence of methicillin resistant *S. aureus*, and the efficacy is based on the streptococcal cause of diffuse non-culturable cellulitis in most cases. The same conclusion is drawn from a large multicenter retrospective cohort study conducted in the United States (Madaras-Kelly et al. 2008). In that study the failure rate of oral ß-lactam and non-ß-lactam antibiotic therapy was assessed in outpatients treated for cellulitis. Patients with purulent infections or chronic ulcers were excluded. There was no statistically significant difference in the efficacy of ß-lactams as compared to other antibiotics. However, adverse reactions were more common in patients treated with other antibiotics (2.2%) than those treated with ß-lactams (0.5%, p=0.04). Also, according to a recent randomised trial (Pallin et al. 2013) (Table 3), there is no need to cover methicillin resistant *S. aureus* (MRSA) in non-purulent cellulitis cases treated as outpatients, even if MRSA is highly prevalent.

Table 3. Controlled trials on antibiotic treatment of non-suppurative cellulitis.

Study	Design	Intervention	N:o of patients	Result
Bernard et al. 1992	Randomised, open, multicenter	Roxithromycin po. vs. penicillin iv.	69 initially hospitalised	Cure without additional antibiotics: roxithromycin 26/31 (84%) vs. penicillin 29/38 (76%) (P = 0.43)
Bernard et al. 2002	Randomised, non-inferiority, open, multicenter	Pristinamycin po. vs. penicillin iv. then po.	289 hospitalised, adult	ITT: cure at follow-up pristinamycin 90/138 (65%) vs. penicillin 79/150 (53%); one sided 97.06% CI for difference (1.7%-∞)
Grayson et al. 2002	Randomized, double-blind equivalence trial	Cefazolin iv. + probenecid vs. ceftriaxone iv. + placebo	134 moderate to severe cellulitis pts, adults	Clinical cure at 1 mo: cefazolin-probenecid 46/56 (82%) vs. ceftriaxone-placebo 50/57 (85%), p=0.55
Zeglaoui et al. 2004	Randomised, open, single centre	Penicillin im. vs. penicillin iv.	112 hospitalised adult pts,	Failure rate penicillin iv. 20% vs. penicillin im. 14% , p=0.40
Hepburn et al 2004	Randomized, placebo-controlled, double-blind, single centre	Levofloxacin 10 d vs. levofloxacin 5 d, then placebo 5 d	87 adult pts	Cure at 28 d: levofloxacin 10 d 42/43 (98%) vs. levofloxacin 5 d 43/44 (98%)
Pallin et al. 2013	Randomized, placebo-controlled, double-blind, multicenter	Cephalexin + TMP-SMX vs. cephalexin + placebo	153 outpatients (age ≥12 mo)	Cure: TMP-SMX 62/73 (85%) vs. controls 60/73 (82%),

ITT, Intention to treat; TMP-SMX, Trimethoprim-sulphamethoxazole

An adjunctive pharmacological therapy in addition to antibiotic treatment has been investigated in two studies. In Sweden, a randomised double-blind placebo-controlled study was conducted on prednisolone therapy added to standard therapy with antibiotics. The study included 112 hospitalised erysipelas patients. The median time to healing and the length of stay in hospital were shorter in the prednisolone group as compared to the placebo group (both 5 days vs. 6 days, respectively, $p<0.01$). In a one year follow-up there was no statistically significant difference in the rate of recurrence between the groups (6/52 and 13/51 in the prednisolone and placebo groups, respectively) (Bergkvist and Sjöbeck 1998).

The role of an anti-inflammatory non-steroidal drug (NSAID) was assessed in a single blind study including 64 patients with upper or lower limb cellulitis (Dall et al. 2005). All patients received the standard antibiotic therapy with initial ceftriaxone followed by oral cephalexin, and 31 patients received ibuprofen 400 mg every 6 hours. The regression of inflammation began in two days in 24 (83%) of 29 patients receiving ibuprofen as compared with 3 (9%) of 33 with standard therapy ($p<0.05$). Also, the time required for complete healing was statistically significantly shorter in the ibuprofen group. No cutaneous adverse events occurred. In reference to the previous concerns of the possibly increased risk for necrotising complications associated with NSAID therapy in cellulitis (Chosidow et al. 1991), the authors suggested a larger study on the efficacy and safety of NSAIDs in cellulitis. However, the association of NSAID use and necrotising infections observed in case reports may also reflect an initial attenuation of the symptoms leading to a delayed diagnosis of necrotising infection rather than actual causal relationship (Aronoff and Bloch 2003).

2.1.2.4 Prevention of recurrent cellulitis

It is a common clinical practice to advise patients with acute cellulitis to take care of the skin integrity, or use compression stockings whenever there is obvious chronic oedema. However, there are no studies on the effectiveness of these non-pharmacological measures in preventing recurrent cellulitis.

Antibiotic prophylaxis has been used since the first reports of the efficacy of penicillin in this use (Duvanel et al. 1985). The optimal indications and drug choice

for, and duration of prophylaxis are yet to be elucidated. The studies on antibiotic prophylaxis for recurrent cellulitis are outlined in Table 4. In the largest, and most recent, study (Thomas et al. 2013) oral penicillin was shown to be effective in preventing recurrent leg cellulitis after at least one recurrence episode. However, after the end of the prophylaxis at one year the risk of recurrence began to rise. Also, it is of note that patients with more than two episodes of cellulitis, those with high BMI, and those with a chronic oedema were more likely to have a recurrence despite ongoing prophylaxis, as compared to other patients (Thomas et al. 2013). Further studies are needed to evaluate the safety and effectiveness of longer periods of prophylactic antibiotic treatment, proper treatment allocation and optimal time to institute prophylaxis.

Table 4. Studies on antibiotic prophylaxis for recurrent cellulitis.

Study		**Setting**	**No. of patients**	**Case definition**	**Exclusion criteria**	**Recurrences (intervention vs. controls)**
Kremer et al 1991	Erythromycin 250 mg x 2 for 18 mo vs. no prophylaxis	Randomised, controlled, open study, Israel	32	≥2 episodes of erysipelas or cellulitis in an extremity during the previous year	Signs of active infection	0/16 (0%) vs. 8/16 (50%) (p<0.001)
Sjöblom et al 1993	Phenoxymethylpenicillin ca. 1,5-3 MU x 2 vs. no treatment	Randomised, controlled, open study, Sweden	40	≥2 episodes of erysipelas during the previous 3 years plus lymphatic congestion/venous insufficiency	Age < 18 yr, HIV infection	2/20 (10%) vs. 8/20 (40%) (p=0.06) (mean follow-up 14 mo)
Chakroun et al 1994[1]	Benzathine penicillin 1,2 MU x 2/mo i.m. vs. no treatment	Randomised, controlled, open study, France	58	Lower extremity erysipelas,		0/18 (0%) vs. 9/26 (35%) (p=0.006)[2] in 1 year
Wang et al 1997	Benzathine penicillin 1,2 MU/mo i.m. vs. no treatment	Controlled, non-randomised, open study, Taiwan	115	Leg cellulitis, presumed streptococcal	Other bacteria cultured, no response to penicillin	4/31 (13%) vs. 16/84 (19%) in 11,6 mo (NS)[3]
Vignes and Dupuy 2006	Benzathine penicillin 2,4 MU/14 days i.m.	Retrospective observational, non-controlled, France	48	Upper extremity lymphoedema, ≥4 episodes of upper extremity erysipelas		Recurrence rate 36% in 2 years
Thomas et al 2013	Phenoxymethylpenicillin ca. 0,4 MU x 2 vs. placebo	Double-blind, randomised, placebo controlled study, multicentre, UK	274	≥2 episodes of leg cellulitis during the previous 3 years,	Age <16 years, dg uncertain, prophylaxis in the previous 6 mo, previous leg ulcer, operation, trauma,	30/136 (22%) vs. 51/138 (37%) in 12 mo (p=0.01)

[1]Article in French
[2]Fisher's test, not reported in the original article
[3]NS = non-significant

2.1.3 Epidemiology of cellulitis

2.1.3.1 Historical overview on the epidemiology of cellulitis

Hippocrates (ca. 460-375 BCE) wrote: *"Early in the spring, at the same time as the cold snaps which occurred, were many malignant cases of erysipelas, some from a known exciting cause and some not. Many died, and many suffered pain in the throat"* (Hippocrates, trans. 1923). It is likely that erysipelas covered also necrotising infections, as Hippocrates continues: *"Flesh, sinews and bones fell away in large quantities. The flux which formed was not like pus but was a different sort of putrefaction with a copious and varied flux."* (Hippocrates, trans. 1923, Descamps et al. 1994).

The most comprehensive historical case series of erysipelas has been published based on Norway's official statistics (Madsen 1973). It describes the notification rate, mortality due to, and case fatality rates of scarlet fever and erysipelas between the years 1880 and 1970. The notification rate of erysipelas, which presumably is lower than its true incidence, was very evenly close to 10 cases per 10 000 inhabitants per year during the reported hundred year period. The only exception were the years 1942-43 when, concomitantly with a scarlet fever epidemic, the rate rose to 24-29/10 000. After the war a steady decline in the rate was recorded until it was 8/10 000 in 1967. In England and Wales, statistics of the incidence of erysipelas are available from 1912 to 1930, when erysipelas was a compulsorily notifiable disease and nationwide records were published by the Registrar-General (Russell 1933). The incidence of erysipelas in England and Wales varied between 321 and 728 per one million inhabitants. The notification rates in Norway, and in England and Wales are well in line with the two more recent investigations, which report the incidence of erysipelas and lower extremity cellulitis to be in the order of 20/10 000/year (Bartholomeeusen et al. 2007, McNamara et al. 2007b). The incidence seems to have been somewhat lower in England and Wales, but may reflect the differences in the notification systems between countries. Also, the recent figures from Belgium and the United States (Bartholomeeusen et al. 2007, McNamara et al. 2007b) are based on systematically collected databases.

In Norway, the case fatality rate in erysipelas was also constantly 26-40/1000 from 1880 until the introduction of sulphonamides in 1937 when the case fatality rate more than halved to around 10/1000. The beginning of the penicillin era nearly eliminated the risk of death due to erysipelas, being less than 1/1000 since 1953. Also, the mortality rate due to erysipelas was less than one per million between the years 1955-1970 (Madsen 1973).

From the pre-antibiotic era, two large patient series from the United States in the early 20th century (Erdman 1913, Hoyne 1935) and one from Finland (Sulamaa 1938) are available, comprising 800, 1193, and 474 cases, respectively. The overall case fatality rate varied between 11.2-16.2% in the reports from the United States, with markedly higher rate observed among infants and elderly. In Hoyne's series the case fatality rate in patients < 1 year of age was 39%, and 15% in the age group 46-55 years, rising to 43% in patients over 75 years of age (Hoyne 1935). In the Finnish series the case fatality rate was 7.4%. overall, and 15% in both age groups <1 year and >70 years (Sulamaa 1938). In all three series 60-85% of the cases were facial, and the case fatality rate was markedly lower in the facial cases than in the other cases. For example, Erdman reports a case fatality rate of 5% in the facial cases, and 27% in cases with leg erysipelas (Erdman 1913). In Sulamaa's series the corresponding figures were 5.4% and 15.0%, respectively (Sulamaa 1938). Sulamaa states that suppurative complications are more common in the extremities than in the face, and gangrenes are encountered frequently in cases involving the genital organs (Sulamaa 1938). Thus, one is tempted to believe that suppurative and necrotising infections, included in non-facial cases of erysipelas, may explain the difference.

A seasonal variation in the incidence of cellulitis has been observed in the early studies. Hippocrates stated (Hippocrates, trans. 1923) that many cases occurred early in the spring when it was cold. Likewise, early studies from Hampshire, England (Smart 1880), Philadelphia, USA (Boston and Blackburn 1907), New York (Erdman 1913), Chicago (Hoyne 1935) have noted the greatest number of erysipelas cases occurring in the early spring and the lowest in the late summer. A careful analysis of the statistics on the notified cases of erysipelas and scarlet fever in England and Wales in 1910-30 shows a very clear seasonal pattern in the rate of notifications, with the highest number of erysipelas cases in January and the lowest in September. However, a shift to the later spring in the peak incidence was observed in the period of 1926-30 (Russell 1933), and

there are different statements of that topic in the early literature, too (Riddell 1935). In the early Finnish study, the number of hospitalisations due to erysipelas was higher during the winter months than in the summer, but no statistical analysis was conducted (Sulamaa 1938).

2.1.3.2 Incidence of cellulitis

The epidemiology of cellulitis during the antibiotic era has been investigated in several studies. Three recent retrospective studies on the incidence of erysipelas or cellulitis have quite similar results (Goettsch et al. 2006, Bartholomeeusen et al. 2007, McNamara et al. 2007b). A study in Belgium using a computerised database of primary care practises comprising the years from 1994 to 2004 and found a rising age-standardised incidence of erysipelas from 1.88 to 2.49 per 1000 patient years in 1994 and 2004, respectively. Also, the incidence was highest in the oldest age group, being 6.8/1000 patient-years in patients aged 75 or older in 2004 (Bartholomeeusen et al. 2007).

A study in the Netherlands using a national database, including all Dutch citizens, found an incidence of 179.6 per 100 000 inhabitants per year for lower extremity cellulitis or erysipelas (Goettsch et al. 2006). Only 7% of the cases were hospitalised.

In a population based study in the United States covering the year 1999, the incidence of leg cellulitis was 199 per 100 000 person-years (McNamara et al. 2007b). Also, as in the Belgian study, the incidence increased with increasing age. The figures in these three studies were well in the same order of magnitude, despite the different case definitions used and the different base populations. In all three studies, the incidence of cellulitis increased significantly with age. Also, consistently in these studies, there was no difference between sexes in the incidence of cellulitis (Goettsch et al. 2006, Bartholomeeusen et al. 2007, McNamara et al. 2007b).

In addition to the three studies cited above, the incidence of cellulitis was investigated in a retrospective study in the United States (Ellis Simonsen et al. 2006). Incidence of cellulitis was 24.6 per 1000 person-years, which is over ten times more than that in the other studies. The most plausible explanation for the discrepancy is that the study probably includes cases with abscesses, wound infections, and diabetic foot infections, which were excluded from the three studies cited above. This reflects the

confusing terminology in the medical literature concerning cellulitis and erysipelas (Bartholomeeusen et al. 2007, McNamara et al. 2007b, Chambers 2013). Observations on seasonality in the more recent studies have not been uniform. In some studies the greatest number of cases have been recorded in the summer (Ronnen et al. 1985, Ellis Simonsen et al. 2006, Bartholomeeusen et al. 2007, Haydock et al. 2007, McNamara et al. 2007b) but also in the winter (Eriksson et al. 1996) In another study no seasonality was observed (Jorup-Rönström 1986). In a recent study in Israel, the greatest numbers of leg erysipelas patients were admitted to hospital in the summer, whereas facial erysipelas was more common during the winter (Pavlotsky et al. 2004). Various possible explanations for the observed seasonality in the incidence of cellulitis have been presented in the studies cited above (skin abrasions in different activities, maceration caused by sweating, worsening of oedema in hot weather), but only speculations can be made. However, it seems likely that not the climate per se causes the variation but human behaviour influenced by the changes in the outdoor air temperature.

In conclusion, based on three register studies in three western countries the incidence of erysipelas and cellulitis is in the order of 200 per 100 000 persons per year, and is even in both sexes. The highest incidence is observed in the oldest age groups. The majority of cellulitis cases are treated as outpatients. Case fatality rate in cellulitis in the antibiotic era is very low.

2.1.3.3 Clinical risk factors for cellulitis

Celsus (ca. 30 BCE – 50) wrote: *"Nam modo super inflammationem rubor ulcus ambit, isque cum dolore procedit (erysipelas Graeci nominant)... Id autem, quod erysipelas vocari dixi non solum vulneri supervenire sed sine hoc quoque oriri consuevit, atque interdum periculum maius adfert, utique si circa cervices aut caput constitit."*

"For sometimes a redness, over and above the inflammation, surrounds the wound, and this spreads with pain (the Greeks term it erysipelas)… But what I have said is called erysipelas, not only follows upon a wound, but is wont also to arise without a wound, and sometimes brings with it some danger, especially when it sets in about the neck or head." (Celsus, trans. 1961)**.**

As indicated above, and also in the citation from Hippocrates in the previous chapter, the observation that skin inflammation often begins from a wound or skin abrasions can be found in the ancient medical writings. Skin breaks for various reasons have been considered a risk factor for cellulitis ever since (Hosford 1938) and have been shown to be associated with cellulitis in controlled studies (Semel and Goldin 1996, Dupuy et al. 1999, Roujeau et al. 2004, Björnsdottir et al. 2005, Mokni et al. 2006, Bartholomeeusen et al. 2007, Halpern et al. 2008). Especially maceration and fungal infection of toe webs, referred to as "athletes foot" by some (Semel and Goldin 1996), has been considered the most important risk factor for cellulitis due to its strong association with cellulitis and also due to its frequency in the population (Dupuy et al. 1999, Roujeau et al. 2004, Mokni et al. 2006, Halpern et al. 2008).

Chronic oedema as a predisposing factor, and as well as a consequence of cellulitis, has also been recognised for long (Sulamaa 1938), and it has also appeared as an independent risk factor for cellulitis in the recent case-control studies (Dupuy et al. 1999, Roujeau et al. 2004, Mokni et al. 2006, Halpern et al. 2008). It has been a common conception, that an attack of cellulitis may irreversibly damage the lymphatic vessels, predisposing the patient to chronic oedema and subsequent recurrences of cellulitis. The evidence of postcellulitic chronic leg oedema is based on clinical observations, and is supported by the recognition of cases with asymmetrical leg oedema without any other explanation for the asymmetry than previous cellulitis (Cox 2006). However, in two lymphoscintigraphic studies on patients with a recent cellulitis attack, an abnormal lymphatic function was revealed not only in the affected leg but also on the contralateral leg with no previous cellulitis (Damstra et al. 2008, Soo et al. 2008). This suggests that pre-existing lymphatic impairment may be a significant predisposing factor for cellulitis.

Of general risk factors diabetes has been suspected (Dupuy et al. 1999, Björnsdottir et al. 2005, Mokni et al. 2006, Halpern et al. 2008, Halpern 2012), but in only one case-control study (Eells et al. 2011) confirmed as a risk factor for cellulitis (OR 3.5 [95% CI 1.4 – 8.9]). In that study fungal infections or toe web maceration were not addressed. Thus, it has been discussed (Halpern 2012) that the possible increased risk for cellulitis among diabetic persons is due to a greater susceptibility to fungal infections of the skin among diabetic than non-diabetic persons. However, in a large prospective cohort study (Muller et al. 2005), diabetes was shown to predispose to

common infections. Adjusted OR for bacterial skin and mucous membrane infections in type II diabetic patients was 1.3 as compared to controls (hypertensive patients without diabetes). Furthermore, incidence of cellulitis was 0.7% among diabetic patients as compared to 0.3% among controls.

Obesity has been shown to be independently associated with acute cellulitis in three previous studies (Dupuy et al. 1999, Roujeau et al. 2004, Bartholomeeusen et al. 2007). The mechanisms behind the susceptibility to cellulitis, and also to other infections, has not been fully elucidated (Falagas and Kompoti 2006). Mechanisms related to impaired balance in lymphatic flow, i.e. overproduction or slow drainage of lymph may be involved (Vasileiou et al. 2011, Greene et al. 2012). Adipose tissue produces a variety of mediators associated with inflammatory reactions. These include leptin, adiponectin, IL-6, and several other factors, which participate in the regulation of inflammatory reactions (Fantuzzi 2005). Obesity is associated with many alterations in skin functions, such as sebum production, sweating, and also in microcirculation, which may impair the barrier function of the skin (Yosipovitch et al. 2007). Obesity also predisposes to other known risk factors for cellulitis, such as diabetes and intertrigo. However, as obesity is associated with cellulitis independently of these diabetes-associated factors, other mechanisms are likely to be involved in this association (Huttunen and Syrjänen 2013). Controlled studies on the risk factors for cellulitis are outlined in Table 5.

2.1.3.4 Clinical risk factors for recurrent cellulitis

It appears logical that the factors predisposing to cellulitis would predispose the patient to its recurrences, too, if constantly present. However, it is also widely believed, that an attack of cellulitis makes one even more prone to subsequent recurrence, thus making up a vicious circle (Cox 2006). The risk factors for recurrent cellulitis in the published studies are outlined in Table 6.

Lewis et al (Lewis et al. 2006) conducted a case-control study based on chart reviews in one hospital in the United States. They found that leg oedema, body mass index (BMI), smoking, and homelessness were independently associated with recurrent cellulitis. Deep venous thrombosis and especially tinea pedis were strongly associated with recurrent cellulitis in the univariate analysis, but with wide confidence intervals.

Thus, they were not included in the final multivariable model because of the possible bias in these variables due to the data collecting method. Diabetes was not statistically significantly associated with recurrent cellulitis (OR 1.54, 95% CI 0.70-3.39).

The risk factors for recurrent cellulitis were the same as for acute cellulitis in the study by Dupuy et al (Dupuy et al. 1999) except that the patients admitted for recurrence were older than those with a primary episode (60.3 vs. 56.5 years, respectively) and had leg surgery done more often (OR 2.2). Björnsdottir et al (Björnsdottir et al. 2005) reported a similar finding: 15 (43%) of 35 patients with previous history of cellulitis had leg surgery as compared to 10 (15%) of 65 patients with no history of previous cellulitis.

Consistent with the finding of leg surgery as a risk factor for recurrent cellulitis, reports have been published of patients with a history of saphenous venectomy for coronary artery bypass operation and recurrent bouts of cellulitis (Baddour and Bisno 1984, Hurwitz and Tisserand 1985, Baddour et al. 1997). Gram positive cocci in chains have been demonstrated in one of such patients in a histological specimen (Hurwitz and Tisserand 1985). Tinea pedis was present in almost all of the published cases (Baddour and Bisno 1984, Hurwitz and Tisserand 1985).

In a retrospective study on hospitalised cellulitis patients (Cox 2006) persistent leg oedema was reported by 49 (60%) of the 81 patients presenting with recurrent cellulitis, as compared to 29 (32%) of the 89 patients with primary episode ($p<0.0002$). Of all cases, 37% reported persistent oedema as a consequence of a cellulitis attack. Thus, it was suggested, that oedema is both a strong risk factor for, and also a consequence of cellulitis, creating a vicious circle. It is of note, that only 15% of the patients reported toe web maceration. As toe web intertrigo was considerably more frequent among cellulitis patients in the controlled studies (66-77%), and in the control populations as well (23-48%) (Dupuy et al. 1999, Björnsdottir et al. 2005), it may be underestimated by the patients themselves.

Two different predictive models of the risk of recurrence of cellulitis after primary episode have been proposed. The first (McNamara et al. 2007a) is based on three risk factors identified in a retrospective population based cohort study (see Table 6), namely tibial area involvement, history of cancer, and ipsilateral dermatitis, each with a hazard ratio of 3 to 5. It was estimated, that if a person has all three risk factors, the probability of recurrence is 84% in one year and 93% in two years. With two risk

factors the figures were 39% and 51%, and with only one risk factor 12% and 17%, respectively. However, the study included only 35 patients with recurrences. Thus, chronic oedema and onychomycosis were statistically significant risk factors in a univariate but not in a multivariable analysis, probably due to a lack of statistical power. In a recent study (Tay et al. 2015) 102 of 225 (45%) inpatients with first cellulitis episode had a recurrence in one year (Table 6). A predictive model was constructed based on the observed risk factors with score points as follows: chronic venous insufficiency (1), deep vein thrombosis (1), lymphoedema (2) and peripheral vascular disease (3). A score of ≥2 had a positive predictive value of 84% for recurrent cellulitis in one year. A score of <2 had a negative predictive value of 68%. Furthermore, a score of ≥3 was associated with a 90% risk of recurrence in one year. The findings of these studies are consistent with the previous Swedish study (Jorup-Rönström and Britton 1987), which showed that 76% of patients with recurrences had at least one supposed risk factor, as compared to 27% of those with no recurrences.

In conclusion, factors predisposing to the primary cellulitis episode obviously predispose to recurrences as well. The effect of the risk factors on the risk of recurrence may be additive. A prior leg surgery seems to be associated especially with recurrences. Of the preventable risk factors, toe web intertrigo may be the most easily treated, but it is probably not recognised by the patients.

Table 5. Controlled studies assessing risk factors for cellulitis. Risk factors given in the order of odds ratios reported, from highest to lowest.

Controlled studies	**Study design, setting**	**Patients/controls**	**Case definition**	**Exclusion criteria**	**Risk factors**
Dupuy et al. 1999	Case-control, prospective, multicentre, France	167 hospitalised patients, acute cellulitis, 294 hospitalised controls	Sudden onset of a well demarcated cutaneous inflammation, with fever	Age < 15 yr, abscess, necrotising infection	Lymphoedema, skin brakes, venous insufficiency, leg oedema, overweight
Roujeau et al. 2004	Case-control, prospective, multicentre, Austria, France, Germany, Iceland	243 hospitalised or outpatients, acute cellulitis, 467 hospitalised controls	Well-demarcated lesion with erythema, warmth and swelling, and fever >38°C or chills	Bilateral cellulitis, abscess, necrotising infection, recent use of antifungals	PH, skin brakes, leg oedema, interdigital tinea, overweight
Björnsdottir et al. 2005	Case-control, prospective, single centre, Iceland	100 hospitalised patients, acute cellulitis, 200 hospitalised controls	Demarcated inflammation, sudden onset with fever, chills or leukocytosis	Age < 18 yrs, abscess, necrotising infection, recent use of antifungals, recent hospitalisation	PH, presence of *S. aureus* or BHS in toe webs, leg erosions or ulcers, prior saphenectomy
Mokni et al. 2006	Case-control, prospective, multicentre, Tunisia	114 hospitalised patients 208 hospitalised controls	Sudden onset, demarcated inflammation, fever >38°C or chills	Age < 15 yr, abscess, necrotising infection, PH	Lymphoedema, skin brakes, leg oedema
Bartholomeeusen et al. 2007	Retrospective cohort, general practice database, Belgium	1336 erysipelas patients in a cohort of 160 000 primary care patients	Diagnosis of erysipelas made in primary care (no formal definition)	None	Chronic ulcer, obesity, thrombophlebitis, heart failure, DM2, dermatophytosis, varicose veins (univariate analysis only)
Halpern et al. 2008	Case-control, prospective, single centre, UK	150 hospitalised patients 300 hospitalised controls	Acute pyogenic inflammation of dermis and subcutis, tender, warm, erythematous, swollen leg, no sharp demarcation	Age <16 yrs, abscesses, necrotising infection	PH, ulceration, eczema, oedema, leg injury, DVT, leg surgery, toe-web disease, dry skin, white ethnicity
Eells et al. 2011	Case-control, prospective, single centre, USA	50 hospitalised patients 100 hospitalised controls	Non-suppurative cellulitis, confirmed by a dermatologist	Abscesses, furuncles, carbuncles, osteomyelitis, necrotising infection	Homelessness, diabetes

PH, Positive history of cellulitis; DM2, Diabetes mellitus, adult type; DVT, Deep venous thrombosis

Table 6. Studies assessing risk factors for recurrent cellulitis. Where appropriate, risk factors given in the order of odds ratios (OR) reported, from highest to lowest. OR for BMI is not comparable with categorical variables.

Reference	Study setting	Patients/controls	Case definition	Exclusion criteria	Risk factors associated with recurrent cellulitis
Tay et al. 2015	Retrospective cohort study, inpatients, Singapore	225 patients with first cellulitis, follow-up 1 year	Lower extremity cellulitis, age ≥18 yr, dg by dermatologist	Necrotising infection, bursitis, arthritis, carbuncles, furuncles	Peripheral vascular disease, lymphoedema, DVT, venous insufficiency
Bartholomeeusen et al. 2007	Retrospective cohort study, general practice database, Belgium	211 patients in a cohort of 1336, primary care	Diagnosis made in primary care of ≥2 erysipelas episodes during the study (no formal definition)	None	Obesity, chronic ulcer, dermatophytosis, thrombophlebitis (univariate analysis only)
McNamara et al. 2007a	Retrospective, population based cohort study, USA	209 patients in a population based database	Primary episode of acute lower extremity cellulitis, expanding area of warm, erythematous skin with local oedema (chart review)	PH, any purulent infection, osteitis, bursitis, necrotising infections, non-infectious conditions	Tibial area location, history of cancer, ipsilateral dermatitis
Lewis et al. 2006	Case-control, chart review, single centre, USA	47 hospitalised patients 94 hospitalised controls	Diagnosis of lower extremity cellulitis with at least 1 previous episode	Leg ulcer, purulent ulcer, necrotising infection, immediate ICU admission	Leg oedema, homelessness, smoking, BMI
Björnsdottir et al. 2005	Case-control, Iceland	35 PH patients, 65 NH patients	See Table 5.	See Table 5.	Prior leg surgery more common in PH than NH cases
Pavlotsky et al. 2004	Retrospective observation, single centre, Israel	569 patients, NH 304 (53%), PH 265 (47%)	Hospitalised, fever, pain, erythema with swelling, induration, sharp demarcation		Obesity, smoking in the past, tinea pedis, venous insufficiency, lymphoedema, acute trauma

Continued

Reference	Study setting	Patients/controls	Case definition	Exclusion criteria	Risk factors associated with recurrent cellulitis
Dupuy 1999	Case-control, France	See Table 5.	See Table 5.	See Table 5.	PH cases older and had more often leg surgery than NH cases
Eriksson et al. 1996	Prospective cohort study, single centre, Sweden	229 patients, follow- up until 1992	Hospitalised, acute onset, fever ≥38, well demarcated, warm erythema	Age<18 yr, HIV infection, wound infection	No statistically significant difference in underlying diseases between recurrent and non-recurrent cases
Jorup-Rönström and Britton 1987	Prospective cohort study, single centre, Sweden	143 patients, 2-4 years follow-up	In- and outpatients, fever, sudden onset, red plaque, distinct border		Venous insufficiency, any vs. no predisposing conditions (arterial or venous insufficiency, paresis, lymphatic congestion, DM, alcoholism, immunosuppression)[1]

[1] Odds ratios not reported

BMI, body mass index; DM, diabetes mellitus; DVT, deep vein thrombosis; ICU, intensive care unit; PH, positive history of cellulitis; NH, negative history of cellulitis

2.1.4 Aetiology and pathogenesis of, and genetic susceptibility to cellulitis

2.1.4.1 Bacteriology of cellulitis

Fehleisen conducted therapeutic experiments aiming at a cure of cancer by inoculation of streptococci in patients' skin. He was able to demonstrate that erysipelas can be brought on by inoculating a pure culture of streptococci, originally cultivated from an erysipelatous lesion, into the skin (Fehleisen 1883). Erysipelas in its classic form is usually considered to be exclusively caused by BHS and especially by GAS (Bernard et al. 1989, Bisno and Stevens 1996, Bonnetblanc and Bedane 2003, Stevens et al. 2005). Bacterial cultures, however, are frequently negative even with invasive sampling techniques (Hook et al. 1986, Newell and Norden 1988, Duvanel et al. 1989, Eriksson et al. 1996).

Streptococci were shown to be present by direct immunofluorescence in 11 of 15 cases of diffuse cellulitis, and in 26 of 27 patients with classic erysipelas (Bernard et al. 1989). BHS are also found in swab samples obtained from toe webs in patients with cellulitis more often than from healthy controls. In a recent case-control study (Björnsdottir et al. 2005) 37 of the 100 cellulitis patients harboured BHS (28 of which were GGS) in their toe webs as compared to four (2%) of the 200 control patients. BHS and/or *S. aureus* were especially common (58%) in patients with toe web intertrigo. (Björnsdottir et al. 2005). Furthermore, in an earlier study (Semel and Goldin 1996) BHS were isolated from toe webs in 17 (85%) of 20 cellulitis cases with athletes foot. GGS was found in 9 cases, GAS and GBS in four and three cases, respectively and GCS in one case, No BHS could be isolated from control patients with athletes foot but without cellulitis ($p<0.01$).

In a cohort study in Sweden including 229 erysipelas patients (Eriksson et al. 1996) GAS was isolated from wounds or ulcers in 42 (35%) of 119 patients. GGS and GCS were isolated in 19 (16%) and 2 of the 119 cases, respectively, and *S. aureus* in 61 (51%) cases. In an earlier Swedish study (Jorup-Rönström 1986) bacterial cultures

were performed from infected ulcers in erysipelas patients. BHS were isolated in 57 (47%) of 122 cases.

Other ß-haemolytic streptococci than GAS have been reported to be associated with cellulitis, especially group G (GGS) (Hugo-Persson and Norlin 1987, Eriksson et al. 1996, Eriksson 1999, Cohen-Poradosu et al. 2004). Group B ß-haemolytic streptococci have occasionally been isolated in cases of acute and also recurrent cellulitis (Baddour and Bisno 1985, Sendi et al. 2007).

The role of *Staphylococcus aureus* has been clearly demonstrated in superficial skin infections (impetigo, folliculitis, furunculosis), and cellulitis associated with a culturable source, e.g. abscess, wound infection, and surgical site infections (Moran et al. 2006, Que and Moreillon 2009). Also, *S. aureus* is frequently found on the skin in patients with non-suppurative cellulitis (Jorup-Rönström 1986, Eriksson et al. 1996), and it has been the most common finding in bacterial cultures from skin breaks in such patients (Chira and Miller 2010, Eells et al. 2011). However, its role in diffuse cellulitis has been controversial (Moran et al. 2006, Jeng et al. 2010). *S. aureus* frequently colonises the skin (Eells et al. 2011), especially when there are breaks. Thus, the presence of *S. aureus* in association with acute cellulitis may represent mere colonisation, given that the bacteriological diagnosis in cellulitis without a culturable source is seldom achieved (Leppard et al. 1985, Jorup-Rönström 1986, Newell and Norden 1988, Bisno and Stevens 1996, Eriksson et al. 1996, Eells et al. 2011). In the study of Semel and Goldin (1996) BHS were found in interdigital spaces in 17/20 (85%) of leg cellulitis patients with athlete's foot but in none of the controls, whereas *S. aureus* was equally present in both groups. However, in some studies *S. aureus* has been isolated from skin biopsies or needle aspirates, or from blood in a small percentage of studied samples (Leppard et al. 1985, Hook et al. 1986, Jorup-Rönström 1986, Duvanel et al. 1989).

Pneumococcal cellulitis is rare, and it is usually associated with an underlying illness such as diabetes, systemic lupus erythematosus or other immunocompromise, or alcohol or substance abuse (Parada and Maslow 2000).

In addition, cellulitis is reported to be caused by various bacteria related to special circumstances such as immunocompromise (*Pseudomonas, Vibrio, E. coli, Klebsiella, Acinetobacter, Clostridium*) (Carey and Dall 1990, Falcon and Pham 2005, Falagas et al. 2007), human or animal bites (*Eikenella corrodens, Pasteurella, Capnocytophaga*

canimorsus) (Goldstein 2009), immersion to fresh or salt water (*Aeromonas, Pseudomonas, Klebsiella, E. coli, Enterobacter, Proteus, Acinetobacter, Moraxella, Vibrio*) (Swartz 2004, Stevens et al. 2005, Lin et al. 2013a). Evidence of other bacterial causes of cellulitis is presented in case reports, e.g. *Streptococcus pneumoniae* (Parada and Maslow 2000), *Yersinia enterocolitica* (Righter 1981), *Klebsiella pneumoniae* (Park et al. 2004). Additionally, cases of fungal cellulitis have been reported, such as cellulitis caused by *Cryptococcus neoformans* in immunocompromised patients, which may resemble bacterial cellulitis by appearance and an acute onset with fever (Van Grieken et al. 2007, Orsini et al. 2009, Vuichard et al. 2011, Nelson et al. 2014).

Blood cultures are only rarely positive in cellulitis. In the study by Björnsdottir et al. (2005) BHS were isolated from blood in 8 of 81 cellulitis cases (4 GAS, 3 GGS and one GBS). In two Swedish studies (Jorup-Rönström 1986, Eriksson et al. 1996) blood cultures in both studies yielded BHS (mainly GAS, followed by GGS) in 5% of the cases with blood cultures performed. In a recent systematic review (Gunderson and Martinello 2012), comprising 28 studies with a total of 2731 patients with erysipelas or cellulitis, 8% of blood cultures were positive. Of these, 24% were GAS, 37 % other BHS, 15% *S. aureus*, 23% gram-negative rods, and 1% other bacteria. The studies included in the review were heterogeneous in respect of the case definition and exclusion criteria. This may explain the finding that gram-negative rods were as frequent blood culture isolates as GAS or *S. aureus* in patients with erysipelas or cellulitis. Blood cultures may have been obtained more frequently in severe and complicated cases, i.e. with recent abdominal surgery, human or animal bites, or severe immunosuppression than in patients with simple cellulitis. Furthermore, data were prospectively collected in only 12 studies comprising 936 patients, which represents one third of the total patient population included in the review.

In conclusion, based on bacterial cultures of superficial and invasive samples, and immunofluorescence study, BHS are commonly present in cases of cellulitis, especially when the skin is broken. *S. aureus* is also commonly present in the skin of cellulitis patients, but it is also associated with skin breaks without cellulitis. Nevertheless, its role as a cause of cellulitis cannot be excluded. Moreover, it is evident, that other bacteria, especially gram negative rods, and rarely also fungi, may cause cellulitis. However, these pathogens are only rarely encountered, and most often they are

associated with immunocompromise or special environmental exposure. The data concerning bacterial aetiology of cellulitis is flawed by often low yield in bacterial cultures, and highly variable case definitions in different studies.

For epidemiological purposes GAS and GGS strains can be further differentiated by serological and molecular typing methods. The classical methods for GAS are T- and M-serotyping (Moody et al. 1965). At present molecular typing methods, such as *emm* gene sequencing and pulsed-field gel electrophoresis (PFGE), are replacing the serological methods in typing of both GAS and GGS (Single and Martin 1992, Beall et al. 1996, Ahmad et al. 2009).

2.1.4.2 Serology in cellulitis

Evidence of recent streptococcal infection may be obtained by serological methods. Assays for antibodies against different extracellular antigens of BHS have been developed, but only antistreptolysin O (ASO) and anti-DNase B (ADN) assays are widely used in clinical practice for diagnosis of recent GAS infections (Wannamaker and Ayoub 1960, Ayoub 1991, Shet and Kaplan 2002). Serological diagnosis of GAS infections has been most important in the setting of rheumatic fever where symptoms appear several weeks after the acute GAS infection, and where throat swabs are frequently negative (Ayoub 1991). In addition, *Streptococcus dysgalactiae* subsp. e*quisimilis* (SDSE), which may be serologically classified as belonging to either group G or group C, produces streptolysin antigenically similar to streptolysin O produced by GAS (Tiesler and Trinks 1982, Gerlach et al. 1993). Thus, rise in ASO titres are likely to be seen following infections by SDSE as well as GAS infections. Rising ASO titres may be detected one week after an acute infection by GAS, and peak titres are usually reached in 3-5 weeks. High titres may remain up to 3 months with a gradual decline to normal values in 6 months after acute infection (Wannamaker and Ayoub 1960, Ayoub 1991). There is a substantial variation between individuals in the ASO response, the cause of which is largely unknown (Wannamaker and Ayoub 1960, Ayoub 1991). For example, patients with rheumatic fever tend to have a stronger antibody response to streptococcal antigens than healthy controls, which may be either an inherent trait or acquired with past BHS infections (Quinn 1957). Also, there may be variation between GAS strains in the amount of streptolysin O produced (Wannamaker and Ayoub 1960).

Moreover, the distribution of ASO titres vary by age being higher in children than in adults (Kaplan et al. 1998, Shet and Kaplan 2002), and by geographical location. The higher ASO titres observed in the less developed countries are thought to reflect the burden of streptococcal impetigo among these populations (Carapetis et al. 2005, Steer et al. 2009).

ASO response has been shown to be lower in superficial skin infections, such as streptococcal pyoderma or impetigo, than in streptococcal tonsillitis (Kaplan et al. 1970); this does not apply to ADN responses. This has been suggested to be associated with a suppression of ASO response by lipid constituents of the skin (Kaplan and Wannamaker 1976), rather than a generalised immunological unresponsiveness in superficial skin infections. In a study conducted on 30 erysipelas patients before the antibiotic era (Spink and Keefer 1936) a rise in ASO titres was seen in all patients, yet, the magnitude varied substantially between individuals. Peak titres were reached in 20 days after the onset of symptoms, and titres remained elevated variably from 40 days up to six months.

Studies on the serology in cellulitis and erysipelas in the last decades have had very similar results regarding ASO and ADN in paired serum samples. In the study by Leppard et al. (Leppard et al. 1985) six of 15 erysipelas patients, and all of the 20 cellulitis patients showed evidence of BHS infection by either ASO or ADN. Thus, 26 (74%) of the 35 patients had serological evidence of BHS infection. However, only three of the nine seronegative patients had a convalescent phase serum sample available. In a Finnish case series positive ASO serology was found in 48% of the patients after one to two weeks from admission to hospital (Hollmen et al. 1980).

In a Swedish study on erysipelas (Hugo-Persson and Norlin 1987), the ASO titre in patients with BHS cultured from a skin swab differed from those with *S. aureus* as a single finding or from those with a negative culture. In the latter patient group, however, there were several cases with a significant rise in the ASO titre, indicative of a recent GAS or SDSE infection. For ADN, the results were similar, except that there were less positive findings overall (Hugo-Persson and Norlin 1987). Similarly, in a more recent study from Sweden, comprising 229 patients with erysipelas, (Eriksson et al. 1996), there was a significant rise in ASO titres between acute and convalescent sera in patients with GAS, GGS or no pathogen in the skin swab specimen. No such increase was observed in groups of patients with *S. aureus* or enterococci in the skin

swab. Overall, an ASO titre of ≥200 U/ml, considered positive, was detected in acute and convalescent phase in 30% and 61% of the erysipelas patients, respectively. Positive ADN titres were recorded in 30% and 51% in the acute and convalescent phase, respectively.

In a recent serological study 70% (126/179) of cellulitis patients were either ASO or ADN seropositive and 35% (63/179) were both ASO and ADN seropositive (Jeng et al. 2010).

Antibodies to staphylococcal α-haemolysin, measured by anti-staphylolysin assay (ASTA) are formed in deep *S. aureus* infections (Larinkari and Valtonen 1984). Positive ASTA values were found in 44% of patients with atopic dermatitis and in 28% of those with infectious eczema (Larinkari 1982). Serological testing may be useful in culture-negative endocarditis, but its value in *S. aureus* soft tissue infections is unclear and has been disputed (Elston et al. 2010).

Overall, in the studies cited, serological evidence of BHS infection was observed in 61-74% of the erysipelas or cellulitis cases. However, there is quite a considerable variation in the serologic response even in the culture-positive cases of cellulitis and erysipelas (Hugo-Persson and Norlin 1987, Jeng et al. 2010). This may be due to a preceding antibiotic treatment (Anderson et al. 1948, Leppard et al. 1985), or differences in the streptolysin O production between BHS strains (Anderson et al. 1948, Wannamaker and Ayoub 1960, Leppard et al. 1985) or patients' genetics (Quinn 1957). Again, substantial variation in the case definitions used impedes the interpretation of the studies. The usefulness of antistaphylococcal serology in cellulitis appears low.

2.1.4.3 Pathogenesis of cellulitis

Much is known about the adhesion and invasion of BHS to mucous membranes and skin (Cunningham 2000, Courtney et al. 2002, Bisno et al. 2003, Johansson et al. 2010, Cole et al. 2011). Beyond that, nevertheless, the pathogenesis of cellulitis is largely unknown. Given the low and often negative yield of bacteria with invasive sampling in studies on cellulitis (Leppard et al. 1985, Hook et al. 1986, Hugo-Persson and Norlin 1987, Newell and Norden 1988, Bernard et al. 1989, Duvanel et al. 1989, Eriksson et al. 1996), it has been hypothesised that cellulitis is a paucibacillary condition with

overwhelming inflammatory response against streptococcal, and probably also fungal, antigens causing the clinical manifestations of cellulitis (Duvanel et al. 1989, Sachs 1991). In contrast to cellulitis, in necrotising infections caused by GAS, bacterial density in the skin is probably much higher (Thulin et al. 2006).

Hypotheses of streptococcal toxins (Hook et al. 1986), or hypersensitivity to them (Baddour et al. 2001), as the cause of local manifestations of cellulitis have been presented. These are based on the clinical observations that the suspected portal of entry of the bacteria, where the bacteria are most abundant, is often distant to the inflammation of the skin, e.g. in toe webs (Duvanel et al. 1989, Semel and Goldin 1996, Björnsdottir et al. 2005). An impaired lymphatic clearance of microbial antigens and inflammatory mediators has been suggested to lead to a self-sustained vicious circle of inflammation (Duvanel et al. 1989). The strong association of cellulitis and chronic oedema, especially lymphoedema, fits well to this hypothesis (Cox 2006).

A recent study assessed the molecular pathology of erysipelas caused by GAS (Linder et al. 2010). The study suggested that the clinical signs of inflammation in erysipelas may be caused by vasoactive substances, such as heparin-binding protein and bradykinin, the production of which is enhanced in the inflamed skin infected by GAS. Furthermore, bacterial cells were found by immunohistochemistry and confocal microscopy throughout the inflamed skin, suggesting that the inflammatory changes are not solely caused by toxins secreted by bacteria distant to the inflamed skin area.

Streptococci interact with extracellular matrix components of tissues, and invade and persist in macrophages, fibroblasts, and epithelial and endothelial cells (LaPenta et al. 1994, Thulin et al. 2006, Hertzen et al. 2012). This ability to survive intracellularly has been proposed to play a role in recurrent tonsillitis (Osterlund et al. 1997), and may possibly contribute to the recurrent nature of cellulitis, too (Sendi et al. 2007).

2.1.4.4 Genetic susceptibility to cellulitis

The highly complex system of human innate and adaptive immunity has evolved in the continuous selective pressure of potentially pathogenic organisms in changing environments (Netea et al. 2012). Of the human genes, the most abundant are those involved with immune mechanisms. Thus, it is apparent, that inherited variation of the

host contributes to the susceptibility to acquire and to survive infections, together with the properties of the pathogen and the environment (Burgner et al. 2006). Genetic traits influencing the susceptibility to infections may be caused by single genes, as in several primary immunodeficiencies, or by multiple genes (Kwiatkowski 2000, Casanova and Abel 2005). A genetic trait advantageous in one environment may be disadvantageous in another. For example, this mechanism has been proposed in TLR4 polymorphism, where a certain allele is protective of cerebral malaria, but increases susceptibility to Gram-negative septic shock (Netea et al. 2012). In contrast, as an example of a complex trait, heterozygosity in the alleles of human leukocyte antigen (HLA) class-II genes is advantageous in clearing hepatitis B infection (Thursz et al. 1997).

An early epidemiological study on adoptees suggested a genetic predisposition to infections to be five times greater than to cancer (Sorensen et al. 1988). The risk of succumbing to infection increased over fivefold, if a biological parent had died of an infectious cause. In contrast, death of the biologic parent from cancer had no influence on the probability of dying from cancer among the adoptees.

The susceptibility to acquire GAS in the throat was suggested to be at least partly explained by inherited factors in an early study on streptococcal carriage in families (Zimmerman 1968). More recent studies have shown differences in cytokine response and HLA class II allelic variation to contribute to the severity and outcome of invasive GAS infection (Norrby-Teglund et al. 2000, Kotb et al. 2002).

Genetic predisposition to cellulitis has been studied recently in a genome-wide linkage study in 52 families with cases of cellulitis in two or more members of the family (Hannula-Jouppi et al. 2013). There was a significant linkage in chromosome 9 in a region which corresponds to a region in mouse genome contributing to susceptibility to GAS infections. The candidate gene sequencing did not, however, reveal any association with cellulitis. Additionally, there was a suggestive linkage in chromosome 3, in which there was a suggestive association with cellulitis in the promoter region of Angiotensin II receptor type I (AGTR1) gene. There was no linkage found in the HLA region associated with the severity and outcome of invasive GAS infections mentioned above. It is likely that multiple genes, probably different in different families, contribute to the susceptibility to cellulitis.

2.2 Inflammatory markers in bacterial infections

Bacterial infections elicit a complex inflammatory response in the human body. Several factors have been identified, the production of which is clearly accelerated during the early phase of bacterial infections. These are called acute phase reactants, and include proteins such as serum amyloid A, haptoglobin, fibrinogen, ferritin, and members of the complement system, to mention but a few (Gabay and Kushner 1999). The production of acute phase reactants is regulated by a network of cytokines and other signal molecules (Mackiewicz et al. 1991, Gabay and Kushner 1999, Volanakis 2001, Mantovani et al. 2008).

2.2.1 C-reactive protein

C-reactive protein (CRP) is an acute phase reactant, which contributes in several ways to the inflammatory response. CRP is synthesised mainly in the liver (Hurlimann et al. 1966). Interleukin-6 (IL-6) is the main stimulator of CRP synthesis, but IL-1 and complement activation products enhance its production (Ganapathi et al. 1991, Volanakis 2001). The biological role of CRP is to participate in the innate immunity to infections (Du Clos and Mold 2001, Szalai 2002), and to contribute to the clearance of necrotic cell remnants (Black et al. 2004). Concentration of CRP in the serum reflects ongoing inflammation or tissue damage, whatever the cause (Pepys and Hirschfield 2003).

The magnitude of the rise in CRP concentration in human blood is dependent on the size and duration of the stimulus (Kushner et al. 1978, Ablij and Meinders 2002, Pepys and Hirschfield 2003). Elevated concentrations after a stimulus can be measured in six hours, and the peak is reached in 48 hours (Gabay and Kushner 1999, Pepys and Hirschfield 2003). The biological half-time of CRP is 19 h after removal of stimulus (Ablij and Meinders 2002). Thus, the rate of decline in serial CRP measurements reflects the rate of CRP synthesis, and therefore is dependent on the persistence of inflammatory stimulus.

The first clinical observations of CRP were made over eighty years ago (Tillett and Francis 1930). Since then, measurement of CRP has become clinical routine for diagnosing infections, monitoring treatment response, and predicting the outcome of

acute infections. Also, CRP is a useful biomarker in various non-infectious conditions, such as rheumatoid arthritis (Otterness 1994, Du Clos and Mold 2001). Albeit often used to differentiate between viral and bacterial infections, no clear distinction differentiation can be made based solely on it (Heiskanen-Kosma and Korppi 2000, van der Meer et al. 2005, Sanders et al. 2008). Likewise, non-infectious inflammatory conditions cannot be distinguished from bacterial infections by CRP (Limper et al. 2010, Rhodes et al. 2011). However, monitoring the activity of all these conditions by serial measurements of CRP has proved useful (Clyne and Olshaker 1999). Moreover, CRP has proved valuable in predicting the severity of infectious conditions in various settings, e.g. meningitis (Peltola 1982), pneumonia (Chalmers et al. 2008), infective endocarditis (Heiro et al. 2007), and bacteraemia (Gradel et al. 2011).

Studies in the context of rheumatologic and cardiovascular diseases have revealed inherited variation between individuals in CRP response to inflammatory stimuli (Perry et al. 2009, Rhodes et al. 2011). In *S. aureus* bacteraemia, the variation in the CRP gene has been shown to contribute partly to the maximal CRP level during the first week of hospitalisation (Mölkänen et al. 2010). In another study (Eklund et al. 2006), polymorphism in the CRP gene promoter region was associated with mortality in *Streptococcus pneumoniae* bacteraemia, but did not correlate with CRP concentrations.

Few studies have assessed CRP in cellulitis. Lazzarini et al. (2005) found the CRP level on admission to be associated with the length of stay in hospital. The mean serum CRP values were 106 mg/l in patients hospitalised for more than ten days as compared to 42 mg/l in those with a shorter stay. Overall, CRP levels were above normal in 150 out of 154 (97%) cellulitis patients on admission. In a recent retrospective study on complicated erysipelas (Krasagakis et al. 2011) increased levels of CRP were associated with local complications (purpura, bullae, abscesses and necrosis) of erysipelas (mean values of 88 mg/l and 43 mg/l for complicated and non-complicated cases, respectively, $p<0.05$). The association, however, disappeared in the multivariable analysis, where only obesity was statistically significantly associated with local complications of erysipelas. In a preceding report of the non-complicated cases of the same patient cohort (Krasagakis et al. 2010), CRP was found to be above normal level (presumably > 10 mg/l) in 27 (77%) of 35 patients. Eriksson et al (1996) reported a mean CRP concentration of 163 mg/l, ranging from <10 to 507 mg/l in 203 hospitalised erysipelas patients. No data concerning the timing of CRP measurement in

relation to admission was reported. The mean CRP concentration seemed to be somewhat lower in cases with facial erysipelas (107 mg/l) than in cases with leg erysipelas (170 mg/l), but no statistical analysis was conducted. The difference probably reflects the larger area of inflammation in the leg than in the face.

In conclusion, there is a wide variation in the CRP response in cellulitis. Again, the interpretation of the data available is hampered by the variation in study design and case definition in the few studies reporting CRP measurements. CRP is elevated in most cases, and high CRP values may predict severe disease or complications, yet the clinical usefulness of the latter observation is uncertain. There may be genetic variation in the CRP response.

2.2.2 Pentraxin-3

Pentraxin-3 (PTX3) and CRP share structural and functional similarities. Both belong to the family of five-subunits containing acute phase proteins called pentraxins. PTX3, recognizes and binds to different pathogens, including bacteria, fungi and viruses, and to altered self-molecules, and contributes to the opsonisation. Thus, like CRP, it is an essential component of the innate immunity and of the clearance of necrotic and apoptotic cells (Agrawal et al. 2009, Bottazzi et al. 2009, Mantovani et al. 2013). Unlike CRP, however, PTX3 is mainly synthesised in mononuclear phagocytes and myeloid dendritic cells. Also, in vitro, endothelial cells, adipocytes, fibroblasts, smooth muscle cells, synovial cells and chondrocytes may produce PTX3 (Luchetti et al. 2000, Garlanda et al. 2005, Doni et al. 2006, Mantovani et al. 2013). The production of PTX is induced by microbial components (e.g. lipopolysaccharide), and inflammatory signals, as Toll-like receptor (TLR) activation, tumour necrosis factor α (TNF- α) and IL-1β (Bottazzi et al. 2009, Inforzato et al. 2013, Mantovani et al. 2013). Interferon-γ (IFNγ) inhibits and IL-10 enhances PTX3 production in dendritic cells (Doni et al. 2006). PTX3 itself may act as a regulator of the inflammatory response by multiple mechanisms, e.g. by inhibiting neutrophils in massive leukocyte activation, and by contributing to angiogenesis and smooth muscle cell activation (Deban et al. 2008, Agrawal et al. 2009, Maugeri et al. 2011, Mantovani et al. 2013).

The kinetics of PTX3 is more rapid than that of CRP, probably owing to the local activation and release of pre-formed PTX3 (Peri et al. 2000, Maugeri et al. 2011,

Mantovani et al. 2013). Peak concentration after an inflammatory stimulus is reached in 6-8 hours (Mantovani et al. 2013). Study on patients with acute myocardial infarction showed a median time of 7.5 hours from the onset of symptoms to peak PTX3 levels (mean peak PTX3 concentration 6.9 ± 11.26 ng/ml), and 24 hours to the peak CRP levels. Elevated PTX levels (>2.01 ng/ml, cut off based on 20 control subjects) were observed at 24 h in 26 (76%) of 34 patients. At 48 h the median PTX3 level was near the cut off value, whereas the CRP levels were at the peak (Peri et al. 2000). Considerably higher PTX3 levels have been reported in viral and bacterial diseases, ranging from the median of 60 ng/ml in dengue fever to 250 in sepsis with the highest values over 1000 ng/ml in septic shock (Muller et al. 2001, Mairuhu et al. 2005).

PTX3 has proved to be a prognostic marker in bacteraemia (Huttunen et al. 2011), community acquired pneumonia (Kao et al. 2013), ventilator associated pneumonia (Lin et al. 2013b), febrile patients presenting in emergency care (de Kruif et al. 2010), febrile neutropenia (Juutilainen et al. 2011), sepsis (Muller et al. 2001), dengue (Mairuhu et al. 2005), and Puumala hantavirus infection (Outinen et al. 2012). It is also associated with the severity of non-infectious conditions, such as polytrauma (Kleber et al. 2013), acute coronary syndrome (Lee et al. 2012), ischemic stroke (Ryu et al. 2012), chronic kidney disease (Tong et al. 2007), and psoriasis (Bevelacqua et al. 2006). Thus, PTX3 produced locally in the site of inflammation, is detectable in the serum very early in the course of the disease, and disappears considerably more rapidly than CRP in the same situation. The concentration of PTX3 in the blood correlates with the severity of the disease in various inflammatory conditions. No studies on PTX3 in cellulitis have been published previously.

3. AIMS OF THE STUDY

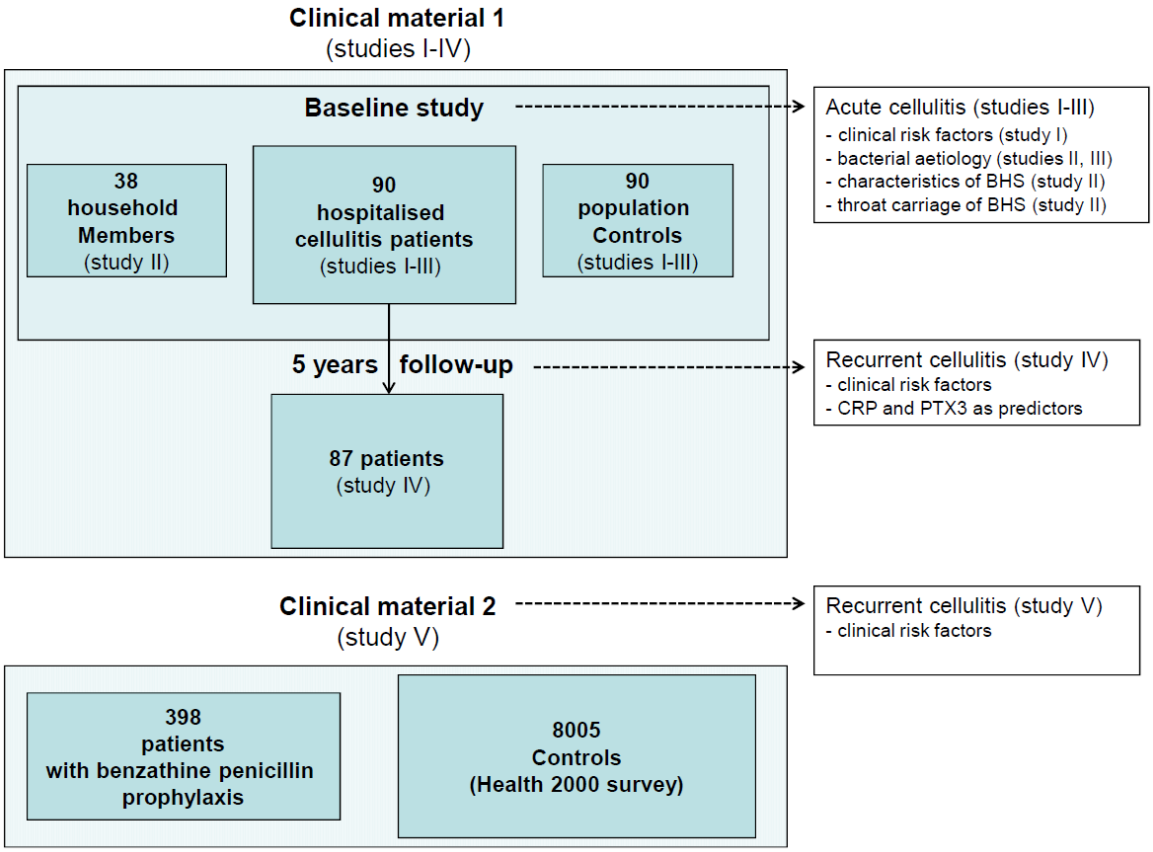
The aims of the present study were:

1. To study the clinical risk factors for acute cellulitis (study I)
2. To study the clinical risk factors for recurrent cellulitis (studies IV, V).
3. To assess the risk of recurrence of acute cellulitis in five years and to evaluate CRP and PTX3 as predictive biomarkers for recurrence (study IV).
4. To evaluate the bacteriological aetiology of cellulitis (studies II, III)
5. To characterise the BHS associated with cellulitis, and to evaluate throat carriage of BHS in cellulitis patients, their household members and controls.

4. SUBJECTS AND METHODS

4.1 Overview of the study

Figure 2. Overview of the study design.



4.2 Clinical material 1, acute cellulitis and five year follow-up (studies I-IV)

4.2.1 Patients and case definition

The study was carried out in two wards in Tampere University Hospital and Hatanpää City Hospital in Tampere between April 2004 and March 2005. Consecutive hospitalised patients presenting with an acute cellulitis were recruited into the study. Case definition was as follows:

- Patient ≥18 years of age, referred by the primary physician with a diagnosis of acute cellulitis
- Skin erythema localised on one extremity or erythematous lesion on the face with well-demarcated border
- Recent history of acute onset of fever or chills (except for cellulitis of the face)

The diagnosis of acute bacterial non-necrotising cellulitis was confirmed within four days after admission, the patients were interviewed, the clinical examination conducted and data on possible risk factors collected by an infectious disease specialist, the author of this thesis (MK).

If, on admission, the skin lesion was described by the attending physician as sharply demarcated, the patients were classified as having "erysipelas".

4.2.2 Patients' household members

The family relations of the patients were also analysed in pursuance of the patient interview. Household members were asked to participate in the study and sent a consent form. Consenting household members were asked to give a throat swab sample.

4.2.3 Controls

One control subject for each patient was recruited. From the Finnish Population Register six control candidates, living in Tampere and matched for sex and age (same birth year and month), were obtained. For each group of six, one person at a time was sent an invitation letter at two weeks' intervals until the first response. A control candidate was excluded if he or she had at any time had an acute cellulitis, and another control candidate was invited. Any further attempts to reach a control candidate were not made in case of not responding in two weeks, so the reason for non-response could not be elucidated.

4.2.4 Study protocol

4.2.4.1 Clinical examination

Patients and controls were weighed and their height was recorded as reported by them. BMI was calculated as weight in kilograms divided by square of height in metres. Data concerning the comorbidities were obtained from the medical records. Alcohol abuse was defined as any health or social condition which was recorded in the medical chart as being caused by excessive alcohol use. Oedema present at the time of the clinical examination was considered chronic based on the medical records or interview. Toe-web intertrigo was considered to be present if the skin in the toe-webs was not entirely intact at the time of the examination. History of skin diseases, traumatic wounds and previous operations were obtained from the medical records or by interview. Fever was defined as tympanic temperature of 37.5°C or higher as measured during the hospital stay or otherwise measured temperature of 37.5°C or higher before admission as reported by the patient.

4.2.4.2 Patient sample collection

Following samples were collected on admission to hospital:

1. Throat swab in duplicate.

2. Skin swab in duplicate from any skin breach on the affected limb, whether in the inflamed area or elsewhere on the same limb, for example in toe web.

3. Blood cultures (aerobic and anaerobic bottles) from all patients by routine method. Whole blood, plasma and serum samples for subsequent analyses were obtained together with the routine clinical sample collection on admission, when possible, or on the next working day. Samples were sent to the THL (The National Institute of Health and Welfare, formerly KTL) laboratory and were stored in aliquots in -20°C. Subsequent leukocyte counts and CRP assays were performed as part of the clinical care on the discretion of the treating physician. Convalescent phase samples were scheduled to be taken four weeks after the admission.

Swabs were sent to the THL on the same day, when appropriate, or stored in +4°C and sent on the next working day. Swabs were cultivated in the THL laboratory. Blood cultures were sent to the local hospital laboratory (Laboratory Centre of Pirkanmaa Hospital District) and cultured according to the routine procedure.

Furthermore, an additional skin swab was collected on the discretion of the attending physician if it was considered necessary in the clinical care of the patient. These were sent and processed according to a standard procedure in the local hospital laboratory.

4.2.4.3 Sample collection from control subjects

Throat swabs in duplicate, as well as whole blood, plasma and serum samples were obtained during a study visit. Swabs were stored in room temperature and sent to the THL on the same day or on the next working day and cultivated there. Whole blood, plasma and serum samples were stored as described above.

4.2.4.4 Sample collection from household members

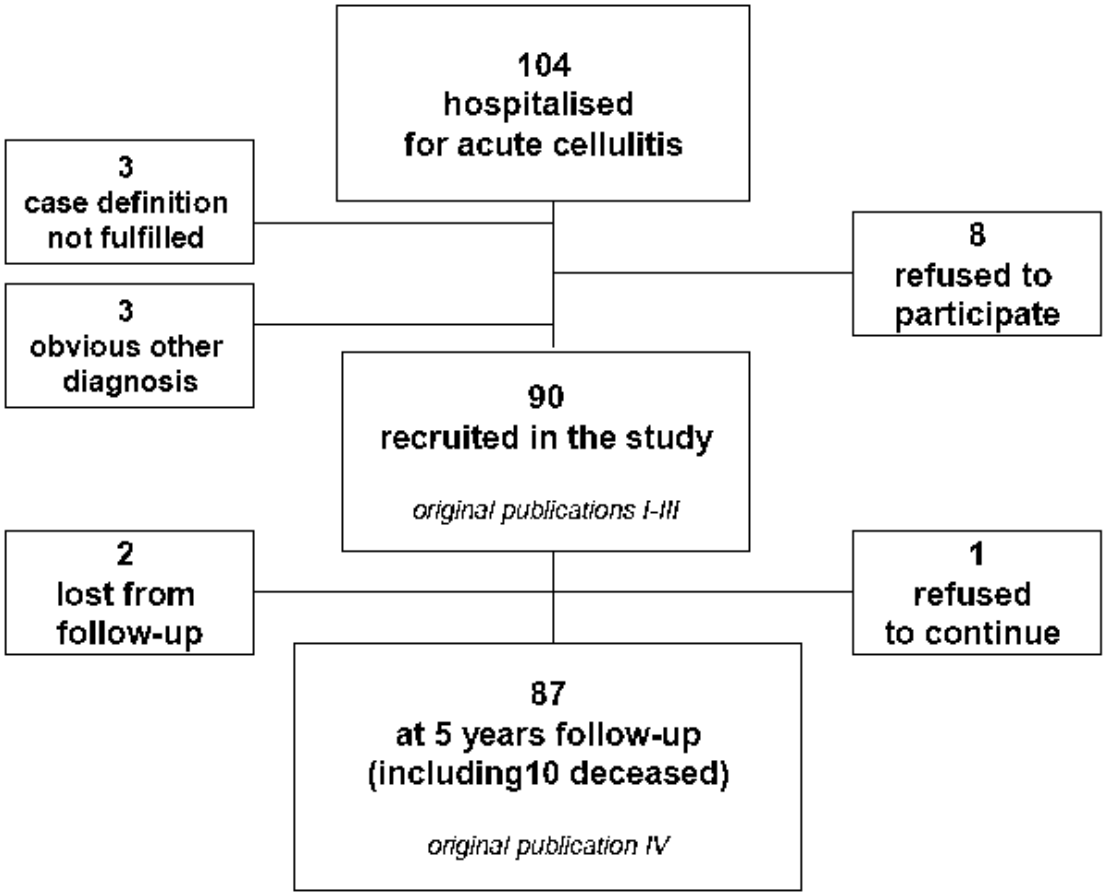
Patients' household members having given consent were sent appropriate sample collection tubes and asked to have the samples taken in the health care centre laboratory. Samples were stored and sent to THL as described above.

Table 7. Study protocol in the clinical study 1. The patients were interviewed and examined on admission to hospital.

	Interview	Clinical examination	Throat swab	Skin swab	Blood culture	Whole blood, serum, plasma
Patient	■	■	■	■	■	■ ■[1]
Control	■	■	■			■
Household member	□[2]		■			■

[1] Convalescent phase
[2] Questionnaire

Figure 3. Flowchart of the patient recruitment in the clinical material 1 (original publications I-IV).

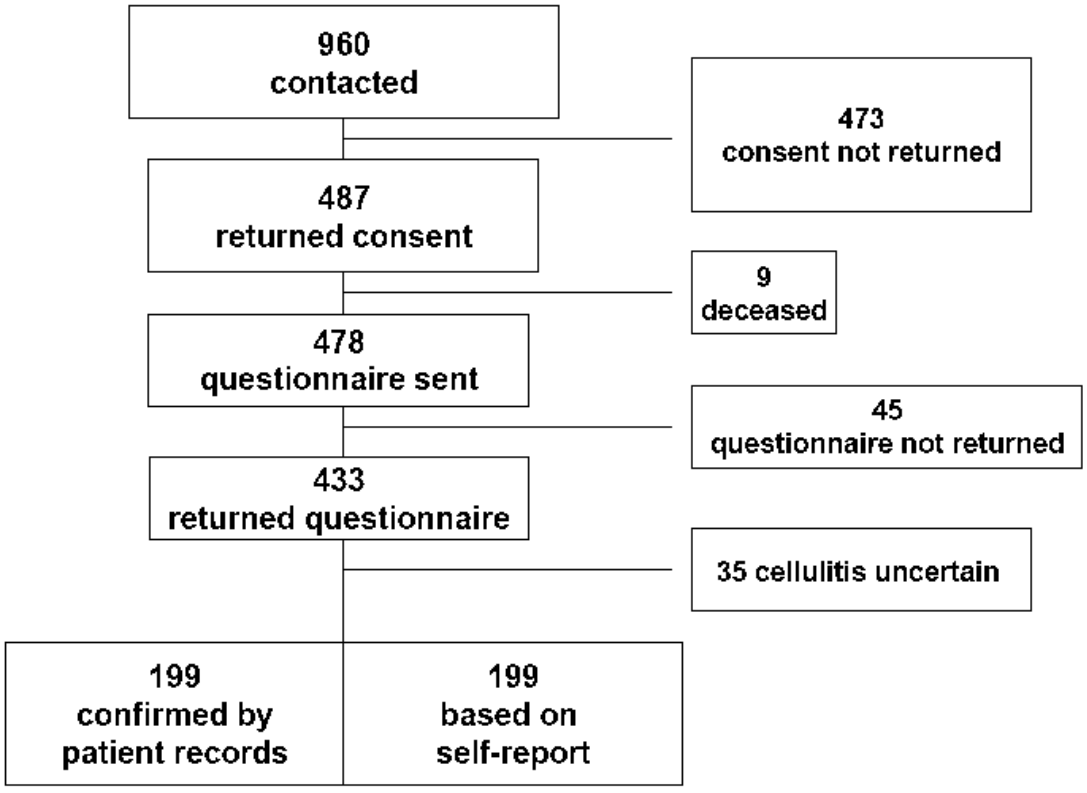


4.3 Clinical material 2, recurrent cellulitis (study V)

4.3.1 Patients and case definition

The clinical material 2 (study V) was comprised of all individuals in Finland who were receiving reimbursement for benzathine penicillin in the year 2000. Patients (n=960) were tracked via National Health Insurance Institution in February 2002 and sent a letter together with a consent form. A questionnaire was sent to the 487 (50%) returning the consent form. Patients were also asked to confirm the indication of benzathine penicillin prescription. Furthermore, 199 patient records were received and reviewed in order to confirm that the indication for a benzathine penicillin treatment was recurrent cellulitis and that there was no reasonable doubt of the correct diagnosis**.**

Figure 4. Flowchart of the patient recruitment in the clinical material 2. (study V) A total of 398 patients and were recruited.



4.3.2 Controls and study protocol

The controls were 8005 Finnish subjects, aged ≥30 years, a randomly drawn representative sample of the Finnish population, who participated in a national, population-based health examination survey (Health 2000, http://www.terveys2000.fi/julkaisut/baseline.pdf). The survey was carried out in the years 2000-2001 by the Finnish National Public Health Institute (present name National Institute for Health and Welfare). A comprehensive database was available, collected in the Health 2000 survey by an interview using a structured set of questions and a health examination of the study subjects. The data corresponding to the variables recorded in the patient questionnaire were drawn from the database.

Following variables were recorded from both the patient questionnaire and Health 2000 database: age, sex, height, weight, diabetes (not known/type 1/type 2), history of tonsillectomy, history of psoriasis and history of other chronic dermatoses.

The data in the Health 2000 survey concerning the histories of diabetes, psoriasis and other chronic dermatoses were considered to correspond to the data collected by the questionnaire for patients. However, the data concerning the history of tonsillectomy were collected in a materially different way. The study subjects in the Health 2000 survey were asked to list all previous surgical operations, whereas the history of tonsillectomy was a distinct question in the patient questionnaire. Furthermore, the controls were weighed and their height was measured in the Health 2000 survey, but weight and height were self-reported by the patients.

4.4 Bacteriological methods

4.4.1 Bacterial cultures

Sterile swabs (Technical Service Consultants) were used for sampling and transportation of both throat and skin swab specimens. First, a primary plate of sheep blood agar was inoculated. The swab was then placed in sterile water. The resulting bacterial suspension was serially diluted and plated on sheep blood agar. Plates were incubated in 5% CO2 at 35°C, and bacterial growth was determined at 24 h and 48 h.

β-haemolytic bacterial growth was visually examined, and the number of colony forming units per millilitre (cfu/ml) was calculated. Up to 10 suspected β-haemolytic streptococcal (BHS) colonies and one suspected *Staphylococcus aureus* colony per sample were chosen for isolation.

The culturing and identification of blood cultures were performed according to the standard procedure using Bactec 9240 (BD Diagnostic Systems) culture systems, and standard culture media. Isolates of BHS were sent on blood agar plates to the THL bacteriologic laboratory as well as BHS isolates from the skin swabs taken on clinical grounds.

4.4.2 Identification and characterisation of isolates

In the THL laboratory, bacitracin sensitivity was tested on suspected BHS. Subsequently, the Lancefield group antigens A, B, C, D, F and G were detected by Streptex latex agglutination test (Remel Europe Ltd). *S. aureus* was identified using the Staph Slidex Plus latex agglutination test (bioMérieux). BHS isolates were identified to species level with the API ID 32 Strep test (bioMérieux). T-serotyping, *emm*-typing and PFGE were used for further characterisation of BHS. The identified bacterial isolates were stored at -70°C.

4.4.2.1 T-serotyping

T-serotyping was performed according to standard procedure (Moody et al. 1965) with five polyvalent and 21 monovalent sera (1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 22, 23, 25, 27, 28, 44, B3264, and Imp19) (Sevac).

4.4.2.2 emm-typing

Primers used in the *emm* gene amplification and sequencing are shown in Table 8. Amplification with primers MF1 and MR1 was performed under the following conditions: initial denaturation at 95°C for 10 min and 94°C for 3 min, 35 cycles of denaturation at 93°C for 30 s, annealing at 54°C for 30 s and extension at 72°C for 2

min, with a final extension step at 72°C for 10 min. Amplification conditions with primer 1 and primer 2 were: initial denaturation at 95°C for 10 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 60 s and extension at 72°C for 2.5 min, with a final extension step at 72°C for 7 min.

PCR products were purified with the QIAquick PCR purification kit (Qiagen), as described by the manufacturer. The emm sequencing reaction was performed with primer MF1 or emmseq2 and BigDye chemistry (Applied Biosystems), with 30 cycles of denaturation at 96°C for 20 s, annealing at 55°C for 20 s, and extension at 60°C for 4 min. Sequence data were analysed with an ABI Prism 310 genetic analyser (Applied Biosystems) and compared with the CDC *Streptococcus pyogenes emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

Table 8. Primers used for *emm*-typing

Primer	**Sequence 5'→ 3'**	**Reference**
MF1 (forward, sequencing)	ATA AGG AGC ATA AAA ATG GCT	(Jasir et al. 2001)
MR1 (reverse)	AGC TTA GTT TTC TTC TTT GCG	(Jasir et al. 2001)
primer 1 (forward)	TAT T(C/G)G CTT AGA AAA TTA A	(Beall et al. 1996, CDC 2009)
primer 2 (reverse)	CGA AGT TCT TCA GCT TGT TT	(Beall et al. 1996, CDC 2009)
emmseq2 (sequencing)	TAT TCG CTT AGA AAA TTA AAA ACA GG	(CDC 2009)

4.5 Serological methods

ASO and ADN titres were determined by a nephelometric method according to the manufacturer's instructions (Behring, Marburg, Germany). The normal values for both are <200 U/ml, according to the manufacturer. For antistaphylolysin (ASTA), a latex agglutination method by the same manufacturer was used. Titre <2 IU/ml was considered normal.

4.6 Inflammatory markers

4.6.1 C-reactive protein assays and leukocyte count

CRP assays and leukocyte counts were performed according to the standard procedures in the Laboratory Centre of Pirkanmaa Hospital District. CRP and leukocyte counts were measured on admission, and further CRP assays were conducted on the discretion of the attending physician during the hospital stay. CRP values measured on hospital days 1-5 (1 = admission) were recorded, and the highest value measured for a given patient is considered as acute phase CRP.

4.6.2 Pentraxin-3 determinations

Plasma samples stored at -20°C were used for PTX3 assays. Commercially available human PTX3 immunoassay (Quantikine, R&D Systems Inc., Minneapolis, MN) was used according to the manufacturer's instructions.

4.7 Statistical methods

To describe the data, median and range, or minimum and maximum values are given for normally distributed and skew-distributed continuous variables, respectively. In study I, a univariate analysis was performed by McNemar's test. A conditional logistic regression analysis (Method Enter) was performed to bring out independent risk factors for cellulitis. The factors emerging as significant in the univariate analysis or otherwise considered to be relevant (diabetes, and cardiovascular and malignant diseases) were included in the multivariable analysis, which at first was undertaken separately for general and local (ipsilateral) risk factors. Finally, all variables, both general and local, that proved to be associated with acute cellulitis were included in the last multivariable analysis.

In Studies II-IV, categorical data were analysed with χ^2 test or Fisher's exact test, where appropriate, except when comparing the bacteriological findings between patients and controls (Study II), when McNemar's test was applied. Univariate analysis

between categorical and continuous variables was performed by Mann-Whitney U-test. Logistic regression analysis (method Forward Stepwise in Study, IV, and method Enter in Study V) was performed to bring out independent risk factors for recurrence. The value of CRP and PTX3 in predicting recurrence of cellulitis was evaluated by ROC curves (Study IV).

Population attributable risks (PAR) were calculated as previously described (Bruzzi et al. 1985, Roujeau et al. 2004) for the risk factors independently associated with acute cellulitis in the clinical material 1 and with recurrent cellulitis in the clinical material 2.

4.8 Ethical considerations

All patients and controls gave their written informed consent before participation in the study. Study protocols have been approved by the Ethical Review Board of Pirkanmaa Health District (clinical study 1) or Ethical Review Board of Epidemiology and Public Health, Hospital District of Helsinki and Uusimaa (clinical study 2).

5. RESULTS

5.1 Characteristics of the study material

5.1.1 Clinical material 1, acute cellulitis and five year follow-up

Ninety patients were ultimately included in the study (Figure 3). Six patients were excluded due to alternative conditions discovered after the initial diagnosis of acute bacterial cellulitis. Three patients had obvious gout, one had *S. aureus* abscess, and one had *S. aureus* wound infection. One patient had no fever or chills in conjunction with erythema in the leg, thus he did not fulfil the case definition.

The clinical characteristics of the patients are shown in Tables 9-11. Four patients had one recurrence, and two patients had two recurrences during the study period of one year. In the analysis only the first episode was included.

Of the 302 matching controls contacted, 210 did not reply and two were excluded because of a history of cellulitis. All patients and controls were of Finnish origin. There was no intravenous drug use or human immunodeficiency virus infection among the patients or controls. All cellulitis lesions healed or improved during the hospital stay and no deaths or admissions to critical care occurred.

Table 9. Characteristics of the patient populations in the clinical material 1 (original publications I-IV) and the clinical material 2 (original publication V).

	Clinical material 1 (N=90)		**Clinical material 2 (N=398)**	
	n (%) unless otherwise indicated			
Female	32	(36)	235	(59)
Age (years)	58	(21-90)[1]	65	(22-92)[1]
BMI[2]	29	(19.6-65.2)[1]	31	(17-65)[1]
Diabetes, type1 or 2	13	(14)	82	(21)
Cardiovascular disease	18	(20)	n/a	
Malignant disease	14	(16)	n/a	
Alcohol abuse	12	(13)	n/a	
Current smoking	32	(36)	23	(5.8)
Any chronic dermatoses	37	(41)	136	(35)
Psoriasis	n/a		29	(8)
Chronic oedema	23	(26)[3]	139	(35)[4]
Toe-web intertrigo	50	(56)[3]	177	(45)[4]
History of tonsillectomy	12	(14)	93	(23)
Localization of cellulitis				
lower extremity	76	(84)	333	(84)[5]
upper extremity	7	(8)	64	(16)[5]
face	7	(8)	28	(7)[5]
other	0		6	(2)[5]

[1] Median (minimum-maximum)
[2] BMI, body mass index
[3] In the same extremity as cellulitis
[4] Patients with cellulitis in the leg only
[5] As reported by the patients, includes multiple locations in 32 (8%) patients.
n/a, data not available

Table 10. Antibiotic treatment in the 90 cases in Clinical study 1.

	n	**(%)**
Antibiotics initiated before admission	26	(29)
Initial antibiotic treatment in hospital		
Benzylpenicillin	39	(43)
Cefuroxime	26	(29)
Clindamycin	24	(27)
Ceftriaxone	1	(1)
Antibiotic treatment changed		
due to initial treatment failure[1]	15	(17)
due to intolerance	4	(4)

[1]As defined by the attending physician: penicillin 9/39 (23%), cefuroxime 5/26 (19%), clindamycin 1/24 (4%)

Table 11. Inflammatory markers and markers of disease severity in the 90 patients in Clinical study 1.

	median	**min-max**
CRP on admission (mg/l)	128	1-317
Peak CRP (mg/l)	161	5 -365
Leukocyte count on admission (10^9/l)	12.1	3.2-26.8
PTX-3 in acute phase (ng/ml, n=89)	5.5	2.1-94.3
PTX-3 in convalescent phase (ng/ml, n=75)	2.5	0.8-11.8
Length of stay in hospital (days)	8	2-27
Duration of fever (days)		
from onset of disease	2	0-11
from admission	1	0-7

5.1.2 Clinical material 2, recurrent cellulitis

Three hundred ninety-eight patients were ultimately recruited in the study. Clinical characteristics of the patients are shown in Table 9.

5.2 Clinical risk factors

5.2.1 Clinical risk factors for acute cellulitis (clinical material 1)

The clinical risk factors for acute cellulitis and PAR calculated for risk factors independently associated with acute cellulitis are shown in Table 12.

5.2.2 Clinical risk factors for recurrent cellulitis (clinical materials 1 and 2)

The risk factors for recurrent cellulitis were analysed in the clinical material 1 in a setting of a prospective cohort study. Patients with and without a recurrence in 5 years follow-up were compared (Study IV). In the clinical material 2, clinical risk factors were assessed in 398 patients with benzathine penicillin prophylaxis for recurrent cellulitis and 8005 control subjects derived from a population based cohort study.

5.2.2.1 Clinical material 1, five year follow-up (study IV)

Seventy-eight patients were alive at followup and 67 were reached by telephone. One patient had declined to participate in the study. Two patients had moved and their health records were not available. Thus, electronic health records were available of 87 patients (Figure 3). The median follow-up time was 4.5 years. For the patients alive at follow-up and for those deceased, the median follow-up time was 4.6 (4.0-5.4) and 2.3 (0.2-5.0) years, respectively. Overall, cellulitis recurred in 36 (41.4%) of the 87 patients.

Risk factors as assessed in the baseline study were studied in relation to recurrence in five years. The univariate and multivariable analysis of clinical risk factors are shown in Tables 13 and 14, respectively. In the multivariable analysis patients with a recurrence (n=30) were compared to those with no recurrence (n=44). Cases with cellulitis of the face (n=6) and upper extremities (n=7) were excluded. Age at the 1st cellulitis episode was omitted, as it could not be objectively assessed.

Table 12. Statistical analysis of clinical risk factors for acute cellulitis in 90 hospitalised patients with acute cellulitis and 90 population controls, and estimates of population attributable risk (PAR) of the risk factors independently associated with acute cellulitis.

Risk factor	**Patients** N (%)	**Controls** N (%)	**Univariate analysis** OR (95% CI)	**Final multivariable analysis**[1] OR (95% CI)	**PAR**
Chronic oedema of the extremity[2]	23 (28)	3 (4)	21.0 (2.8-156.1)	11.5 (1.2-114.4)	30%
Disruption of cutaneous barrier[3,4]	67 (86)	35 (46)	11.3 (4.0-31.3)	6.2 (1.9-20.2)	71%
Obesity	37 (41)	15 (17)	4.7 (1.9-11.3)	5.2 (1.3-20.9)	43%
Malignant disease	14 (16)	6 (7)	2.6 (0.9-7.3)	2.0 (0.5-8.9)	
Current smoking	32 (36)	16 (18)	3.0 (1.3-6.7)	1.4 (0.4-5.3)	
Alcohol abuse	12 (13)	2 (2)	6.0 (1.3-26.8)		
Cardiovascular disease	18 (20)	9 (10)	2.5 (1.0-6.4)		
Diabetes	13 (14)	9 (10)	1.7 (0.6-4.6)		
Skin disease	29 (32)	12 (13)	3.8 (1.6-9.4)		
Chronic ulcer	6 (7)	0	∞		
Toe-web intertrigo[4]	50 (66)	25 (33)	3.5 (1.7-7.1)		
Traumatic wound <1 month	15 (17)	4 (4)	3.8 (1.2-11.3)		
Previous operation >1 month	39 (43)	22 (24)	2.4 (1.2-4.7)		
Previous tonsillectomy[5]	12 (14)	13 (15)	1.2 (0.2-6.1)		

[1] A multivariable analysis was first conducted separately for the local and general risk factors. Variables appearing independently associated with acute cellulitis were included in the final multivariable analysis.
[2]Cellulitis of the face excluded (n=83)
[3]Combined variable (traumatic wound <1 month, skin disease, toe-web intertrigo, and chronic ulcer)
[4]Calculated for lower extremities (n=76)
[5]Data available in 88 cases and controls

Table 13. Univariate analysis of risk factors for cellulitis recurrence in 5 years follow-up.

Risk factors as assessed in the baseline study	**Recurrence in 5 years follow-up**		**p-value**	**OR**	**95% CI**
General risk factors	Yes (n=36)	No (n=51)			
Previous cellulitis episode at baseline	25 (69)	19 (37)	**0.003**	3.8	1.5 - 9.5
Median age at the baseline study, years	56.7	63.3	0.079	0.98	0.95-1.01
Median age at the 1st cellulitis episode, years	48.9	58.3	**0.008**	0.96	0.93-0.99
Alcohol abuse	3 (8)	7 (14)	0.513	0.6	0.1 - 2.4
Obesity (BMI ≥ 30)	19 (53)	17 (34)	0.082	2.1	0.9 - 5.2
Current smoking	10 (29)	21 (41)	0.232	0.6	0.2 - 1.4
Malignant disease	8 (22)	5 (10)	0.110	2.6	0.8 - 8.8
Cardiovascular disease	4 (6)	12 (20)	0.141	0.4	0.1 - 1.4
Diabetes	6 (17)	6 (12)	0.542	1.5	0.4 - 5.1
Tonsillectomy	3 (9)	9 (18)	0.242	0.4	0.1- 1.7
Antibiotic treatment before admission	10 (28)	15 (29)	0.868	0.9	0.4 - 2.4
Local risk factors					
Chronic oedema of the extremity[1]	13 (38)	10 (21)	0.095	2.3	0.9 - 6.1
Disruption of cutaneous barrier[2]	28 (93)	38 (86)	0.461	2.2	0.4 - 11.8
-traumatic wound < 1 mo	5 (14)	10 (20)	0.487	0.7	0.2 - 2.1
-skin diseases	14 (39)	14 (28)	0.261	1.7	0.7 - 4.2
-toe-web intertrigo[2]	20 (67)	29 (66)	0.946	1.0	0.3 - 2.8
-chronic ulcer	4 (11)	2 (4)	0.226	3.1	0.5 - 17.7
Previous operation	19 (53)	19 (37)	0.151	1.9	0.8 - 4.5
Markers of inflammation					
Peak CRP > 218 mg/l[3]	10 (28)	12 (24)	0.653	1.3	0.5 - 3.3
Peak leukocyte count > 16.9×10^9/l[3]	11 (31)	11 (22)	0.342	1.6	0.6 - 4.2
Duration of fever > 3 days after admission to hospital	3 (8)	7 (14)	0.513	0.6	0.1 - 2.4
Length of stay in hospital > 7 days	17 (47)	30 (59)	0.285	0.6	0.3 - 1.5

[1]Cellulitis of the face (n=6) excluded

[2]Cellulitis of the face (n=6) and upper extremities (n=7) excluded; disruption of cutaneous barrier comprises traumatic wounds < 1 month, skin disease, toe-web intertrigo, and chronic ulcers.

[3]75^{th} percentile

Table 14. Multivariable analysis (logistic regression, method enter) of clinical risk factors for cellulitis recurrence in 5 years follow-up in 74 patients hospitalised with acute cellulitis..

Risk factors as assessed in the baseline study	**OR**	**95% CI**
Previous episode at the baseline (PH)	3.8	1.3-11.1
Diabetes	1.0	0.2-4.0
BMI	1.06[1]	0.99-1.14
Chronic oedema of the extremity	1.3	0.4-4.2
Disruption of the cutaneous barrier[2]	1.9	0.3-11.4

[1]Per one unit increase
[2]Disruption of cutaneous barrier comprises traumatic wounds < 1 month, skin disease, toe-web intertrigo, and chronic ulcers

5.2.2.2 Clinical material 2, recurrent cellulitis (study V)

Table 15 shows the multivariable analysis of risk factors for recurrent cellulitis with benzathine penicillin prophylaxis in the clinical material 2. All corresponding variables that could be derived from the patient and control data are included. Thus, toe web intertrigo and chronic oedema could not be included in the case control analysis, because these variables could not be obtained from the controls.

One hundred fifty-eight (40%) of the 398 patients reported a prophylaxis failure i.e. an acute cellulitis attack during benzathine penicillin prophylaxis. There was no association between prophylaxis failure and toe-web intertrigo (p=0.49), chronic leg oedema (p=0.38) or BMI (p=0.83). Associations concerning toe web intertrigo and chronic leg oedema with prophylaxis failure were analysed for leg cellulitis cases only (n=305), but association of BMI was analysed for all cases.

Table 15. Multivariable analysis of risk factors for recurrent cellulitis with benzathine penicillin prophylaxis in 398 patients and 8005 controls.

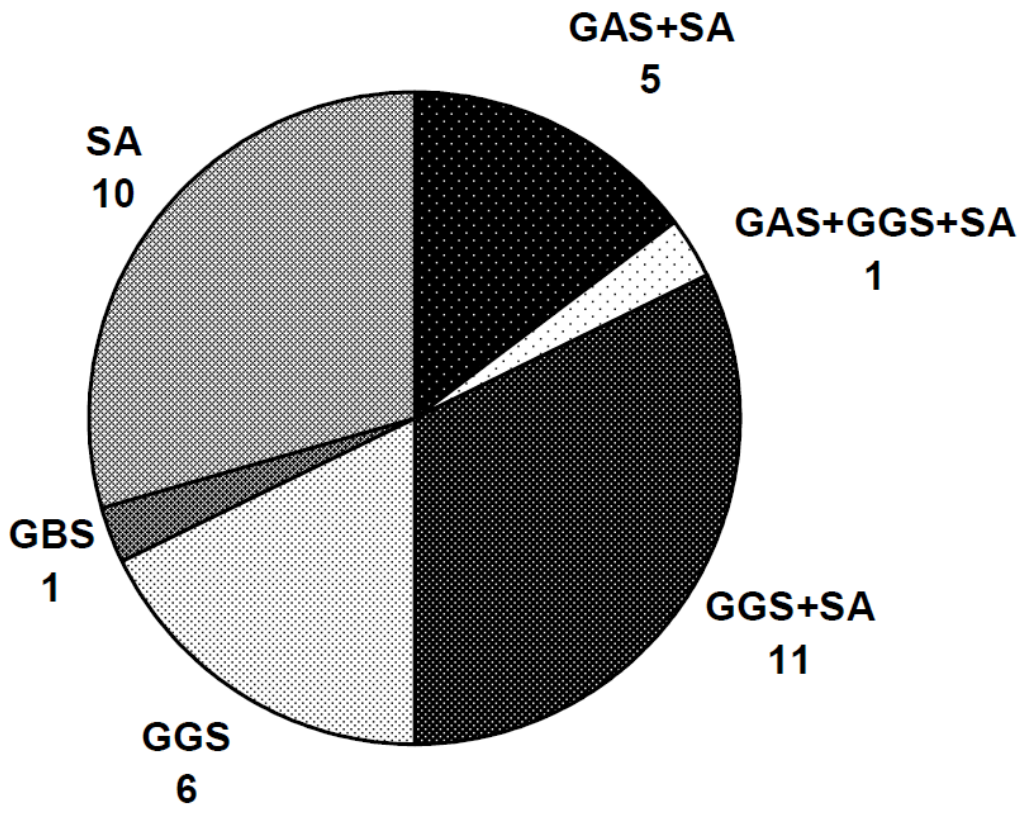
Risk factor	**Patients**	**Controls**	**OR**	**95% CI**
	n (%)	n (%)		
Male	163 (41)	3626 (45)	0.9	0.7-1.2
Age, years (median)	64.9	51.0	1.06[1]	1.05-1.07
BMI (median)	31.2	26.3	1.17[2]	1.15-1.19
Diabetes	82 (20.6)	451 (6.1)	1.7	1.2-2.3
Chronic dermatoses[3]	136 (34.5)	804 (11.6)	4.1	3.1-5.5
Psoriasis	29 (7.4)	156 (2.2)	3.7	2.3-6.1
Tonsillectomy	93 (23.6)	475 (5.9)	6.8	5.0-9.3

[1]Per one year
[2]Per one unit increase
[3]Excluding psoriasis

5.3 Bacterial findings in acute cellulitis (study II)

Skin swabs were examined in 73 cellulitis episodes in 66 patients. Of these, skin swabs were taken from a wound, intertriginous toe web or otherwise affected skin outside the cellulitis lesion (i.e. suspected portal of entry) in 39 patients and from the cellulitis lesion in 27 patients. BHS were isolated in 24 (36%) of the 66 patients, *S. aureus* concomitantly with BHS in 17 cases and alone in 10 cases (Figure 5).

Figure 5. Bacterial isolates in skin swabs in 66 patients hospitalised with acute cellulitis.



SA, *Staphylococcus aureus*

Throat swabs were obtained from 89 patients, 38 household members, and 90 control subjects. Bacterial findings from patients in relation to serogroups and sampling sites are shown in Table 16. Bacterial findings from the throat swabs are shown in Table 17.

All but two of the 31 GGS isolates (skin swabs, throat swabs and blood cultures from patients, and throat swabs from household members) were SDSE. *S. anginosus* was isolated in one patient's throat swab and a non-typeable GGS from another patient's throat swab. No GGS were isolated from the control subjects.

Table 16. Skin swab isolates in relation to sampling site, and throat swab isolates from patients

Site	**GAS**	**GGS**	**Other BHS**	**Total n:o of patients**
Infection focus	4	5	1 GBS	27
Site of entry	2	13		39
Skin isolates, total	6	18	1 GBS	66

Table 17. Throat swab isolates from 89 patients, 38 household members and 90 control subjects

Serogroup	**Patients (n=89)**	**Household members (n=38)**	**Control subjects (n=90)**
GGS	6	5[1]	
GAS	2		2
GBS		2	1
GCS	1		3
GFS	2		2
GDS	1		
Non-groupable		1	1
Total	12	8	9

[1]Two identical clones according to *emm* and PFGE typing from the nursing home cluster

There were eight recurrent cellulitis episodes during the study period (one recurrence in four and two recurrences in two patients). In two patients the same GGS strain (according to the emm typing and PFGE) was cultured from the skin swab during consecutive episodes (Table 18). The interval between the two successive episodes with the same GGS strain recovered was 58 and 62 days, respectively, as compared to the mean interval of 105 (range 46-156) days between the other recurrent episodes. The difference was, however, not statistically significant. There were no recurrent cases with two different BHS found in consecutive episodes.

A GAS strain was recovered in six skin swabs in six patients (Table 19). Three patients were living in the same household and harboured the same GAS strain according to *emm* typing and PFGE. Other three GAS strains were different according to the PFGE typing.

Blood cultures were obtained from 88 patients. In two cases GGS was isolated from blood.

There was a cluster of three cellulitis cases among residents of a small nursing home. Throat swabs were obtained from eight residents and staff members. Identical GAS clone, together with *S. aureus*, was isolated from the skin swabs in all three patients. Bacterial findings in samples from the patients in the cluster and the nursing home household are presented in Table 20.

Table 18. The *emm* types and identical PFGE patterns of the GGS isolates from patient samples

***emm* type**	**No. of isolates**	**Sample sites**	**No. of isolates with identical PFGE pattern**
stG6.0	3	skin	2 from recurrent episodes in 1 patient
stG11.0	2	skin	2 from recurrent episodes in 1 patient
stG245.0	3	skin, throat	2 from the same patient's skin and throat
stG480.0	4	skin, throat	See footnote 1
stG643.0	4	skin, throat	
stC6979.0	2	blood, skin[2]	
stG485.0	2	blood, skin[2]	
stG6.1	2	skin	
stG166b.0	2	skin	
stG5420.0	1	skin	
stC74A.0	1	skin	

[1]Identical strain according to the *emm* and PFGE typing was isolated from a household member's throat swab
[2] Blood and skin isolates from different patients

Table 19. The *emm* types and identical PFGE patterns the GAS isolates from patient samples

emm type	**No. of isolates**	**Sample sites**	**No. of isolates with identical PFGE pattern**
emm11.0	1	throat	
emm12.0	1	throat	
emm28.0	1	skin	
emm73.0	1	skin	
emm81.0	3[1]	skin	3
emm85.0	1	skin	

[1]Nursing home cluster, see Table 20

Table 20. Bacterial findings among patients of the nursing home cluster and their household

		Throat	Skin	*emm* type
Patients (n=3)	GAS	-	3	emm81.0[1]
	GGS	-	1	stC6979.0
Household (n=8)	GAS	-	n/a	
	GGS	2		stG6.1[2]
	GBS	1		

[1]All three identical PFGE profile, one patient harboured also GGS
[2]Identical PFGE profile

5.4 Serological findings in acute and recurrent cellulitis (study III)

Paired sera were available from 77 patients. Median interval between acute and convalescent phase sera was 31 days, ranging from 12 to 118 days. One patient declined to participate in the study after initial recruitment and 12 patients did not return to the convalescent phase sampling.

5.4.1 Streptococcal serology

A total of 53 (69%) patients were ASO seropositive and 6 (8%) were ADN seropositive. All six ADN seropositive patients were also ASO seropositive. Thus, streptococcal serology was positive in 69% of the 77 patients with paired sera available. In acute phase the ASO titres ranged from 22 IU to 4398 IU and in the convalescent phase from 35 IU to 3674 IU. Values for ADN ranged from 70 IU (background threshold) to 726 IU and 841 IU in the acute and convalescent phases, respectively. Positive ASO serology was found already in the acute phase in 59% (31/53) of ASO seropositive patients. The mean positive (≥200 IU) values for ASO in the acute and convalescent phase were 428 IU and 922 IU, respectively, and for ADN 461 IU and 707 IU, respectively. Antibiotic therapy had been initiated in the primary

care in 22 (29%) of the 77 cases before admission. Findings of streptococcal serology in relation to prior antibiotic therapy and bacterial findings are shown in Tables 21 and 22, respectively.

Table 21. Positive ASO and ADN serology in relation to prior antibiotic therapy in 77 patients with serological data available

Serological finding	**Antibiotic therapy prior to admission**		
	Yes (N=22) n (%)	No (N=55) n (%)	Total (N=77) n (%)
ASO +	11 (50)[1]	42 (76)[1]	53 (69)
ADN +	1 (5)[2]	5 (9)[2]	6 (8)

[1]χ2 test p=0.024
[2]Fisher's test p=0.69
ASO+ and ADN+, positive serology for ASO and ADN, respectively

Table 22. Serological findings in relation to bacterial isolates in 77 patients hospitalised with acute non-necrotising cellulitis

Serological finding	**Bacterial isolate**			
	S. aureus (n=23) n (%)	*S. aureus* only (n=9) n (%)	GAS (n=4) n (%)	GGS n=(18)[1] n (%)
ASO+	18 (78)	5 (56)	4 (100)	16 (89)
ADN+	4 (17)	0	3 (75)	2 (13)
ASTA+	1 (4)	0	0	1 (6)

[1] GGS in two patients from blood culture only, in one skin swab both GGS and GAS
ASO+, ADN+, and ASTA+, positive serology for ASO, and and ASTA respectively

Both of the two patients with GGS isolated in blood culture, were ASO seropositive, but ADN seronegative. Altogether, of the 53 patients with positive serology for ASO, 21 (40%) had GAS or GGS isolated in skin swab or blood, and 32 (60%) had no BHS isolated.

Of the 77 patients with serological data available, 16 had a skin lesion with a distinct border and thus could be classified as having "erysipelas". In the remaining 61 patients, the lesion was more diffuse, thus representing "cellulitis". ASO seropositivity was more common in the former than in the latter group [13/16 (81%) and 40/61 (66%), respectively], yet the difference was not statistically significant (p=0.36).

Sera of five of the six patients with a recurrence during the initial study period were available for a serological analysis. Three of the five were ASO seropositive and two were ADN seropositive. Of the three patients in the nursing home cellulitis cluster all were ASO seropositive and two were ADN seropositive. There was no statistically significant difference in the median ASO titres between patients with a negative history of cellulitis (NH) and patients with a positive history of cellulitis (PH) in acute or convalescent phase. Similarly, there was no difference in ASO values between those with a recurrence in five years follow-up and those without (data not shown).

Ten (11%) of the 89 control subjects had ASO titre ≥200 U/ml, with highest value of 464 U/ml, and three (3%) had ADN titre ≥200 U/ml, with highest value of 458 U/ml.

5.4.2 ASTA serology

Three (4%) patients were ASTA seropositive with highest ASTA titre of 2 units. However, they were also ASO seropositive with high ASO titres in the convalescent phase (701 IU, 2117 IU, and 3674 IU, respectively). Of the 89 controls, 11 (12%) were ASTA seropositive with titre of 8 IU/ml in one, 4 IU/ml in four and 2 IU/ml in six control subjects. Furthermore, three of the ASTA seropositive controls had ASO titre ≥200 IU/ml.

5.5 Antibiotic treatment choices in relation to serological and bacterial findings

For the 90 acute cellulitis patients the initial antibiotic choice was penicillin G in 39 (43%), cefuroxime in 26 (29%), clindamycin in 24 (27%) cases and ceftriaxone in one case. The antibiotic therapy was switched due to a suspected inadequate treatment response in 17% (15/90) of the cases, penicillin G in 23% (9/39), cefuroxime in 19% (5/26) and clindamycin in 4% (1/24). Table 23 shows the initial antibiotic treatment choices and the decisions to switch to another antibiotic in relation to the serological findings. Of the 77 patients with serological data available 11 patients with *S. aureus* were initially treated with penicillin G. Of these, penicillin was switched to another antibiotic due to suspected inadequate response in four cases. However, all four cases also had positive streptococcal serology.

Table 23. Initial antibiotic treatment and suspected inadequate response in relation to bacterial and serological findings in 77 patients with serological data available

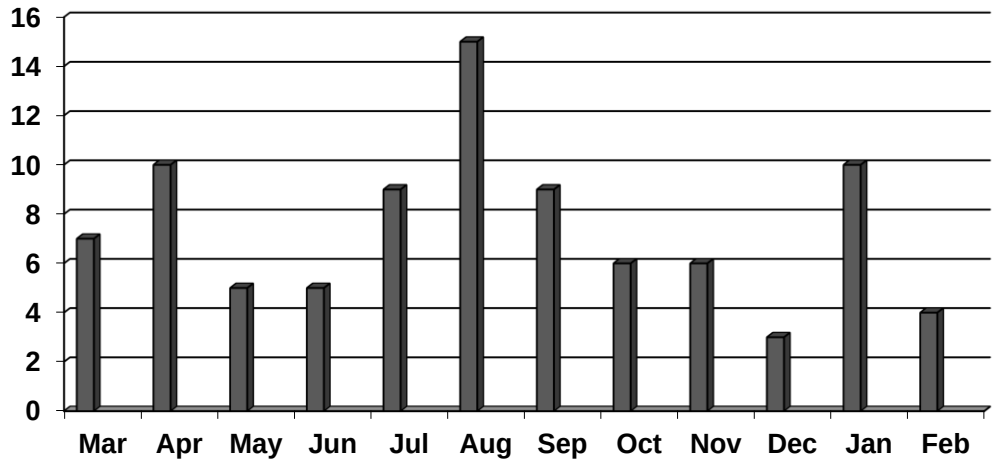
	Antibiotic switched due to suspected inadequate treatment response, n (%)	
Antibiotic initiated on admission	positive streptococcal serology (n=53)	negative streptococcal serology (n=24)
penicillin	6/24 (25%)	0/10
other	3/29 (10%)	1/14 (7%)

5.6 Seasonal variation in acute cellulitis (study II)

During the seven months when the average temperature in Tampere in the year 2004 (http://ilmatieteenlaitos.fi/vuosi-2004) was over 0°C (April - October) 59 patients (8.4/month) were recruited in to the study as compared to 30 patients (6.0/month)

recruited between November and March. Monthly numbers of recruited patients between March 2004 and February 2005 (n=89) are presented in Figure 6.

Figure 6. Number of patients recruited per month between March 2004 and February 2005



5.7 C-reactive protein and pentraxin-3 in acute bacterial non-necrotising cellulitis (studies I and IV)

5.7.1 C-reactive protein in acute bacterial non-necrotising cellulitis

For all 90 patients CRP was measured on admission (day 1) and in 82 cases at least on two of the subsequent four days. In eight patients CRP was measured only twice during the days 1-5. The days of the highest CRP value (peak CRP) for a given patient are shown in Figure 7.

Peak CRP was elevated (>10 mg/l) in all but one patient. Mean acute phase peak CRP was 164 mg/l, SD 85.2 (min 5 mg/l, max 365 mg/l). Mean CRP value on day 1 (admission) was 128 mg/l (min 1 mg/l, max 350 mg/l). CRP was normal (<10 mg/l) on admission in three patients. The one patient with normal CRP was a 56 year old woman

with breast carcinoma operated six years earlier. She had suffered four previous cellulitis episodes in the chronically oedematous right upper extremity and had been prescribed procaine penicillin for prophylaxis, instead of benzathine penicillin (most probably by mistake). She had also taken 1125 mg of cephalexin in the morning before being admitted to the hospital due to cellulitis in the right upper extremity.

Figure 7. Distribution of the hospital days on which the highest CRP value for a given patient (peak CRP) was recorded in 90 patients hospitalised with cellulitis (day 1=admission).

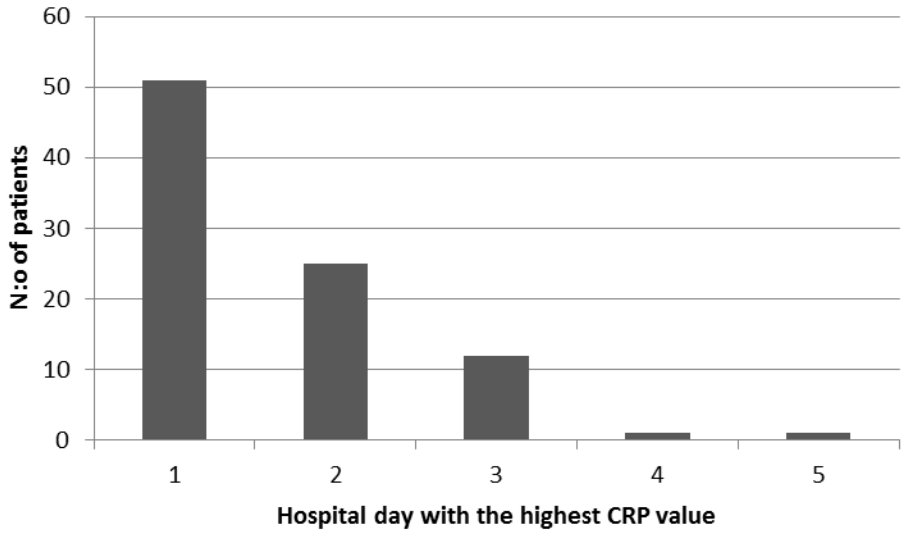
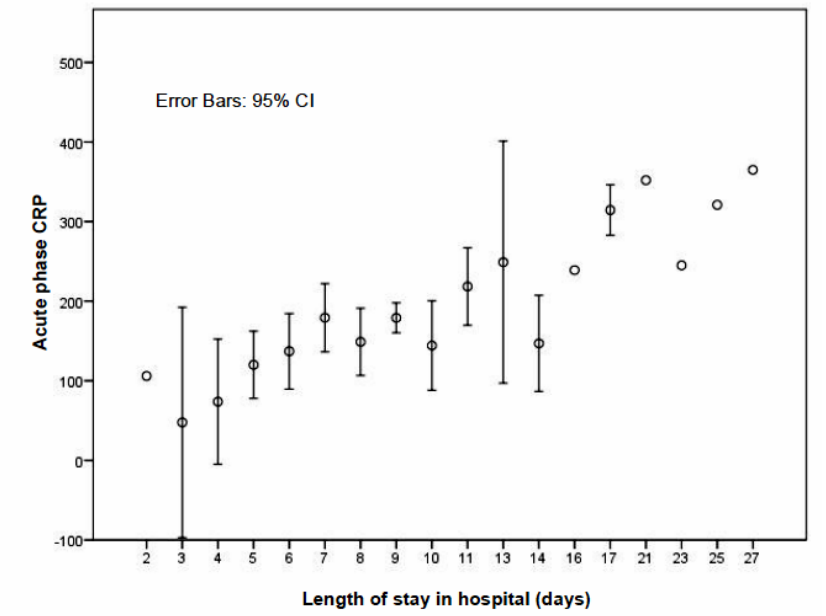


Figure 8. Length of stay in hospital in relation to highest CRP value in days 1-5 (1=admission) in 90 patients hospitalised with cellulitis (Spearman's correlation r_s=0.52, p<0.001).



High peak CRP (≥75th percentile, 218 mg/l) was statistically significantly associated with PH in the univariate analysis (p=0.037, study I). Also, high peak leukocyte count [(≥75th percentile, 16.9 x 10^9) p=0.037], duration of fever >3 days after admission (p=0.007) and length of stay in hospital (LOS) >7 days (p=0.019) were associated with PH. These are considered as markers of inflammatory reaction, and they are strongly associated with each other (data not shown). Furthermore, obesity (p=0.014) and no traumatic wound less than 1 month before admission (p=0.014) were associated with PH in the univariate model. In the multivariable analysis (Table 24), high peak CRP, obesity and no traumatic wound <1 month remained statistically significantly associated with PH.

Table 24. Logistic regression analysis (method forward stepwise) of risk factors associated with positive history of cellulitis (PH).

	Patients with positive history of cellulitis (n=44)	**Patients with negative history of cellulitis (n=46)**		
	n (%)	n (%)	OR	95% CI
High peak CRP[1]	15 (34)	7 (15)	3.5	1.1-10.8
Obesity (BMI≥30)	24 (55)	13 (28)	3.0	1.2-7.6
Traumatic wound <1 mo	3 (7)	12 (26)	0.2	0.05-0.9
Variables offered but not entered in the equation[2]				
High peak leukocyte count[1]	15 (34)	7 (15)		
Length of stay in hospital >7 days	29 (66)	19 (41)		

[1]75th percentile corresponding CRP 218 mg/l and leukocyte count 16.8 x 10^9
[2]Duration of fever >3 days after admission was omitted because of small numbers and wide (95%) CI

5.7.2 Pentraxin-3 in acute cellulitis

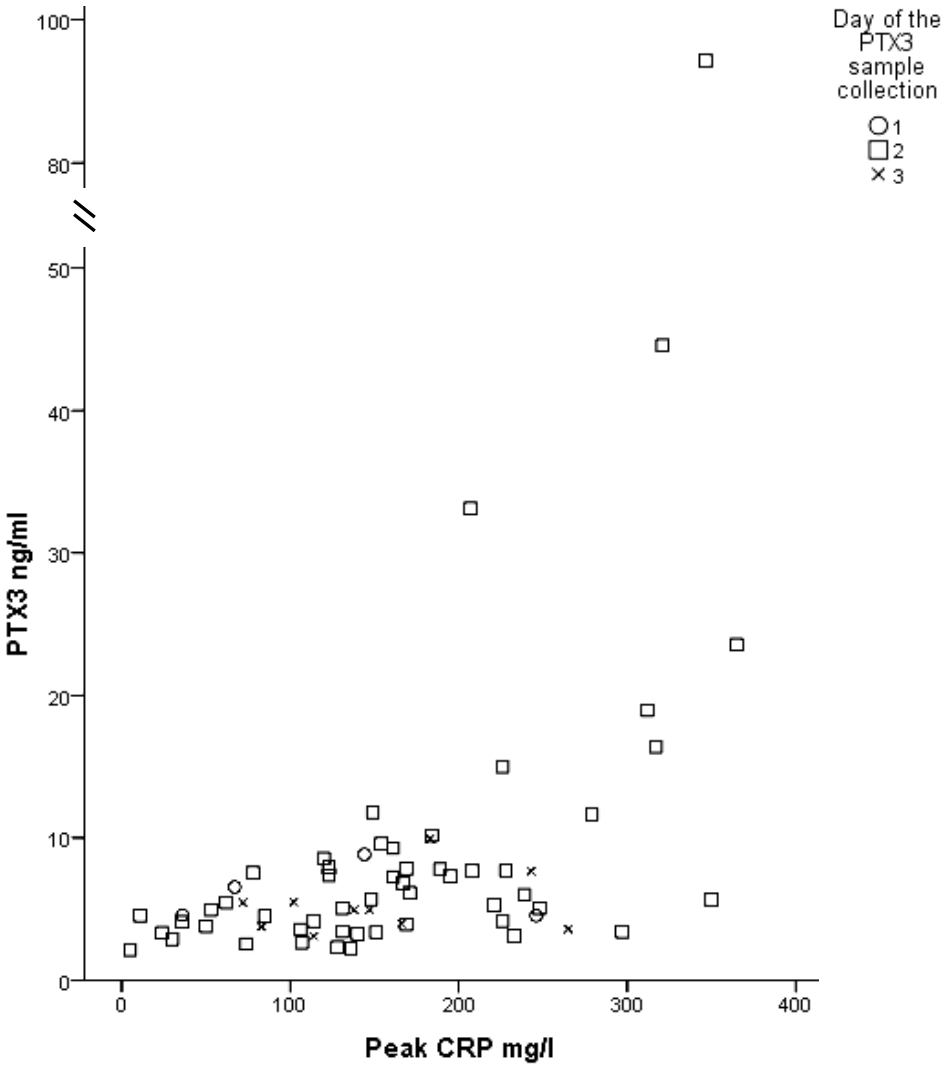
Acute phase sera for PTX3 analyses were collected and stored from 89 patients on hospital days 1-6 (day 1=admission) in 4, 52, 10, 10, 9 and 4 cases, respectively. PTX3 concentrations in relation to peak CRP concentration and day of PTX3 measurement

are shown in Figure 9. Convalescent phase sera were obtained from 73 patients one month after admission (median 31 days, range 12 to 67 days, except for one patient 118 days).

Mean acute phase PTX3 concentration among 89 patients was 8.0 ng/ml. Because only one PTX3 measurement in acute phase was available, statistical analyses were conducted for the 66 cases with acute phase sample collected on days 1-3. For these 66 cases mean PTX3 concentration was 8.7 ng/ml (median 5.5 ng/ml, range 2.1-94.3 ng/ml). Mean convalescent phase PTX3 concentration was 2.9 ng/ml (median 2.5 ng/ml, range 0.8-11.8, n=75).

PTX3 values showed a statistically significant correlation with peak CRP (r_s 0.50, $p<0.01$, Figure 9). In contrast to CRP, however, there was no statistically significant association between PTX3 and PH (Mann-Whitney U-test, p=0.58).

Figure 9. Peak CRP concentration on hospital days 1-5 (1=admission) and PTX3 concentrations in relation to the day of PTX3 sample collection.



5.7.3 C-reactive protein and pentraxin-3 as predictors of cellulitis recurrence

As CRP was associated with PH, its value, together with PTX3, in predicting cellulitis recurrence in five years was studied using receiver operating characteristic (ROC) analysis. CRP or PTX3 did not predict recurrence of cellulitis in five years. ROC curves for peak CRP and PTX3 in 87 and 65 patients, respectively, are shown in Figures 10 and 11, respectively. Area under the ROC curve for CRP [AUC(ROC)] = 0.499 (CI 0.371-0.626, p=0.98), and for PTX3 [AUC(ROC)]=0.535 (95% CI 0.39-0.68, p=0.64).

Figure 10. Receiver operating characteristic (ROC) curve for peak acute phase C-reactive protein (CRP) level on hospital days 1-5 (1 = admission) in relation to five year follow-up (n=87). Straight line = reference line for ROC(AUC)=0.500.

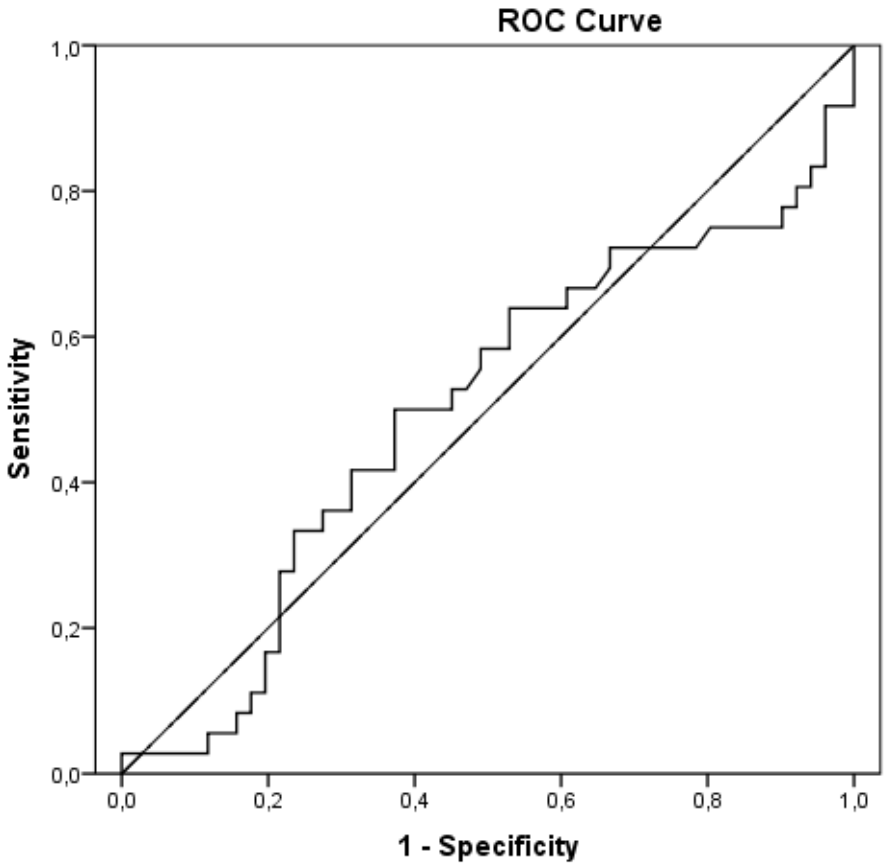
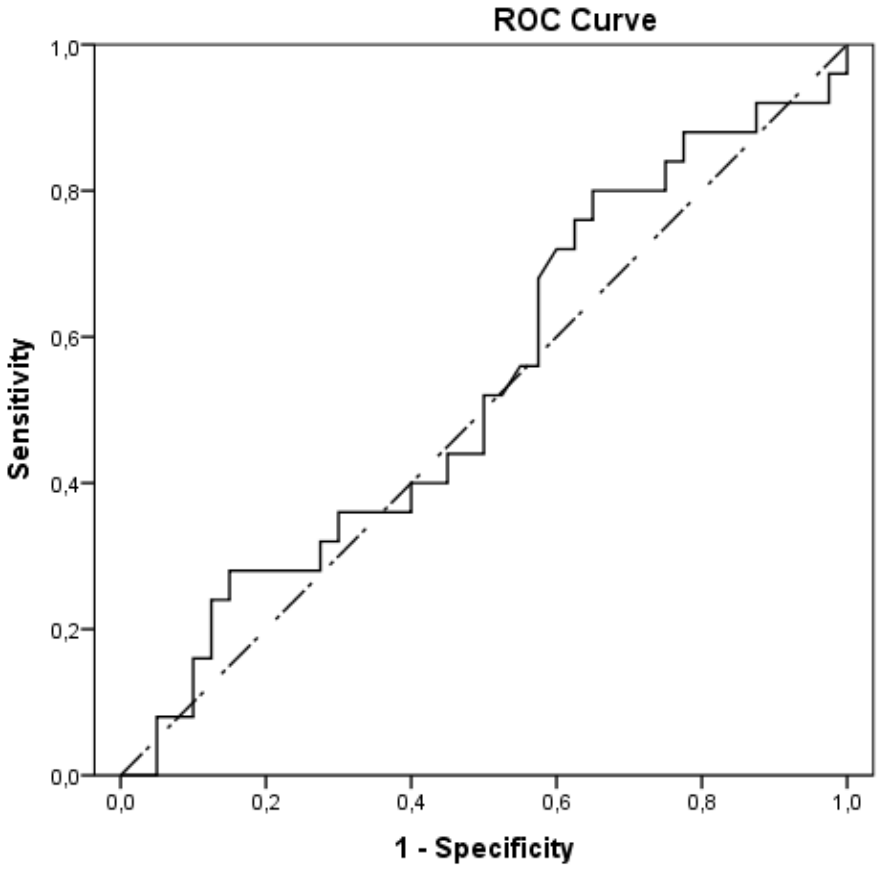


Figure 11. Receiver operating characteristic (ROC) curve for acute phase pentraxin-3 (PTX3) level measured on hospital days 1-3 (1 = admission, n=65) in relation to cellulitis recurrence in five year follow-up. Straight line = reference line for ROC(AUC)=0.500.



6. DISCUSSION

6.1 Clinical risk factors for acute cellulitis and recurrent cellulitis

6.1.1 Clinical risk factors for acute cellulitis (study I)

Chronic oedema of the extremity, disruption of the cutaneous barrier and obesity were independently associated with acute cellulitis (Study I, Table 12), which is in accordance with previous controlled (Dupuy et al. 1999, Roujeau et al. 2004, Björnsdottir et al. 2005, Mokni et al. 2006, Bartholomeeusen et al. 2007, Halpern et al. 2008, Eells et al. 2011) and non-controlled studies (Jorup-Rönström 1986, Eriksson et al. 1996, Lazzarini et al. 2005, Cox 2006). Skin breaks are considered to serve as portals of entry to the pathogens. Indeed, it has been shown that pathogenic bacteria are abundantly present in macerated toe webs in patients with acute cellulitis (Semel and Goldin 1996, Björnsdottir et al. 2005, Hilmarsdottir and Valsdottir 2007) and this was found in the present study as well (Table 16).

In one previous study (Dupuy et al. 1999) lymphoedema, analysed separately from other leg oedema, showed the strongest association with acute cellulitis (OR 71 for lymphoedema vs. OR 2.5 for other leg oedema). In the present study lymphoedema was not recorded separately from other causes of chronic oedema as it is not always possible to make a clear distinction (Cox 2006). The mechanism by which chronic oedema predisposes to cellulitis is unsolved as yet. The disturbance in lymphatic flow is associated with susceptibility to infection (Drinker 1938). The accumulation of antigens, protracted trafficking of dendritic cells, and diminished clearance of inflammatory mediators may have a role (Alitalo et al. 2005, Angeli and Randolph 2006, Damstra et al. 2008). Further, various other factors (Table 5), have been found to associate with cellulitis also in the previous studies. Thus, it is evident that a susceptibility to acute cellulitis is multifactorial.

More patients were recruited in the study during the warm months from April to October than during the cold months November to March (8.4 and 6.0 per month, respectively, Study II). This is in accordance with some previous studies reporting more cellulitis cases during the summer than during the winter (Ellis Simonsen et al. 2006, Bartholomeeusen et al. 2007, Haydock et al. 2007, McNamara et al. 2007b). However, the present study included only hospitalised patients, and was not primarily designed to study the incidence of cellulitis. Thus, no conclusions can be made based on the findings concerning seasonality. Moreover, no clinically relevant information could be derived from such observations until a plausible and proven explanation for seasonality of cellulitis incidence is at hand.

6.1.2 Clinical risk factors for recurrent cellulitis (studies I, IV, V)

6.1.2.1 Previous cellulitis

When treating a cellulitis patient, two questions arise in the clinician's mind: Does this patient have a high or a low risk for recurrence? What can be done to prevent a recurrence? An answer for the first question was sought in the present study. The only risk factor found associated with recurrence after an attack of acute cellulitis was previous cellulitis (Study IV). The risk for a recurrence in the PH patients was more than twice that of the NH patients (57% vs. 26%, respectively). Overall, cellulitis recurred in 41% of the patients in five years. In previous studies the risk of recurrence has been in the order of 10% per year (Table 2), and the findings of the present study fit well into that frame. Also, the association of previous cellulitis with subsequent recurrences is in accordance with previous studies (Roujeau et al. 2004, Björnsdottir et al. 2005, Halpern et al. 2008). The association could be explained by either an inherited or an acquired predisposition to cellulitis. The latter seems evident in the cases of upper extremity cellulitis, appearing after mastectomy and axillary evacuation (El Saghir et al. 2005, Vignes and Dupuy 2006). Nevertheless, there is some evidence of a pre-existing susceptibility to leg cellulitis. In two lymphoscintigraphic studies on patients with a primary attack of leg cellulitis, the lymphatic flow was impaired not only in the affected, but also in the non-affected leg with no clinical lymphoedema (Damstra et al.

2008, Soo et al. 2008). It is plausible, however, that cellulitis itself makes one more susceptible to subsequent attacks, as the persistent clinical oedema appears frequently in the ipsilateral leg after the first attack of cellulitis (Cox 2006).

Whether the susceptibility to recurrent cellulitis is acquired or inherited, cannot be concluded on the basis of the present study. Interestingly, however, the PH patients had been younger during their first cellulitis episode than the NH patients (Study I). Furthermore, there was no difference in the median age of PH and NH patients (58 years for both), as one would expect PH patients to be older. One explanation for that would be an inherited susceptibility of PH patients to infections in general or to cellulitis particularly. Nonetheless, in another case-control study (Dupuy et al. 1999), patients with recurrent cellulitis were older than those with their first episode (mean age 60.3 and 56.5 years, respectively). The patient population in the present study included only hospitalised patients, which may skew the data. It is possible that patients' age and the number of previous episodes of cellulitis influence the decision between hospitalisation and outpatient treatment.

6.1.2.2 Obesity

Obesity was more common among PH than NH patients in the present study (Study I), which is in line with the findings of the previous studies (Dupuy et al. 1999, Björnsdottir et al. 2005, Lewis et al. 2006, Bartholomeeusen et al. 2007). Also, obesity was associated with recurrent cellulitis with benzathine penicillin prophylaxis (Study V). However, in the five year follow-up study (Study IV) only the history of previous cellulitis at baseline, but not obesity, was associated with the risk of recurrence. This may reflect the role of obesity as a predisposing factor for cellulitis in general, as obesity was common among both NH and PH patients in Study I. Obesity was associated with cellulitis and recurrent cellulitis with benzathine penicillin prophylaxis, independently of diabetes (Studies I and V) and chronic oedema (Study I), which frequently occur together. The mechanisms predisposing to various infections remain unclear, yet one would expect that losing weight, or reducing the burden of obesity on the population, would reduce the burden of cellulitis. As yet, no studies have addressed this in a clinical setting.

The patients with PH had more often been operated on the ipsilateral leg than those with NH (study I). Saphenous venectomy, leading to lymphatic compromise, is suggested to predispose to recurrent cellulitis by case series (Greenberg et al. 1982, Baddour and Bisno 1984, Baddour and Bisno 1985) and shown to be associated with acute cellulitis in a case-control study (Björnsdottir et al. 2005). Based on the present study and the two previous studies comparing the risk factors between recurrent cases and cases with first episodes (Dupuy et al. 1999, Björnsdottir et al. 2005) it seems evident that all previous operations on the ipsilateral site predispose to recurrent cellulitis as well. However, in the present study and in that by Dupuy et al (Dupuy et al. 1999) all leg surgery was grouped together, thus saphenous venectomies were not distinguished from other surgery.

6.1.2.3 Traumatic wound

Previous traumatic wound was more common in NH patients than in PH patients (OR 6.0, Study I). However, in the five year follow-up there was no significant difference in the recurrence risk between patients with recent trauma and those without (Table 13). It has been discussed previously that trauma as a risk factor for cellulitis may be more temporary than other, more persistent risk factors. Some studies have shown that the risk of recurrence of cellulitis in a patient with no other predisposing factors is probably low (Björnsdottir et al. 2005), while others have suggested that primary cellulitis itself is a risk factor for recurrence (Cox 2006) Our study supports the latter view. Nonetheless, it is not possible to draw definite conclusions on causal relationships on the basis of the present study.

6.1.2.4 Diabetes

In our data recurrent cellulitis in patients with benzathine penicillin prophylaxis was associated with diabetes (OR 1.65, Study V), in line with previous studies exploring the role of diabetes as a risk factor for common infections. In a retrospective cohort study in Ontario, Canada the risk ratio for cellulitis was 1.81 (99% CI 1.76–1.86) (Shah and Hux 2003), which corresponds well to the OR for diabetes observed in the present study. However, in that study the definition of cellulitis may have included also

abscesses and infections of chronic wounds, making the comparison to the present study unreliable. In a prospective cohort study in the Netherlands, (Muller et al. 2005) the incidence of cellulitis was 0.7% per year in diabetic patients as compared to 0.3% per year in non-diabetic hypertensive patients in general practice. Likewise, abscesses and other suppurative infections were included in a case-control study on cellulitis (Mokni et al. 2006), and another on recurrent cellulitis (Lewis et al. 2006) These reported OR 1.3 and OR 1.54, for diabetes, respectively, but neither was statistically significant. Similarly, in the present study OR 1.7 for diabetes in the acute cellulitis study (Study 1) did not reach statistical significance. This may reflect the small size of the study population with 90 cases in the present study and 114 (Mokni et al. 2006) and 47 (Lewis et al. 2006) in the other two. In the most recent case-control study (Eells et al. 2011) with 50 hospitalised patients and 100 controls diabetes was independently associated with acute cellulitis. Tinea pedis and toe web maceration were not assessed in that study nor ours (Study V). The role of diabetes as an independent risk factor for cellulitis has been disputed (Halpern 2012), emphasising that fungal infection is a more important, and easily treated, predisposing factor. Diabetic patients are prone to fungal infections and other skin problems, which may lay behind the association with cellulitis (Muller et al. 2005, Bristow 2008).

6.1.2.5 Age

In addition to obesity and diabetes, increasing age was associated with recurrent cellulitis in patients with benzathine penicillin prophylaxis (Study V). This may partly be explained by the recurring nature of the infection and partly by the declining immune mechanisms in senescence. In a previous population based study (McNamara et al. 2007b) the incidence of cellulitis increased with age (3.7% per year of age).

6.1.2.6 Chronic dermatoses

Chronic dermatoses, and especially psoriasis, were associated with recurrent cellulitis with benzathine penicillin prophylaxis (Study V). The onset of a certain type of psoriasis (guttate psoriasis) is known to be strongly associated with streptococcal throat infections (Mälkönen and Suomela 2011). Also, exacerbation of plaque psoriasis is

associated with streptococcal throat infections, which, in turn are more common among psoriatic patients than non-psoriatic controls (Gudjonsson et al. 2003). There are no previous reports of the association of recurrent cellulitis and psoriasis, or other streptococcal infections than tonsillitis (Mälkönen and Suomela 2011). The association of recurrent cellulitis and psoriasis in the present study may be due to broken skin integrity in psoriatic plaques. These plaques are, however, characterised by hyperkeratosis, in contrast to skin conditions disrupting the cutaneous barrier previously recognised to be associated with cellulitis.

6.1.2.7 Previous tonsillectomy

Previous tonsillectomy was strongly associated with recurrent cellulitis (Study V). This could implicate an elevated susceptibility to streptococcal infections or to infections in general. On the other hand, tonsillectomy could predispose an individual to other streptococcal infections than tonsillitis. However, due to a methodological weakness, this finding must be interpreted with caution. The question regarding tonsillectomy was different in the patient and control questionnaires, which may lead to an underestimation of the frequency of a previous tonsillectomy in the control population. Moreover, the history of tonsillectomy was not statistically significantly associated with acute cellulitis in the clinical material 1 (Table 12).

6.1.3 Susceptibility to cellulitis and prevention of recurrences

In conclusion, chronic oedema, disruption of the cutaneous barrier, and obesity are risk factors for acute cellulitis leading to hospitalisation. The susceptibility to recurrent cellulitis is obviously multifactorial. The risk may be low if the patient has no known risk factors (Jorup-Rönström and Britton 1987), but increases along cumulating risk factors (McNamara et al. 2007a, Tay et al. 2015). Diabetes, obesity, increasing age, psoriasis and other chronic dermatoses, and particularly previous cellulitis are risk factors for recurrence. A single episode of cellulitis probably makes one more vulnerable to subsequent attack, but inherited susceptibility to infections, and to cellulitis in particular, may play a role. Thus, to answer the first question presented above, the risk of recurrence after the primary cellulitis attack is one in four during the

next five years. After the first recurrence the risk for subsequent recurrence is over 50%.

The answer to the second question, whether the recurrences of cellulitis can be prevented, is less clear. Apart from the studies on prophylactic antibiotics, there are no intervention trials on the prevention of recurrent cellulitis. Risk factors that can be cured or alleviated include toe web maceration, tinea pedis, and chronic oedema of the extremity, as has been discussed previously (Baddour and Bisno 1984, Dupuy et al. 1999, Pavlotsky et al. 2004, Roujeau et al. 2004, Björnsdottir et al. 2005, Lewis et al. 2006, Mokni et al. 2006, Bartholomeeusen et al. 2007, Halpern et al. 2008, Halpern 2012). However, these risk factors are frequently overlooked by the patients themselves, and probably also by treating physicians (Cox 2006). Whether it would be beneficial to start a prophylactic antibiotic treatment already after the first cellulitis attack remains to be elucidated. It is of interest, however, that the recent, most comprehensive study on antibiotic prophylaxis for prevention of cellulitis recurrences (Thomas et al. 2013) included patients with at least two episodes of cellulitis. In a previous study by the same trialists (Thomas et al. 2012), where the majority of the patients had had only one episode of cellulitis, the number needed to treat to prevent one recurrence was higher (8 vs. 5). However, the previous trial suffered from a slow recruitment and did not reach enough statistical power. It is likely, however, that the more risk factors are present the more susceptible the patient is to a recurrence of cellulitis. After the primary attack of cellulitis, a young healthy patient with cellulitis arising from a trauma probably has a lower risk of recurrence than an obese diabetic patient with oedematous legs and macerated toe webs. The latter patient may benefit more from an antibiotic prophylaxis than the former, and intervention in the predisposing conditions can be attempted. The risk scores proposed in previous studies, predicting recurrence after a primary cellulitis episode (McNamara et al. 2007a, Tay et al. 2015), may be of help in the decision about antibiotic prophylaxis. However, they may lack sensitivity, as the former obviously excludes important risk factors, such as chronic oedema and obesity, and data on obesity was missing in half of the cases in the latter.

6.2 Bacterial aetiology of cellulitis

Microbiological diagnostics of cellulitis is very challenging as the skin may be intact with no sites for bacterial cultures available; furthermore, the bacteria identified may only represent local findings. In the present study bacterial aetiology of acute cellulitis was investigated by bacterial cultures (Study II) and serology (Study III). Based on these methods, streptococcal origin was confirmed in 56 (73%) of the 77 patients with acute and convalescent phase sera available. This finding is in line with previous studies in which streptococcal aetiology was demonstrated by culture, serology, or direct immunofluorescence in 61-88% of erysipelas and cellulitis patients (Leppard et al. 1985, Hugo-Persson and Norlin 1987, Bernard et al. 1989, Eriksson et al. 1996, Jeng et al. 2010).

In the present study BHS were isolated in 26 (29%) of the 90 patients (Study II). Two patients had SDSE in a blood culture and in 24 patients BHS were isolated from a skin swab specimen, which were obtained in 66 patients. The low yield of BHS in the skin swabs, and also in samples collected by invasive methods, has uniformly been reported in the previous studies (Leppard et al. 1985, Hook et al. 1986, Jorup-Rönström 1986, Hugo-Persson and Norlin 1987, Newell and Norden 1988, Duvanel et al. 1989, Eriksson et al. 1996, Björnsdottir et al. 2005). Only in the study by Semel and Goldin (1996), BHS was isolated from toe webs in 17 (85%) of 20 patients with cellulitis associated with athlete's foot.

Streptococcal serology, assessed by ASO and ADN in paired sera, was positive in 53 (69%) of the 77 cases with both serum samples available. All patients with GAS and 14 of the 16 with GGS isolated in skin swabs were ASO seropositive. One of the two GGS positive, but seronegative had cephalexin treatment initiated before admission. Only 40% of the seropositive patients had BHS isolated from skin or blood. Thus, a negative culture does not rule out BHS as the causative agent in acute cellulitis. On the other hand, mere presence of BHS on the skin of a cellulitis patient doesn't prove aetiology. The serological response, however, is a more plausible proof of streptococcal aetiology.

There was a statistically significant difference in the ASO seropositivity between the patients with prescribed antibiotics before admission and those without (50% vs. 76%, respectively, Study III). Antibiotic treatment may attenuate the serological response in

streptococcal disease (Anderson et al. 1948, Leppard et al. 1985), which is a plausible explanation for this difference. Prior antibiotic therapy did not, however, have a significant effect on the bacterial findings (Study II).

Blood cultures were positive in two patients (2%) in the present study. Both yielded SDSE. In previous studies positive blood cultures have been reported in 5-10% in prospectively collected materials (Jorup-Rönström 1986, Eriksson et al. 1996, Björnsdottir et al. 2005). In a recent retrospective study (Perl et al. 1999), blood cultures yielded BHS in 8 (1%) of 553 cases. *S. aureus* (postoperative infection), *Morganella* (haemodialysis) and *Vibrio* (fishbone injury) were isolated in one case each. In another large retrospective study (Peralta et al. 2006), 57 (19%) of the 308 limb cellulitis patients were bacteremic, and gram-negative rods were isolated in 14 (5%) patients. In a previous Finnish case series one of 30 blood cultures was positive and yielded GGS (Ohela 1978). The variation between studies most probably reflects differences in case definitions and the study design. Finally, in a systematic analysis of studies on erysipelas and cellulitis (Gunderson and Martinello 2012), 6.5% of the 2731 patients were bacteremic, 61% with BHS, 15% with *S. aureus*, and 23% with gram-negative bacteria. Again, the heterogeneity of the included cases limited the conclusions. The role of *S. aureus* in cellulitis is controversial. A distinction between erysipelas and cellulitis has been considered relevant, because of the presumed role of *S. aureus* as an aetiological agent in cellulitis but not in erysipelas (Leppard et al. 1985, Hook et al. 1986, Jorup-Rönström 1986, Bernard et al. 1989, Duvanel et al. 1989, Bisno and Stevens 1996). However, in a recent study there was no difference in the distribution of blood culture findings between cases classified as erysipelas or cellulitis (Gunderson and Martinello 2012). In the present study *S. aureus* was isolated in 27 (32%) of the 90 patients (Study II). In 10 patients, it was the only bacterial finding, and BHS was isolated concomitantly in 17 patients. However, 78% of the patients with *S. aureus* were ASO or ADN seropositive, and 56% of those with *S. aureus* only. Moreover, of the 77 patients with serological data available, 11 patients with *S. aureus* were initially treated with penicillin. Seven of these were successfully treated with penicillin alone. Yet all four patients who were switched to another antibiotic due to suspected inadequate response were ASO seropositive (Study III). Response to penicillin is indirect and by no means conclusive evidence of streptococcal or non-staphylococcal aetiology of cellulitis. Nevertheless, it adds to the evidence gained from

serology and bacterial culture, both of which lack full sensitivity. The majority of clinical *S. aureus* isolates produce penicillinase (Jeng et al. 2010). Thus, an inadequate treatment response with penicillin would be expected in staphylococcal cellulitis.

S. aureus is a frequent coloniser of chronic ulcers (Jockenhofer et al. 2013). Of the six patients with a chronic ulcer in the ipsilateral site in the present study, three harboured *S. aureus*. However, three patients had evidence of BHS infection based on culture or serology. Paired sera for serology were available in only two of the six patients.

ASTA gave positive findings, with low titres, in only three cases. Furthermore, only one patient with *S. aureus* had positive ASTA serology and none of those with *S. aureus* only in the skin swab. This finding adds to the evidence of streptococcal aetiology of cellulitis even if S. *aureus* is concomitantly present. ASTA serology was positive more often in controls (14%) than cases (4%). Thus, it is concluded, that ASTA has no role in the serodiagnosis of cellulitis, as has been discussed previously (Elston et al. 2010).

Taken together, the findings of the present study suggest that *S. aureus* may represent merely a colonisation instead of a true infection in the great majority of acute cellulitis cases, as is also shown in recent studies on non-suppurative cellulitis (Jeng et al. 2010, Eells et al. 2011). However, in some previous studies *S. aureus* has been isolated from blood of cellulitis patients (Jorup-Rönström 1986). These studies have included patients with abscesses, bursitis, septic arthritis, osteomyelitis, and necrotic infections, which differ from diffuse, non-suppurative cellulitis. Non-suppurative cellulitis, which the patients of the present study represent, may be considered to be caused by BHS, and thus penicillin may be considered as the treatment of choice. Caution should be exerted, however, when a wound or a chronic ulcer, especially in a diabetic patient, or an abscess is present, or when the patient is immunosuppressed. Staphylococci, gram-negative rods, or other microbes are important to consider in these cases (Carey and Dall 1990, Swartz 2004, Chira and Miller 2010, Finkelstein and Oren 2011). Also, it must be kept in mind, that the patient population in the present study included hospitalised patients, but not the most severely ill. Also, there were no diabetic foot infections, although one of the 13 diabetic patients had a chronic ulcer in the ipsilateral leg.

In the present study 82% of the patients had skin erythema with a non-clear margin and could therefore be classified as cellulitis rather than classic erysipelas. The common conception has been that *S. aureus* may be involved in cellulitis but very rarely in erysipelas. However, the majority of our cases showed evidence of streptococcal origin. Thus, in the clinical point of view it is not important to make a distinction between erysipelas and cellulitis, as has been discussed previously (Gunderson and Martinello 2012).

In conclusion, the present study suggests causal association of BHS in the majority of cases of acute cellulitis leading to hospitalisation. However, the role of *S. aureus* cannot be excluded in this setting. Patients with chronic ulcers may be prone to staphylococcal infections, but this issue cannot be fully elucidated based on the present study. Also, as shown in previous studies and case reports, other bacteria may be involved in cellulitis. However, in these cases a distinct predisposing condition, such as immunosuppression, or a specific environmental factor is usually present. If there is pus, *S. aureus* should be suspected.

6.3 Characterisation of β-haemolytic streptococci in acute non-necrotising cellulitis

GGS was isolated in skin samples in 18 patients, GAS in 6, and GBS in one patient. One patient harboured both GAS and GGS (Figure 5). GAS is usually considered to be the main streptococcal cause of cellulitis, but GGS is also found associated with cellulitis (Hugo-Persson and Norlin 1987, Eriksson 1999, Björnsdottir et al. 2005). A Finnish case series from the 1970s (Ohela 1978) is one of the earliest reports of the association of GGS with cellulitis. GGS has also been increasingly found in invasive infections, particularly among the elderly (Cohen-Poradosu et al. 2004, Brandt and Spellerberg 2009, Rantala et al. 2010).

All GGS skin isolates were SDSE, and they fell into 11 different emm types. According to the emm typing and PFGE pattern, identical GGS strains were isolated in two cases from recurrent episodes. In a third case, an identical GGS strain was isolated in both the skin and throat swabs from the same patient and in a fourth case from the patient's skin and from a household member's throat. All other GGS isolates showed

different PFGE patterns (Table 18). Three most common strains found in non-related cases were SDSE emm types stG480, stG6 (subtypes stG6.0 and stG6.1), and stG643, which were also among the four most common blood isolates in bacteremic SDSE infections in the population-based survey during 1995-2004 in Finland (Rantala et al. 2010). These *emm* types were also among the five most common types in a subsequent survey of clinical GGS strains in the Pirkanmaa Health District during 2008-9 (Vähäkuopus et al. 2012). .

Of the GAS isolates, all but the three isolated in the nursing home cluster (emm81.0) were of different emm types (Table 19). The most common GAS emm type during the years 2004-2007 in Finland was emm28 (Siljander et al. 2010), which was isolated in one patient. During the years 2008-9 the most common GAS strain in the Pirkanmaa Health District was *emm*77 (Vähäkuopus et al. 2012), which was not found in the present study.

Epidemics of erysipelas were not uncommon in the past; typically occurring in isolated settings like ships (Smart 1880). Reports of outbreaks of GAS diseases, including cellulitis and erysipelas have been published also in the last decades, mainly occurring at nursing homes or other similar facilities (Dowsett et al. 1975, Schwartz and Ussery 1992). A carrier state has been implicated in previous reports, based on culture findings and the temporal distance of cases. In the small cluster encountered in the present study (Table 20), no throat carriers were identified, and the cases appeared within a short time. Thus, direct patient to patient transmission is the most plausible mechanism involved.

The same GGS strain was found in consecutive cellulitis episodes in two patients, with a two months interval between the episodes in both cases. There were, however, no cases with different strains recovered in the subsequent episodes in a given individual. In only one case the same GGS strain was isolated both from the skin and the throat. Anal colonisation of GGS has been suggested to constitute the reservoir in a case series of recurrent erysipelas (Eriksson 1999). This was unfortunately not investigated in the present study. There is also evidence of a prolonged survival of GAS in macrophages and epithelial cells, despite an antibiotic treatment (Kaplan et al. 2006, Thulin et al. 2006). One could hypothesise that a recurrence within a relatively short time could be a consequence of viable intracellular bacteria escaping the immunological response and antibiotic treatment. It has been suggested that a

prolonged antibiotic treatment of acute cellulitis episode could reduce the risk for recurrence (Cox 2006), but more robust evidence is needed. Based on expert opinions, the Finnish guideline recommends a three weeks course of antibiotic in acute cellulitis and six weeks in recurrent cases (Bacterial Skin Infections: Current Care Summary, 2010).

Pharyngeal BHS colonisation was equally common in patients and control subjects (13%, 10%, respectively). The distribution of BHS serotypes was, however, different (7% vs. no GGS in patients and control subjects, respectively). In contrast, 21% of the household members harboured BHS in the throat, and the distribution of serotypes resembled that of the patients. This may suggest that BHS causing cellulitis are circulating in the households of the patients, due to factors predisposing to the carrier state of BHS. Cellulitis may then attack the member of the household with an accumulation of risk factors for cellulitis. However, in only one case, the same strain was recovered from the patient's skin and in the household, and in only one case from both the skin and the pharynx of a patient. The low yield of BHS in the skin swabs makes it difficult to show the association between throat carriage and cellulitis. The observed 2% rate of pharyngeal GAS colonisation in the controls is in the expected range in this adult population.

In conclusion, GGS instead of GAS was the predominant streptococcal finding in acute cellulitis. There was no clear predominance of any particular *emm* type among GAS or GGS isolates. The same strains were isolated in two recurrent cases within a short interval, implicating a relapse of the preceding infection. The concomitant colonisation of the skin and throat by the same BHS strain was rare. The throat may not be the reservoir of BHS in the majority of cellulitis cases, but throat carriage in the household may contribute to the susceptibility for cellulitis in the persons with other risk factors for cellulitis.

6.4 C-reactive protein and pentraxin-3 in acute cellulitis and recurrent cellulitis

In the present study CRP was elevated during the hospitalisation in all but one, and on admission in 97% (87/90) of the patients. The mean CRP value on admission (128

mg/l) was somewhat higher than values reported in two previous studies: In a study on hospitalised cellulitis patients only 77% had on admission an elevated CRP value (Krasagakis et al. 2011); the mean CRP was 88 mg/l in complicated and 43 mg/l in non-complicated cases, respectively. In another study the mean CRP values were 42 mg/l and 106 mg/l in patients with LOS ≤10 days and >10 days, respectively (Lazzarini et al. 2005). Fever was present in only 71% of cases in that study. Indeed, the fact that a fever was not part of the case definition in these two retrospective studies leaves open the possibility that various other, non-bacterial conditions clinically resembling erysipelas could have been included in the study material (Falagas and Vergidis 2005, Gunderson 2011, Hirschmann and Raugi 2012b).

Variables reflecting the intensity of inflammation, i.e. high peak CRP, high peak leukocyte count, and duration of fever, were associated with a previous history of cellulitis (PH) in the univariate analysis. Also, PH was associated with LOS, independently of age and diabetic status (Study I). LOS, in turn, correlated positively with CRP values, as was noted also in a previous study (Lazzarini et al. 2005). As the length of stay in hospital is partly a matter of a subjective decision of the attending physician, it may be influenced by high CRP values. Therefore, high peak CRP (≥75th percentile, 218 mg/l) was used as a marker of strong inflammatory response in another multivariable analysis including all risk factors which were associated with PH in the univariate model [obesity, recent traumatic wound, high peak leukocyte count, and LOS >7 days (Study I)]. Duration of fever >3 days was omitted, however, because of the small numbers and wide (95%) CI. In this multivariable analysis high peak CRP (OR 3.5), together with obesity and recent traumatic wound, was strongly and independently associated with PH. Based on this finding, the value of CRP and PTX3 as predictors of recurrence was investigated in the five year follow-up study (Study IV). However, measured at the baseline, these parameters had no association with further recurrence of cellulitis. There are no previous studies on the association of CRP with a risk of recurrence of cellulitis. In a study on complications of cellulitis (Krasagakis et al. 2011) high CRP was associated with local complications, but the association disappeared in the multivariable analysis. PTX3 has not been assessed in cellulitis previously.

In the majority (57%) of patients peak CRP was measured on day 1. Given the differences in the kinetics of CRP and PTX3, it is likely that the peak PTX3 had been

reached on the day 1 also in the majority of patients. The distribution of PTX3 values in acute cellulitis as assessed in the present study (mean 8.7 ng/ml, median 5.5 ng/ml, range 2.1-94.3 ng/ml) is in the same range with survivors of bacteraemia (Huttunen et al. 2011) and sepsis patients without organ failure (Uusitalo-Seppälä et al. 2013). In pneumonia patients (Kao et al. 2013) the PTX3 values were somewhat higher (12 ng/ml), but also the mean PTX3 value in healthy controls was higher than the convalescent phase values in the present study (6.1 ng/ml vs. 2.9 ng/ml).

In conclusion, CRP is elevated in practically all hospitalised cellulitis patients, yet it shows great variability between individuals. CRP values are correlated with the length of hospital stay. CRP thus appears to reflect the severity of the illness, consistently with previous studies on cellulitis (Lazzarini et al. 2005, Krasagakis et al. 2011) and several other conditions (Peltola 1982, Pepys and Hirschfield 2003, Heiro et al. 2007, Chalmers et al. 2008, Rhodes et al. 2011). On the other hand, CRP levels may as such contribute to the physicians' decisions in regard to the need of hospitalisation. Patients with PH had higher CRP values as compared to patients with NH. In contrast to our initial hypothesis neither high CRP or PTX3 could predict further recurrences..

6.5 Strengths and weaknesses of the study

Population controls were recruited in the clinical study 1. This adds to the knowledge concerning the risk factors for acute cellulitis, since in the previous studies control populations were comprised of hospitalised patients. A selection bias may affect the control population, as some of the risk factors studied may influence the willingness of a control candidate to participate. Moreover, those who participated may have been more health conscious than those who declined. However, the bias is likely to be different and acting in the opposite direction in the present study as compared to the previous studies using hospitalised controls.

A case-control study is retrospective by definition. Nevertheless, the design of the studies I and II (clinical material 1) may be described as "prospective", as many other authors have done (Dupuy et al. 1999, Roujeau et al. 2004, Björnsdottir et al. 2005, Mokni et al. 2006, Halpern et al. 2008, Eells et al. 2011), because the data concerning the clinical risk factors, treatment and outcome were systematically collected on the

admission to hospital. As a difference to a typical retrospective setting, blood samples and bacterial swabs were also systematically collected for research purposes. Age at the first cellulitis episode could not be ascertained from the patient charts, and is therefore prone to recall bias. Furthermore, a distinction between chronic oedema and oedema caused by the acute cellulitis itself could only be made on the basis of the interview. However, interobserver variation was avoided, as the same person (MK) interviewed and clinically examined all the patients and the control subjects.

It must be emphasised that the results of the studies on the clinical material 1 may not be generalizable to all patients with cellulitis, as the present study included only hospitalised patients. The majority of cellulitis patients may be treated as outpatients (Ellis Simonsen et al. 2006, McNamara et al. 2007b), but no data is available on that ratio in Finland. Moreover, the most severe cases, e.g. those admitted to an intensive care, were excluded.

The strength of the clinical material 2 (study V) is the large number of patients and controls. Due to the statistical power, it is possible to demonstrate relatively weak associations, such as that between recurrent cellulitis and diabetes. The main disadvantage of the clinical material 2 is that it represents only patients with benzathine penicillin prophylaxis, which may be offered more likely to those with comorbidities than to those without. Moreover, the 50% response rate raises the question of a selection bias, the direction of which could not be evaluated, as the reasons for not responding could not be assessed.

Individuals with a history of recurrent cellulitis could not be excluded from the control population in the clinical material 2. Given the relative rarity of cellulitis, as compared to obesity or diabetes, for example, bias due to this flaw is likely to be negligible. Moreover, it would rather weaken the observed association instead of falsely producing statistically significant associations.

Some variables were recorded by different methods in the patient and control populations: height and weight were measured in the controls, but these were self-reported by the patients. Again, this merely weakens the observed association, as a person's own weight is frequently underestimated rather than overestimated. As a consequence, the calculated BMI values would be lower than the true values in the patient population. (Gorber et al. 2007).

Finally, due to a methodological weakness, the frequency of tonsillectomies in the control population may be an underestimate and thus, previous tonsillectomy as a risk factor for recurrent cellulitis should be interpreted with caution. The data on highest CRP values during hospitalisation is valid, because CRP was measured more than twice in the great majority of patients. PTX3 was, however, measured only once, and in the majority of cases on day 2. The highest CRP value for a given patient was measured on day 1 in half of the patients. Thus, it is likely, that PTX3 values have already been declining at the time of the measurement.

6.6 Future considerations

A recurrence can be considered as the main complication of cellulitis. Even though the same clinical risk factors predispose to acute and recurrent cellulitis, it is likely, that cellulitis itself is a predisposing factor, making attempts to prevent recurrences a high priority. It is obvious, that treating toe web maceration and chronic oedema, losing weight if obese, quitting smoking, or supporting hygiene among the homeless (Raoult et al. 2001, Lewis et al. 2006) are of benefit in any case, even if recurrences of cellulitis could not be fully prevented. Thus, antibiotic prophylaxis probably remains the most important research area in recurrent cellulitis. It is not clear, whether prophylaxis should be offered already after the primary cellulitis episode, and what is the most effective mode of dosing the antibiotic or the duration of prophylaxis. Furthermore, a carefully designed study on the effect of treatment duration in acute cellulitis on the risk of recurrences would be important, given the great variation in treatment practices.

A biomarker distinguishing the cellulitis patients with low and high risk of recurrence would help direct antibiotic prophylaxis. Promising candidates are not in sight at the moment. However, meticulously collected study materials, with valid case definitions and appropriate samples stored for subsequent biochemical, serological or genetic studies would be of great value. Research in this area would be promoted by revising the diagnostic coding of cellulitis. At present, abscesses, wound infections and other suppurative skin infections fall in the same category as non-suppurative cellulitis, which evidently is a distinct disease entity (Chambers 2013).

Studies are needed to explore the hypothesis that a tonsillectomy predisposes to recurrent cellulitis. Alternatively recurrent tonsillitis, leading to tonsillectomy, could be a marker of inherent susceptibility to infections, especially streptococcal infections.

SUMMARY AND CONCLUSIONS

The main findings of the present study are summarised as follows:

1. Chronic oedema, disruption of cutaneous barrier, and obesity were independently associated with cellulitis. Chronic oedema showed the strongest association and disruption of the cutaneous barrier had the highest population attributable risk.
2. Psoriasis, other chronic dermatoses, diabetes, increasing body mass index, increasing age, and history of previous tonsillectomy were independently associated with recurrent cellulitis in patients with benzathine penicillin prophylaxis.
3. Recurrence of cellulitis was a strong risk factor for subsequent recurrence. The risk of recurrence in five years was 26% for NH patients and 57% for PH patients.
4. There was bacteriological or serological evidence of streptococcal infection in 73% of patients hospitalised with cellulitis. Antibiotic therapy attenuates the serological response to streptococci.
5. Of the BHS associated with cellulitis GGS was most common. GGS was also found in the pharynx of the cases and their family members, although throat carriage rate was low. No GGS was found among the control subjects.
6. According to *emm* and PFGE typing, no specific GAS or GGS strain was associated with cellulitis, except for a small household cluster of cases with a common GAS strain.
7. Inflammatory response was stronger in PH patients than in NH patients. However, acute phase CRP or PTX3 levels did not predict further recurrences.

The bacterial cause of a disease cannot be proven by the mere presence of bacteria. However, serological response to bacterial antigens during the course of the illness more plausibly demonstrates the causal relation of the bacteria with the disease. Taken

together, the bacteriological and serological findings, from the experiments of Fehleisen in 1880's to the recent controlled studies and the present study, support the causal association of BHS in most cases of cellulitis. However, occasionally other bacteria, especially *S. aureus*, may cause cellulitis, which might not be clinically distinguishable from a streptococcal infection.

Only the clinical risk factors were associated with a recurrent cellulitis or a risk of recurrence. The first recurrence more than doubles the risk of a subsequent recurrence. It must be kept in mind that, due to the design of the present and previous studies, only associations can be proved, not causality. There are no trials assessing the efficacy of interventions aimed at clinical risk factors, e.g. chronic oedema, toe web maceration or other skin breaks, or obesity. It seems, nevertheless, wise to educate patients on the association of these risk factors and attempt to reduce the burden of those in patients with acute or recurrent cellulitis.

The current evidence supports an antibiotic prophylaxis administered after the first recurrence (Thomas et al. 2013). If a bout of acute cellulitis makes one ever more susceptible to a further recurrence, prophylaxis might be considered already after the first attack of cellulitis, especially if the patient has several risk factors. However, the risk of recurrence is considerably lower after the first attack of cellulitis than after a recurrence, as is shown in the present study. This finding supports the current Finnish practice that pharmacological prevention of cellulitis is generally not considered until a recurrence. Nevertheless, the most appropriate moment for considering antibiotic prophylaxis, would be worthwhile to assess in a formal study. Targeting preventive measures to cellulitis patients, not the population, is obviously the most effective course of action. Nonetheless, fighting obesity, which is considered to be one of the main health issues in the 21st century, could probably reduce the burden of cellulitis in the population.

Although the present study is not an intervention study, some conclusions can be made concerning the antibiotic treatment of acute cellulitis, excluding cases with diabetic foot, necrotizing infections, or treatment at an intensive care unit. Penicillin is the drug of choice for acute cellulitis in most cases, because the disease is mostly caused by BHS and BHS, bacteria known to be uniformly sensitive to penicillin. However, *S. aureus* may be important to cover in an initial antibiotic choice when cellulitis is associated with a chronic leg ulcer, suppuration or abscess.

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Matti Karppelin

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Factors predisposing to acute and recurrent bacterial non-necrotizing cellulitis in hospitalized patients: a prospective case–control study

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Abstract

Acute non-necrotizing cellulitis is a skin infection with a tendency to recur. Both general and local risk factors for erysipelas or cellulitis have been recognized in previous studies using hospitalized controls. The aim of this study was to identify risk factors for cellulitis using controls recruited from the general population. We also compared patients with a history of previous cellulitis with those suffering a single episode, with regard to the risk factors: length of stay in hospital, duration of fever, and inflammatory response as measured by C-reactive protein (CRP) level and leukocyte count. Ninety hospitalized cellulitis patients and 90 population controls matched for age and sex were interviewed and clinically examined during the period April 2004 to March 2005. In multivariate analysis, chronic oedema of the extremity, disruption of the cutaneous barrier and obesity were independently associated with acute cellulitis. Forty-four (49%) patients had a positive history (PH) of at least one cellulitis episode before entering the study. Obesity and previous ipsilateral surgical procedure were statistically significantly more common in PH patients, whereas a recent (<1 month) traumatic wound was more common in patients with a negative history (NH) of cellulitis. PH patients had longer duration of fever and hospital stay, and their CRP and leukocyte values more often peaked at a high level than those of NH patients. Oedema, broken skin and obesity are risk factors for acute cellulitis. The inflammatory response as indicated by CRP level and leukocyte count is statistically significantly more severe in PH than NH patients.

Keywords: Case–control study, cellulitis, erysipelas, inflammation, risk factor
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Introduction

Bacterial non-necrotizing cellulitis and erysipelas are acute infections of the skin and subcutaneous tissue. Erysipelas is often considered to be a superficial form of acute cellulitis. The typical clinical presentation of classic erysipelas is an acute onset of fever or chills together with localized skin inflammation that is sharply demarcated from the surrounding normal skin. Cellulitis usually comprises more deeply situated skin infection. The distinction between erysipelas and cellulitis is not clear-cut [1]. In clinical practice, especially in northern Europe, the term erysipelas is often used for cases beyond those meeting the strict definition, reflecting the common clinical features of these entities: sudden onset, usually high fever, response to similar treatment, and a tendency to recur. The most important causative organisms are group A and group G β-haemolytic streptococci and *Staphylococcus aureus* [2–7].

Chronic leg oedema, obesity, history of previous erysipelas, prior saphenectomy, skin lesions as possible sites of bacterial entry and bacterial colonization of toe-webs have been recognized in previous case–control studies as risk factors for erysipelas or cellulitis of the leg [8–11]. In previous studies on recurrent cellulitis, overweight, venous insufficiency, chronic oedema, smoking, homelessness, prior malignancy, trauma or

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previous surgical intervention, ipsilateral dermatitis and tinea pedis have been recognized as risk factors [12–15].

The aim of the present prospective study was to evaluate the risk factors for acute infectious non-necrotizing cellulitis in hospitalized patients in comparison with age-matched and sex-matched community controls. We also compared the risk factors and inflammatory response as measured by the level of C-reactive protein (CRP), leukocyte count and duration of fever between patients with and without a previous history of cellulitis.

Patients and Methods

Study design

Consecutive patients ≥18 years of age hospitalized for acute cellulitis were recruited into the study between April 2004 and March 2005 in two infectious diseases wards in Tampere University Hospital and Hatanpää City Hospital, Tampere, which together serve the 500 000 population living in the city of Tampere and its surroundings. Patients referred by the primary physician to the two wards with a diagnosis of acute cellulitis or erysipelas were eligible to participate in the study. Ward nurses obtained informed consent and took the bacteriological samples on admission. The diagnosis of acute bacterial non-necrotizing cellulitis was confirmed by a specialist physician (MK) within 4 days from admission, and patients were subsequently interviewed and clinically examined. Data were collected concerning possible underlying general and local risk factors. The study was approved by the Ethical Review Board of Pirkanmaa District.

The bacteriological findings have been published elsewhere [2]. For all 90 patients, serum CRP level and leukocyte count were measured on admission (day 1) and during the next 4 days (days 2–5). The CRP level was measured two to five times, until it was declining. The CRP level was measured by the Roche Diagnostics CRP method using a Cobas Integra analyser (F. Hoffman-La Roche, Basel, Switzerland). Leukocyte counts were measured by the standard laboratory method. High CRP level and high leukocyte value were defined as CRP level or leukocyte count above the 75th percentile of those of the whole patient population.

Case and control definition

Acute bacterial non-necrotizing cellulitis was defined by a recent history of acute onset of fever or chills and localized erythema of the skin on one extremity or the typical appearance of a well-demarcated skin lesion on the face, with or without fever or chills.

For each patient, one control subject living in Tampere and matched for age (same year and month of birth) and sex was recruited. For a given patient, six control candidates were randomly sampled from the records of the Finnish Population Register Centre and, in random order, asked by letter to participate in the study, at 2-week intervals until the first response was received. The reasons for non-participation could not be established. A recruited control was excluded if he or she had suffered from any cellulitis episode, and a new control was asked to participate. Control subjects were interviewed and examined by MK.

Alcohol abuse was defined as any social or medical problem recorded as being related to overuse of alcohol in a patient's or control subject's medical history. Obesity was defined as body mass index ≥30. Data on cardiovascular disease, diabetes and other underlying diseases were obtained from the medical records of the patients and control subjects, and based on diagnoses made by a physician. Chronic oedema was defined as any oedema (venous or lymphatic) present at the time of examination and considered to be chronic in the medical record or interview. Traumatic wounds and chronic ulcers, skin diseases and any previous surgical operations on the extremities or head were recorded by clinical examination and interview. Toe-web intertrigo was defined as any alteration of normal skin integrity in the toe-web space observed upon examination. Duration of fever after admission to hospital was defined as number of days on which a tympanic temperature ≥37.5°C was recorded for a given patient. On the basis of interview, the number of days with temperature ≥37.5°C before admission to hospital was also recorded. The elderly were defined as those aged >65 years.

Statistical analysis

To describe the data, median and range or minimum and maximum values are given for skew-distributed continuous variables. Univariate analysis was performed by McNemar's test in order to identify factors associated with cellulitis. The main univariate analyses included calculation of ORs and their 95% CIs. A conditional logistic regression analysis (Method Enter) was performed to bring out independent risk factors for cellulitis. Factors emerging as significant in the univariate analysis or otherwise considered to be relevant (diabetes, and cardiovascular and malignant diseases) were included in the multivariate analysis, which at first was undertaken separately for general and local (ipsilateral) risk factors. Finally, all variables, both general and local, that proved to be associated with acute cellulitis were included in the last multivariate analysis.

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For each patient and the corresponding control, a local risk factor was considered to be present if it was situated on the same anatomical (ipsilateral) site as the cellulitis lesion. When studying local risk factors, we created a new variable, disruption of the cutaneous barrier, including toe-web intertrigo, chronic dermatitis, and a traumatic wound within the last month.

Correlations between two continuous variables (one or both variables non-normally distributed) were calculated using Spearman (r_S) bivariate correlation. Associations between categorical variables and continuous non-normally distributed variables were calculated using the Mann–Whitney *U*-test. Relationships between categorical variables were analysed by chi-squared or Fisher's exact test when appropriate. A logistic regression analysis (Method Enter) was performed in order to study the effect of a positive previous history of cellulitis on the length of stay in hospital over 7 days by adjusting for the effect of being elderly or diabetic.

Results

Of the 104 patients identified, 90 were included in the study, eight refused, and six were excluded after recruitment because of obvious alternative diagnoses or non-fulfilment of the case definition (gout, three patients; *S. aureus* abscess, one patient; *S. aureus* wound infection, one patient; no fever or chills in conjunction with erythema in one leg, one patient). Of the 302 matching controls contacted, 90 were included, two were excluded because they had suffered from cellulitis, and 210 did not reply. All patients and controls were of Finnish origin. There was no intravenous drug use or human immunodeficiency virus infection among the patients or controls. Fifty-eight of the 90 patients (64%) were male. The median age of the subjects was 58 years (range 21–90 years), and the median body mass index values of patients and controls were 29.1 (range 19.6–65.2) and 26.5 (range 17.4–40.7), respectively. The cellulitis was localized in the lower extremity in 76 (84%) of the 90 patients, in the upper extremity in seven (8%), and in the face in seven (8%). Four patients had one recurrence, and two patients two recurrences, during the study period, but the analysis included the first episode only.

TABLE 1. Univariate analysis of general and local (ipsilateral) risk factors for acute cellulitis among 90 hospitalized patients and 90 control subjects

	Patients	Controls	Univariate analysis
Risk factor	***N* (%)**	***N* (%)**	**OR (95% CI)**
General			
Alcohol abuse	12 (13)	2 (2)	6.0 (1.3–26.8)
Obesity (BMI ≥30)	37 (41)	15 (17)	4.7 (1.9–11.3)
Current smoking	32 (36)	16 (18)	3.0 (1.3–6.7)
Malignant disease	14 (16)[a]	6 (7)[b]	2.6 (0.9–7.3)
Cardiovascular disease	18 (20)	9 (10)	2.5 (1.0–6.4)
Diabetes[c]	13 (14)	9 (10)	1.7 (0.6–4.6)
Local			
Chronic oedema of the extremity (n = 83)[d]	23 (28)	3 (4)	21.0 (2.8–156.1)
Traumatic wound <1 month	15 (17)	4 (4)	3.8 (1.2–11.3)
Skin disease	29 (32)	12 (13)	3.8 (1.6–9.4)
Toe-web intertrigo (n = 76)[e]	50 (66)	25 (33)	3.5 (1.7–7.1)
Previous operation >1 month	39 (43)	22 (24)	2.4 (1.2–4.7)
Chronic ulcer	6 (7)	0	∞
Disruption of cutaneous barrier (n = 76)[e,f]	67 (86)	35 (46)	11.3 (4.0–31.3)

BMI, body mass index.
[a]Breast cancer (six patients), prostate cancer (two patients), cancer of the bladder, vulva, kidney and throat (one patient each), osteosarcoma (one patient), and lymphoma (one patient). Of the six patients with breast cancer, three had cellulitis on the upper extremity.
[b]Prostate cancer (three control patients), cancer of the breast, colon and thyroid (one control patient each).
[c]Type 2, except for one patient with type 1.
[d]Calculated for lower and upper extremities.
[e]Calculated for lower extremities only.
[f]Disruption of cutaneous barrier comprises traumatic wounds <1 month, skin disease, toe-web intertrigo, and chronic ulcers. This combined variable was used in multivariate analysis.

TABLE 2. Final multivariate analysis of all risk factors found to be significant in the previous separate multivariate analyses; only lower-limb cellulitis patients are included (n = 76)

Risk factor	OR	95% CI
Chronic oedema of the extremity	11.5	1.2–114.4
Disruption of cutaneous barrier[a]	6.2	1.9–20.2
Obesity (BMI ≥30)	5.2	1.3–20.9
Malignant disease	2.0	0.5–8.9
Current smoking	1.4	0.4–5.3

BMI, body mass index.
[a]Traumatic wounds <1 month, skin disease, toe-web intertrigo, and chronic ulcers.

Risk factors for acute cellulitis

Table 1 shows the results of univariate analysis of all—both general and local (ipsilateral)—risk factors studied. Multivariate analysis was at first performed separately for general and local risk factors, and those appearing as significant were included in the final multivariate analysis (Table 2). The final analysis included only lower-limb cellulitis patients, because the combined variable 'disruption of cutaneous barrier' is not relevant in the upper limb or the face.

Patients with a positive history of cellulitis as compared with those with a negative history

Forty-four of the 90 patients had a positive history (PH) and 46 a negative history (NH) of at least one previous cellulitis episode before recruitment to the study (median, one epi-

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TABLE 3. Univariate analysis of risk factors, laboratory parameters, length of stay and duration of fever from admission to hospital among 44 patients with a positive history of previous cellulitis and 46 patients with a negative history of previous cellulitis

	Patients with positive history of cellulitis (n = 44)	Patients with negative history of cellulitis (n = 46)	p-value (chi-squared test)
	N (%)	*N* (%)	
Alcohol abuse	5 (11)	7 (15)	0.591
Obesity (BMI ≥30)	24 (55)	13 (28)	0.014
Current smoking	15 (36)	17 (37)	0.839
Malignant disease	6 (14)	8 (17)	0.501
Cardiovascular disease	6 (14)	12 (26)	0.14
Diabetes	8 (18)	5 (11)	0.324
Chronic oedema of the extremity[a]	14 (33)	9 (23)	0.306
Disruption of the cutaneous barrier[b]	37 (88)	30 (88)	0.985
Traumatic wound <1 month	3 (7)	12 (26)	0.014
Skin diseases	13 (30)	16 (35)	0.595
Toe-web intertrigo[b]	30 (71)	20 (59)	0.249
Chronic ulcer	5 (11)	1 (2)	0.107[c]
Previous operation	24 (55)	15 (33)	0.036
Antibiotic treatment before admission	16 (36)	10 (22)	0.126
High peak CRP level, >218 mg/L[d]	15 (34)	7 (15)	0.037
High peak leukocyte count[d]	15 (34)	7 (15)	0.037
Duration of fever >3 days after admission to hospital[e]	9 (20)	1 (2)	0.007[c]
Length of stay in hospital >7 days[f]	29 (66)	19 (41)	0.019

BMI, body mass index; CRP, C-reactive protein.
[a]Cellulitis of the face excluded.
[b]Cellulitis of the face and upper extremities excluded; disruption of cutaneous barrier comprises traumatic wounds < 1 month, skin disease, toe-web intertrigo, and chronic ulcers. This combined variable was used in multivariate analysis.
[c]Fisher's exact test.
[d] Seventy-fifth percentile of patients, coresponding to a CRP level of 218 mg/L and a leukocyte count of 16.9×10^9/L.
[e]Median duration of fever after admission to hospital was 1 day (range 0–7 days) for patients with a positive history and 1 day (range 0–4 days) for patients with a negative history.
[f]Median length of stay in hospital was 9 days (range 3–27 days) for patients with a positive history and 7 days (range 2–25 days) for patients with a negative history.

sode; range, one to eight episodes). Six PH patients also experienced a recurrence during the study period, whereas no recurrences occurred among NH patients. The median age of both the PH and NH patients was 58 years (ranges 21–90 years and 27–84 years, respectively) at the time of the study. On the basis of interview, PH patients were found to have been younger than NH patients at the time of their first cellulitis episode (median 49 years, range 12–78 years vs. median 58 years, range 28–84 years, respectively, p 0.004).

We compared PH and NH patients with regard to general and local risk factors (Table 3). In univariate analysis of PH patients as compared with their corresponding controls, the OR for obesity was 9.5 (95% CI 2.2–40.8), the OR for previous operation was 3.4 (95% CI 1.3–9.2), and the OR for traumatic wound was 1.5 (95% CI 0.25–9.0). Similarly, for NH patients, the OR for obesity was 2.3 (95% CI 0.69–7.3), the OR for previous operation was 1.7 (95% CI 0.67–4.4), and the OR for traumatic wound was 6.0 (95% CI 1.3–26.8).

Among the CRP estimations, the peak was recorded on day 1 in 51 (57%) patients, and on days 2, 3 and 4 in 25 (28%), 12 (13%) and two (2%) patients, respectively. The peak CRP value ranged from 5 to 365 mg/L (median 161 mg/L). Patients with PH had longer hospital stay and showed a stronger inflammatory response than those with NH, as shown by peak CRP level, peak leukocyte count, and fever after admission to hospital (Table 3). The median duration of fever prior to admission was 1 day for both PH patients (range 0–5) and NH patients (range 0–4).

As length of stay (LOS) was associated with patients' age, diabetic status, and history of cellulitis (data not shown), we studied the effect of a history of cellulitis on an LOS of more than 7 days by adjusting the effect of being elderly and diabetic in a multivariate model. In this model, the effect of PH remained significant (p 0.02). ORs for LOS over 7 days were 3.1 for PH (95% CI 1.2–8.1), 5.4 for being elderly (95% CI 1.9–15.4), and 3.0 for being diabetic (95% CI 0.7–12.8).

Discussion

Our findings suggest that chronic oedema of the extremity, disruption of the cutaneous barrier and obesity are independent risk factors for acute cellulitis leading to hospitalization, which is in line with the results of earlier studies [8,9,11]. Patients who had a history of previous cellulitis tended to be more overweight, had previous operations at the ipsilateral site more often, and showed a stronger inflammatory response as measured by CRP level and leukocyte count. To our knowledge, this is the first study on cellulitis with the

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controls recruited from the general population instead of from hospitalized patients. Furthermore, the association of CRP level, leukocyte count and duration of fever and hospital stay with recurrences of cellulitis has not been previously reported.

The patients in the present study represent hospitalized cellulitis cases. However, the most severe cases, e.g. patients requiring treatment in the intensive-care unit, were not included in this study. The proportion of cellulitis patients treated as outpatients is not known. However, it is likely that hospitalized cellulitis patients are older and have more comorbidities than those treated as outpatients. A selection bias might have affected the control population of the present study, because those control candidates responding to invitation might be, for example, more health-conscious and, as a consequence, less likely to be obese, alcohol abusers or smokers than the general population. On the other hand, a hospitalized control population may be biased in the opposite direction. Inter-observer bias was avoided, as the same investigator examined all patients and controls. However, the distinction between chronic oedema and swelling caused by cellulitis itself was based on interview and thus could not be objectively assessed.

Risk factors for leg cellulitis may differ from those for arm or face cellulitis. We therefore analysed risk factors with regard to their relevance in a particular site, i.e. chronic oedema in the extremities only, and toe-web intertrigo as well as disruption of the cutaneous barrier, which included the former, in the lower extremities only.

The median age of PH patients did not differ from that of NH patients. Moreover, PH patients had been significantly younger at the time of their first cellulitis episode than NH patients. If the predisposing factors are the same for a single cellulitis episode and for recurrences, one would expect PH patients to be older than NH patients. In two previous studies, this was indeed the case, in contrast to our findings [8,13]. However, up to 50% of NH patients may suffer a recurrence [14,16] and thus actually belong to the PH group, a fact that detracts from the validity of the conclusions. This issue will be addressed in a subsequent follow-up study.

The LOS in hospital is determined by the subjective decision of the treating physician, and obviously depends on clinical signs of disease activity. It may also depend on the age and social circumstances of the patient, as well as on comorbidities [17,18]. In the present study, the LOS was associated with recurrent cellulitis independently of the age or diabetic status of the patient.

In conclusion, the present findings support those of earlier case–control studies, in that chronic oedema, disruption of the cutaneous barrier and obesity proved to be risk factors for hospitalization due to acute non-necrotizing cellulitis. In addition, obesity and a previous ipsilateral surgical procedure were statistically significantly more common in patients with a PH of cellulitis, whereas a recent (<1 month) traumatic wound was more common in patients with an NH of cellulitis. PH patients had longer duration of fever and hospital stay, and their CRP and leukocyte values more often peaked at a high level, as compared with NH patients.

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Transparency Declaration

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Acute Bacterial, Nonnecrotizing Cellulitis in Finland: Microbiological Findings

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Background. Bacterial, nonnecrotizing cellulitis is a localized and often recurrent infection of the skin. The aim of this study was to identify the β-hemolytic streptococci that cause acute nonnecrotizing cellulitis infection in Finland.

Methods. A case-control study of 90 patients hospitalized for acute cellulitis and 90 control subjects was conducted during the period of April 2004–March 2005. Bacterial swab samples were obtained from skin lesions or any abrasion or fissured toe web. Blood culture samples were taken for detection of bacteremia. The patients, their household members, and control subjects were assessed for pharyngeal carrier status. β-Hemolytic streptococci and *Staphylococcus aureus* were isolated and identified, and group A and G streptococcal isolates were further analyzed by T serotyping and *emm* and pulsed-field gel electrophoresis typing.

Results. β-Hemolytic streptococci were isolated from 26 (29%) of 90 patients, 2 isolates of which were blood-culture positive for group G streptococci, and 24 patients had culture-positive skin lesions. Group G *Streptococcus (Streptococcus dysgalactiae* subsp. *equisimilis)* was found most often and was isolated from 22% of patient samples of either skin lesions or blood, followed by group A *Streptococcus,* which was found in 7% of patients. Group G streptococci were also carried in the pharynx of 7% of patients and 13% of household members but was missing from control subjects. Several *emm* and pulsed-field gel electrophoresis types were present among the isolates. Six patients (7%) had recurrent infections during the study. In 2 patients, the group G streptococcal isolates recovered from skin lesions during 2 consecutive episodes had identical *emm* and pulsed-field gel electrophoresis types.

Conclusions. Group G streptococci, instead of group A streptococci, predominated in bacterial cellulitis. No clear predominance of a specific *emm* type was seen. The recurrent nature of cellulitis became evident during this study.

Bacterial cellulitis and erysipelas refer to diffuse, spreading skin infections, excluding infections associated with underlying suppurative foci, such as cutaneous abscesses, necrotizing fasciitis, septic arthritis, and osteomyelitis [1]. Cellulitis usually refers to a more deeply situated skin infection, and erysipelas can be considered to be a superficial form of it. However, the distinction between these entities is not clear cut, and the 2 conditions share the typical clinical features—for example, sudden onset, usually with a high fever, and the tendency to recur. *Streptococcus pyogenes* (group A *Streptococcus* [GAS]) has been considered to be the main causative agent of these infections, although streptococci of group G (GGS), group C (most importantly, *Streptococcus dysgalactiae* subsp. *equisimilis*), group B, and, rarely, staphylococci can also cause these diseases [2–4].

The predominant infection site is on the lower extremities, and the face or arms are more rarely affected [2, 3]. Lymphedema and disruption of the cutaneous barrier, which serves as a site of entry for the pathogens, are risk factors for infections [5–8]. Twenty percent to 30% of patients have a recurrence during a 3-year follow-up period [4, 9]. Results of patient blood cultures are usually positive for β-hemolytic streptococci in <5% of cases [2–4]. Although cellulitis is usually not a

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Table 1. Acute bacterial cellulitis episodes, patients, samples, and bacterial findings, by 3-month study periods.

Study period	No. of episodes	No. of recruited patients[a]	No. of samples							
			Taken for bacterial culture[a]		With culture positive for BHS or *Staphylococcus aureus*[a]		With positive skin swab culture result[a]			
			Blood	Skin swabs	Blood	Skin swabs	BHS	GGS	GAS	*S. aureus*
April–June 2004	29	28	28	18	1	12	8	6	1	8
July–September 2004	34	29	29	25	0	11	10	8	3	11
October–December 2004	17	17	17	11	0	4	2	1	1	4
January–March 2005	18	16	14	12	1	7	4	3	1	6
Total	98	90	88	66	2	34	24	18	6	29

NOTE. BHS, β-hemolytic streptococci; GAS, group A streptococcus *(Streptococcus pyogenes);* GGS, group G streptococcus *(Streptococcus dysgalactiae* subsp. *equisimilis).*

[a] Includes only 1 episode of patients with recurrent episodes and the corresponding samples and isolates of that episode.

life-threatening disease, it causes remarkable morbidity, especially in elderly persons [10]. This clinical study aims for a better understanding of acute bacterial cellulitis infections and focuses specifically on the characterization of β-hemolytic streptococci involved in the infection, infection recurrences, and pharyngeal carriage.

METHODS

Study design and population. During 1 year (April 2004–March 2005), patients (age, ⩾18 years) hospitalized for acute bacterial cellulitis were recruited into the study from 2 infectious diseases wards, 1 at Tampere University Hospital (Tampere, Finland) and 1 at Hatanpää City Hospital (Tampere, Finland). After receiving informed consent, each patient's diagnosis of cellulitis was confirmed by a specialist of infectious diseases (M.K.) within 4 days after admission to the hospital. The patients were subsequently interviewed and were clinically examined.

For each patient, 1 control subject living in Tampere who was matched for age (same year and month of birth) and sex was recruited. For each patient, as many as 6 control candidates were randomly sampled from the Finnish Population Register Centre and, in random order, asked by letter, sent at 2-week intervals until the first response was obtained, to participate in the study. The recruited control subject was excluded if he or she had been affected with any cellulitis episode, and a new control subject was asked to participate. The reason for nonparticipation was not recorded. Interview and examination procedures were the same for control subjects as for patients.

To study the pharyngeal carriage and possible transmission of β-hemolytic streptococci, family members sharing the same household with the patients were recruited. The study was approved by the Ethical Review Board of Pirkanmaa District, Tampere, Finland.

Case definition and exclusion criteria. Nonnecrotizing bacterial cellulitis was defined (1) as an acute onset of fever or chills and a localized, more-or-less well-demarcated erythema of the skin in 1 extremity or (2) as a typical appearance of well-demarcated skin eruption on the face, with or without fever or chills. Thus, the case definition includes acute bacterial cellulitis and the superficial form of cellulitis (erysipelas). Patients with cutaneous abscesses, necrotizing fasciitis, septic arthritis, and osteomyelitis were excluded.

Sample collection and culture and isolation of bacteria. Samples were collected from the patients at admission to the hospital. Sterile swabs (Technical Service Consultants) were used for sampling and transportation. Samples were taken in duplicate from any existing wound or blister in the affected skin or, if the infection area was intact, from any abrasion or fissured toe web. Furthermore, a throat swab culture specimen was taken from all patients, household members, and control subjects.

The sample swab was first inoculated on a primary plate of sheep blood agar and then was placed in sterile water, to obtain a bacterial suspension, which was serially diluted and plated on sheep blood agar. Plates were incubated in 5% CO_2 at 35°C, and bacterial growth was determined at 24 h and 48 h.

β-Hemolytic bacterial growth was visually examined, and the number of colony-forming units per milliliter (cfu/mL) was calculated. Up to 10 suspected β-hemolytic streptococcal colonies and 1 suspected *Staphylococcus aureus* colony per sample were chosen for isolation.

In addition to the swabs, 2 sets (for an aerobic bottle and an anaerobic bottle) of blood samples were drawn from each patient. The culturing and identification of blood cultures were performed using Bactec 9240 (BD Diagnostic Systems) culture systems, with standard culture media.

***Identification of β-hemolytic streptococci and* S. aureus.** β-Hemolytic streptococcal isolates were tested for sensitivity to bacitracin and were identified by detection of Lancefield group

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Table 2. Molecular characteristics of group G *(Streptococcus dysgalactiae* subsp. *equisimilis)* and group A *(Streptococcus pyogenes)* streptococci isolated from patients with acute bacterial cellulitis.

Group antigen and *emm* type	No. of isolates	PFGE type	Sample site(s)
G			
stC74A.0	1	Unique	Skin
stC6979.0	1	Unique	Blood
stC6979.0	1	Unique	Skin
stG6.0	2[a]	I	Skin
stG6.0	1	Unique	Skin
stG6.1	2	Unique	Skin
stG11.0	2[a]	IV	Skin
stG166b.0	2	Unique	Skin
stG245.0	2[b]	III	Skin and throat
stG245.0	1	Unique	Skin
stG480.0	2	Unique	Skin
stG480.0	1[c]	II	Throat
stG480.0	1	Unique	Throat
stG485.0	1	Unique	Blood
stG485.0	1	Unique	Skin
stG643.0	3	Unique	Skin
stG643.0	1	Unique	Throat
stG5420.0	1	Unique	Skin
A			
emm11.0	1	Unique	Throat
emm12.0	1	Unique	Throat
emm28.0	1	Unique	Skin
emm73.0	1	Unique	Skin
emm81.0	3[d]	A	Skin
emm85.0	1	Unique	Skin

[a] Isolates from consecutive episodes in the same patient.
[b] Isolates from skin and throat swabs of the same patient.
[c] Identical with a household member's isolate.
[d] A cluster of cases in members of the same household.

antigens A, B, C, D, F, and G, with use of the Streptex latex agglutination test (Remel Europe). Antimicrobial susceptibility testing of blood isolates was performed according to the Clinical Laboratory Standards Institute (the former National Committee on Clinical Laboratory Standards) guidelines. *S. aureus* was identified by the Staph Slidex Plus latex agglutination test (bioMérieux). The API ID 32 Strep test (bioMérieux) was used to determine the species of groups A, B, and G streptococci. Isolates identified as β-hemolytic streptococci and *S. aureus* were stored at −70°C. Group A (*S. pyogenes*) and group G (*S. dysgalactiae* subsp. *equisimilis*) isolates were further analyzed by T serotyping, *emm* typing, and PFGE.

T serotyping. T serotyping was performed using anti–T-agglutination sera (Sevac), as described elsewhere [11, 12]. With multiple isolates per sample, isolates with the same T serotype were considered to be a single strain, and 1 isolate of each serotype was selected for further analysis.

emm *Typing.* The *emm* gene was amplified using primers MF1 and MR1, as described elsewhere [11], or primer 1 (5′-TAT T(C/G)G CTT AGA AAA TTA A-3′) and primer 2 (5′-GCA AGT TCT TCA GCT TGT TT-3′) [13]. With primer 1 and primer 2, PCR conditions were as follows: at 95°C for 10 min; 30 cycles at 94°C for 1 min, at 46°C for 1 min, and at 72°C for 2.5 min; and 1 cycle at 72°C for 7 min [14]. PCR products were purified with QIAquick PCR purification Kit (Qiagen). *emm* Sequencing was performed with primer MF1 [11] or emmseq2 [13] with use of BigDye chemistry (Applied Biosystems), with cycling times of 30 cycles at 96°C for 20 s, at 55°C for 20 s, and at 60°C for 4 min. Sequence data were analyzed with ABI Prism 310 Genetic Analyzer (Applied Biosystems) and were compared against the Centers for Disease Control and Prevention *Streptococcus emm* sequence database to assign *emm* types [15].

PFGE. PFGE was performed using standard methods [16]. DNA was digested with *Sma*I (Roche) and was separated with CHEF-DR II (Bio-Rad), with pulse times of 10–35 s for 23 h. Restriction profiles were analyzed using Bionumerics software (Applied Maths). Strains with ⩾90% similarity were considered to be the same PFGE type. Types including ⩾2 strains were designated by Roman numerals (for GGS) or uppercase letters (for GAS). Individual strains were named "unique."

Data handling and statistical analysis. For calculating

Table 3. Bacterial findings for patients who had recurrent infections during this study.

Patient	Sex	Bacterial findings from skin swabs (*emm* type, PFGE type)			Time between episodes, days	Throat carrier status during the study
		Episode 1	Episode 2	Episode 3		
1	Male	Negative	GGS (stG6.0, I)[a]	GGS (stG6.0, I)[a]	89, 62	Negative
2	Male	Negative	Negative	Negative	101, 46	Group D streptococcus
3	Male	Group B streptococcus	Negative	NA	134	Negative
4	Female	GGS (stG11.0, IV)	GGS (stG11.0, IV)[a]	NA	58	*Staphylococcus aureus*
5	Male	*S. aureus*	GGS (stG643.0, unique)[a]	NA	156	Negative

NOTE. Samples were taken from 5 of 6 patients with recurrences; 2 patients had 3 disease episodes. GGS, group G streptococcus *(Streptococcus dysgalactiae* subsp. *equisimilis);* NA, not applicable (no disease episode); negative, sample was culture negative for β-hemolytic streptococci and *S. aureus.*

[a] Concomitantly with *S. aureus.*

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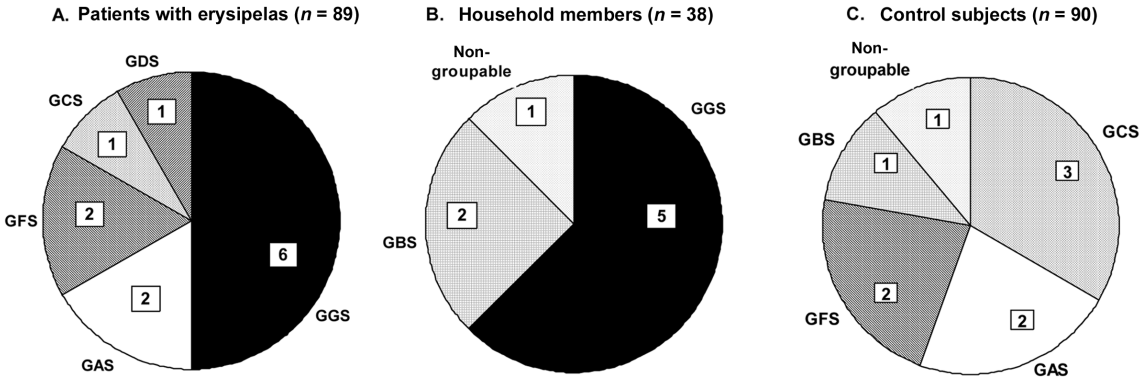

Figure 1. Throat swab samples that were culture positive for β-hemolytic streptococcus in different study groups. n, Total number of samples taken in each study group, including only 1 sample from patients with recurrent episodes. The total number of isolates for each bacterial group is shown. GAS, GBS, GCS, GDS, GFS, and GGS, group A, B, C, D, F, and G *Streptococcus,* respectively.

percentages, 1 episode per patient was considered, unless otherwise specified. A patient was considered to be culture positive for a given bacterial group if the patient culture sample was positive for that bacterial group at any time during the study. This rule was applied separately for clinical and pharyngeal data.

Data were analyzed using Intercooled Stata 9.1 for Windows (StataCorp). Categorical data were compared using the χ^2 test with Stata, GraphPad software [17], or an interactive calculation tool for χ^2 tests [18]. McNemar's test was used in comparing differences between the findings of patients and control subjects. Differences were considered to be significant when $P < .05$.

RESULTS

Research subjects and disease episodes. A total of 104 patients received the diagnosis of acute bacterial cellulitis during the study period. Eight eligible patients refused to participate (the reason for refusal was not recorded). Six patients were excluded from the study after recruitment because of obvious alternative diagnosis (3 patients) or not fulfilling the case definition (3 patients). Therefore, 90 patients (58 men and 32 women) were included in the study. Correspondingly, 90 control subjects and 38 family members were recruited. Of the control subjects, 34 (38%) of 90 were the first invited individuals of 6 eligible candidates. Six patients had recurrences during the study period; therefore, a total of 98 disease episodes were recorded (table 1). Sixteen of these 98 cellulitis episodes could be classified as classic erysipelas with a sharply demarcated area of inflammation. 44 (49%) of the 90 patients had a history of cellulitis infection before our study. The median age of the patients was 58 years (range, 21–90 years). More episodes occurred in July–September than in other periods ($P \leq .05$).

Bacteriological findings of clinical samples. A skin swab sample was taken for culture from 66 patients, who presented with 73 disease episodes (table 1). β-Hemolytic streptococci were isolated from 24 patients. The most common finding was GGS *(S. dysgalactiae* subsp. *equisimilis),* which was recovered from 18 (20%) of the 90 patients, 12 of whom also harbored *S. aureus.* GAS *(S. pyogenes)* was isolated from 6 patients (7%), always concomitantly with *S. aureus.* Group B streptococcus (*S. agalactiae* [GBS]) was isolated from 1 patient. *S. aureus* was isolated as the only bacterium from 10 patients. A blood culture sample was obtained from 88 (98%) of the patients, 2 (2%) of whom had a blood culture result positive for GGS *(S. dysgalactiae* subsp. *equisimilis).* The median ages of patients whose cultures were positive for GGS and GAS were 58 and 65 years, respectively.

From 9 (33%) of 27 patients, β-hemolytic streptococci were isolated from the infection focus; from 15 (38%) of 39 patients, they were isolated from a suspected site of entry—for example, from a wound, intertrigo, or between the toes. Isolates from the infection foci were diverse, yielding 5 GGS isolates, 4 GAS isolates, and 1 GBS isolate, whereas isolates from the probable portals of entry were more uniform, with 13 GGS and 2 GAS isolates. In 27 episodes, antibiotic treatment (penicillin, cephalexin, or clindamycin) had been started before admission to the hospital, but the treatment did not significantly affect the amount of culture-positive findings (data not shown).

β-Hemolytic streptococcal growth could be quantitated in 23 samples. The viable counts in samples with a GGS isolate had a range of 10^3–10^7 cfu/mL (mean, 3×10^6 cfu/mL) and, with a GAS isolate, 10^3–10^5 cfu/mL (mean, 2×10^5 cfu/mL).

Eleven *emm* types among GGS isolates and 4 *emm* types among GAS isolates were found (table 2). Three patients harbored the most common *emm* type of GGS, stG643.0. We identified a cluster of 3 cellulitis cases among patients in a nursing home, and the patients had the same clone of GAS in their

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affected skin: *emm*81.0 with PFGE profile type A. One of these patients also harbored a GGS *emm* type stC6979.0 at the infection site.

Recurrent infections. Six patients (median age, 48 years) had recurrences during the study period; 4 patients had 2, and 2 patients had 3 disease episodes (table 3). The median time between recurrences was 81 days. All of these patients had a history of at least 1 cellulitis infection before the time of this study. The infection site remained the same in all episodes, but the site of sampling varied. GGS combined with *S. aureus* was isolated from 3 patients, none of whom had any visible abrasion or wound at the infection site, and the sample was taken from another site, such as the toe area or heel. In 2 patients, the GGS recovered from cutaneous swabs in 2 consecutive episodes had identical *emm* (stG6.0 and stG11.0) and PFGE types (table 2). All of these patients had negative blood culture results.

Pharyngeal findings. A total of 225 throat swab samples were taken: 97 samples from 89 patients, 38 from household members, and 90 from control subjects. β-Hemolytic streptococci were carried in the pharynx by 12 (13%) of the 89 patients, 8 (21%) of the 38 household members, and 9 (10%) of the 90 control subjects (figure 1). GGS was significantly more commonly found in patients (7%) and household members (13%) than in control subjects (0%) ($P \leqslant .04$, by McNemar's test). *S. aureus* was present in ⩽10% of the samples of these groups (data not shown).

Of the GGS isolated from patients, 4 of 6 isolates were *S. dysgalactiae* subsp. *equisimilis* (table 2), 1 was *S. anginosus,* and 1 could not be characterized. Two patients harbored GAS *(S. pyogenes)* strains. The household members harbored 5 GGS isolates *(S. dysgalactiae* subsp. *equisimilis),* with *emm* types stG6.1 (2 isolates), stG166b.0, stG480.0, and stG652.0. On 2 occasions, the same strain was shared within the household: 1 patient and a household member harbored the same clone of GGS: *emm* type stG480.0 with PFGE type II. Two household members of the nursing home cluster carried an identical clone of a GGS strain: *emm* type stG6.1.

One of the 90 patients had the same streptococcal strain (GGS, *emm* type stG245.0 with an identical PFGE type III) in the pharynx and affected skin (toe web; not the actual infection site).

DISCUSSION

To our knowledge, this is the first case-control study of acute bacterial cellulitis in Finland. Within 1 year, 90 patients presenting with 98 disease episodes were included in the study. Strikingly, GGS *(S. dysgalactiae* subsp. *equisimilis)* instead of GAS was the most common finding. Some patients and household members also carried GGS in the pharynx, whereas it was not detected in the control subjects. We could also confirm in 2 cases of recurrences that the consecutive episodes were caused by the same clone of GGS.

A limitation of our study is that the patient population comprised hospitalized patients with cellulitis of intermediate severity. The proportion of patients treated on an outpatient basis is not known. However, a Finnish treatment recommendation is to hospitalize febrile patients with cellulitis, for initial parenteral antibiotic treatment. The most severe cases—for example, patients requiring intensive care treatment or surgery—were treated in other wards and therefore were not eligible for this study. This may have decreased the observed rate of bacteremia, as well as the rate of recurrence, which may also be underestimated because of the short study period and lack of follow-up data [19].

GGS was isolated either from skin lesion or blood from 22% of patients, whereas GAS was isolated from 7% of patients, in proportions similar to those reported in a recent case-control study [5]. The proportion of patients with a positive blood culture result (2%) was in the expected range for this disease, with GGS as the cause of bacteremia. GAS has been regarded as the main causative agent of streptococcal cellulitis, as well as the cause of bacteremia in patients with cellulitis [3, 4]. Nonetheless, a stronger role of GGS in cellulitis [4, 5, 20] and, with increasing recent frequency, in nonfocal bacteremia [21–24] has been noticed.

With a noninvasive sampling method, we could isolate β-hemolytic streptococci from one-third of the samples. We cannot exclude the possibility that the choice of sampling method and, in some cases, antibiotic treatment before our sampling may have had an effect on the findings. The findings differed by sampling site, and more than one-half of the isolates were obtained from the suspected site of entry, which may or may not be the actual site of entry of the pathogen. Nevertheless, recent findings support the role of toe webs as a potential site of entry, and colonization of toe webs by pathogens is a risk factor for lower-limb cellulitis [5, 25].

Only 1 patient harbored the same streptococcal clone in both the pharynx and affected skin. The skin is a more likely origin of the infection than is the respiratory tract, and presence of streptococci on the intact skin before cellulitis onset has been reported [26]. The causal relationship with anal GGS colonization and bacterial cellulitis has also been studied [27].

There was no clear predominance of a specific *emm* type in GGS or GAS that associated with the disease, although it is difficult to draw conclusions from relatively few isolates. Of the *emm* types in GAS isolates, *emm*28 was common among Finnish invasive strains during the same time period [11]. In contrast, very little is known of the *emm* types of GGS that cause cellulitis. Many of the *emm* types that we found in GGS isolates have been related to invasive disease, bacteremia, and toxic shock syndrome [28–31].

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Of patients with bacterial cellulitis, 20%–30% are prone to recurrences [4, 9]. Even within this short study period, 7% of the patients had a recurrence, and 50% of all patients reported having previous cellulitis infections. In 2 patients, the GGS strains that were isolated in the consecutive episodes only 2 months apart had identical *emm* and PFGE types, suggesting that these infections were relapses. Recurrent GGS bacteremia has also been reported [28]. The pathogen's persistence in the tissue, despite antibiotic treatment, contributes to the rate of recurrence. The question remains as to whether recurrences are specifically associated with a streptococcal species or strain. The median age of patients with recurrences was 10 years younger than the median age of the other case patients. Younger patients may be at a high risk of recurrence, and a previous cellulitis infection seems to be a strong predisposing factor to future episodes [4, 5, 8, 32]. Various general and local risk factors play a role in recurrences, as does the patient's increased susceptibility to infection, such as the inability of the immune system to clear the bacteria from the infection site.

Relatively little is known of the unique characteristics of GGS in relation to its pathogenic potential. The bacterial load and the magnitude and type of cytokine expression correlate with the severity of GAS soft-tissue infection [33]. Toxins have a critical role in streptococcal pathogenesis, and their distribution varies among GAS strains [2, 33, 34]. There is strong support that horizontal transfer of virulence genes between GAS and GGS occurs and may lead to clones with enhanced pathogenic potential [35–40]. Thus, further research targeted to the group A and group G streptococcal virulence determinants and genome is warranted.

Group G streptococci, instead of group A streptococci, seem to predominate in skin lesions of patients with bacterial cellulitis. A predominance of GGS was also seen in the throat of patients and their family members, whereas it was not detected in control subjects. Several *emm* types were present in GGS and GAS isolates, with no clear predominance of a specific type. The recurrent nature of cellulitis became evident during this study.

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ARTICLE

Evidence of streptococcal origin of acute non-necrotising cellulitis: a serological study

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Abstract Bacteriological diagnosis is rarely achieved in acute cellulitis. Beta-haemolytic streptococci and *Staphylococcus aureus* are considered the main pathogens. The role of the latter is, however, unclear in cases of non-suppurative cellulitis. We conducted a serological study to investigate the bacterial aetiology of acute non-necrotising cellulitis. Anti-streptolysin O (ASO), anti-deoxyribonuclease B (ADN) and anti-staphylolysin (ASTA) titres were measured from acute and convalescent phase sera of 77 patients hospitalised because of acute bacterial non-necrotising cellulitis and from the serum samples of 89 control subjects matched for age and sex. Antibiotic treatment decisions were also reviewed. Streptococcal serology was positive in 53 (69 %) of the 77 cases. Furthermore, ten cases without serological evidence of streptococcal infection were successfully treated with penicillin. Positive ASO and ADN titres were detected in ten (11 %) and three (3 %) of the 89 controls, respectively, and ASTA was elevated in three patients and 11 controls. Our findings suggest that acute non-necrotising cellulitis without pus formation is mostly of streptococcal origin and that penicillin can be used as the first-line therapy for most patients.

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Introduction

The bacterial aetiology of acute non-necrotising cellulitis without pus formation is not possible to ascertain in most cases. Beta-haemolytic streptococci (BHS), mainly group A streptococci (GAS) and group G streptococci (GGS) or group C streptococci BHS, as well as *Staphylococcus aureus*, have been considered as the main causative bacteria [1, 2]. A variety of other bacterial species are associated with acute cellulitis in rare cases, mainly in patients with severe comorbidity [3]. The clinical question as to whether *S. aureus* has to be covered in the initial antibiotic choice in acute non-necrotising cellulitis has become more important with the emergence of methicillin-resistant *S. aureus* (MRSA). In a recent study in the USA, serology and blood cultures confirmed BHS as causative agents in 73 % of the 179 cellulitis cases [4]. As *S. aureus* is a common finding in skin lesions [5, 6], it may be found in skin swabs taken in acute cellulitis cases, but its role as a causative agent remains unclear.

We have previously conducted a case–control study on clinical risk factors and microbiological findings in acute bacterial non-necrotising cellulitis with controls derived from the general population [6]. BHS were isolated in 26 (29 %) of

90 patients. GGS was the most common streptococcal finding, followed by GAS (22 % and 7 % of patients, respectively). However, *S. aureus* was also isolated in 29 (32 %) of the 90 patients, but no MRSA was found. The current study was an observational study of patients hospitalised with acute cellulitis, to describe the bacterial aetiology of diffuse non-culturable cellulitis. Clinical antibiotic use during the initial study was also reviewed.

Methods

The patient population was described previously [7]. In short, 90 patients (aged ≥18 years) admitted to two infectious diseases wards at Tampere University Hospital (Finland) and Hatanpää City Hospital (Tampere, Finland) with acute non-necrotising cellulitis were recruited. The patient population represented diffuse non-culturable cellulitis and wound infections, abscesses and human or animal bites were excluded. The control subjects (living in Tampere, Finland, matched for age and sex) were randomly sampled from the Finnish Population Register Centre. Acute phase sera were collected on admission or on the next working day and convalescent phase sera at 4 weeks after admission. Additionally, serum samples were obtained from 89 control subjects, matched for age and sex as described earlier [7]. Anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADN) titres were determined by a nephelometric method according to the manufacturer's instructions (Behring, Marburg, Germany). The normal values for both are <200 U/mL, according to the manufacturer. For anti-staphylolysin (ASTA), a latex agglutination method by the same manufacturer was used. A titre <2 IU/mL was considered normal. Positive serology for ASO and ADN was determined as a 0.2 log rise in a titre between acute and convalescent phase sera [8], and with a titre of ≥200 U/mL in convalescent samples, or ≥200 U/mL in both samples [4]. Positive serology for ASTA was determined as a titre of ≥2 U/mL in convalescent phase samples. For controls, a titre of ≥200 U/mL in the serum sample was considered positive for ASO and ADN and ≥2 U/mL was considered positive for ASTA.

Data concerning antibiotic treatment during the study period and immediately before admission were collected from patient charts and by patient interview, as described in the previous study [7].

Results

Both acute and convalescent phase sera were available in 77 cases (the median time between samples was 31 days, range 12–118 days). Serological findings in relation to preceding antibiotic therapy are shown in Table 1. Overall, on the basis of serology, there was evidence of streptococcal aetiology in 53 (69 %) of the 77 hospitalised patients with acute non-necrotising bacterial cellulitis. All patients with positive serology for ADN also had positive serology for ASO.

In 89 control subjects, the median ASO titre was 61 U/mL (maximum 464 U/mL), and the median ADN titre was 72 U/mL (maximum 458 U/mL). ASO and ADN titres of ≥200 U/mL were measured in ten (11 %) and three (3 %) of the 89 controls, respectively. For ASTA, three patients (titre 2 U/mL in all) and 11 controls (titre 8 U/mL in one, 4 U/ml in four and 2 U/ml in six) were seropositive. The difference in positive ASTA serology between patients and controls was not statistically significant (McNemar's test, $p=0.15$).

Table 2 summarises the antibiotic treatment decisions in hospital in relation to serological findings in the 77 patients. Overall, in the original patient population ($n=90$), initial antibiotic treatment was penicillin in 39 cases (43 %), cefuroxime in 26 cases (29 %), clindamycin in 24 cases (27 %) and ceftriaxone in one case. Initial penicillin treatment was changed because of suspected poor treatment response in 9/39 cases (23 %), cefuroxime in 5/26 cases (19 %) and clindamycin in 1/24 cases (4 %).

Table 1 Relation of preceding antibiotic therapy with serological findings in 77 patients hospitalised with acute cellulitis

Clinical characteristic	Serology		
	ASO+[a]	ADN+[b]	ASTA+[c]
All patients ($n=77$)	53 (69 %)	6 (8 %)	3 (4 %)
Antibiotic therapy before admission ($n=22$)	11 (50 %)[d]	1 (5 %)	2 (9 %)
No antibiotic therapy before admission ($n=55$)	42 (76 %)[d]	5 (9 %)	1 (2 %)

[a] Positive serology for anti-streptolysin O

[b] Positive serology for anti-deoxyribonuclease B (all six patients also ASO+)

[c] Positive serology for anti-staphylolysin

[d] Difference was statistically significant (χ^2 test, $p=0.024$)

Table 2 Initial antibiotic treatment and suspected inadequate response in relation to serological findings in 77 patients hospitalised with acute cellulitis

	Antibiotic therapy switched due to suspected inadequate treatment response	
Antibiotic initiated on admission[a]	ASO+[b] (n=53)	ASO–[c] (n=24)
Penicillin	6/24 (25 %)	0/10
Anti-staphylococcal antibiotics[d]	3/29 (10 %)	1/14 (7 %)

[a] All intravenous, usual doses as follows: penicillin 2–4 mU q 4–6 h, cefuroxime 1.5 g q 8 h, clindamycin 300–600 mg q 6–8 h

[b] Positive streptococcal serology (all patients with positive ADN had positive ASO serology)

[c] Negative streptococcal serology

[d] Cefuroxime, ceftriaxone or clindamycin

Fifty (79 %) of the 63 patients with either positive streptococcal serology (n=53) or successful treatment with penicillin without serological evidence of streptococcal infection (n=10) were classified as cellulitis because of the non-clear margin of the erythema.

Discussion

In the present study, 53 of the 77 patients (69 %) hospitalised with acute bacterial non-necrotising cellulitis had positive streptococcal serology. A recent study in the USA by Jeng et al. [4] showed that, based on serology and blood cultures, 73 % of non-culturable cellulitis cases were of streptococcal origin. The prospective study design, case definition and serological methods in the present study were similar to those in that larger study. Furthermore, in both studies, two-thirds of the patients were male, and the cellulitis was located in the lower extremity in the majority of cases. The patients in our study were older (mean age 57 years vs. 48 years) and obesity (42 % vs. 10 %), lymphoedema (25 % vs. 16 %) and recurrent cellulitis (51 % vs. 19 %) were more common in our patient population than in the study by Jeng et al. In contrast, the proportion of patients with diabetes mellitus (13 % vs. 27 %) and liver cirrhosis (1 % vs. 12 %) was smaller in our study. Thus, the present study confirms the findings of the study by Jeng et al. in a different geographical setting and with a different distribution of risk factors that the majority of diffuse non-culturable cellulitis cases are caused by BHS.

Ten patients with negative streptococcal serology were successfully treated with penicillin alone, suggesting non-staphylococcal aetiology. Thus, 63 (82 %) of the 77 patients had either serological evidence of streptococcal origin of the infection or were successfully treated with penicillin. Although not prospectively studied, our findings, together with the study by Jeng et al. [4], support the recent findings and conclusions [4, 9, 10] that β-lactam antibiotics, including penicillin, are effective in this setting, even if MRSA is more prevalent than in our area during the present study. This has been demonstrated in a randomised, double-blind, placebo-controlled study in which there was no benefit of combining an anti-MRSA antibiotic (trimethoprim–sulphamethoxazole) with cephalosporin for the treatment of uncomplicated cellulitis in outpatients [10].

Streptococcal serology was significantly less often positive in those patients who had received antibiotic treatment as outpatients immediately before hospitalisation than in those who had not (Table 1), which is in line with earlier findings [11]. Therefore, it is likely that streptococci are the aetiological agents, at least in some of the seronegative cases.

Additionally, 11 % of the control subjects were ASO seropositive. This is most likely owing to a previous streptococcal throat infection, because those patients with a history of cellulitis were excluded from the control population in the initial study [6, 7]. Unfortunately, we lack the data concerning sore throat in the previous months. However, according to our earlier study [6], BHS were cultured from throat swabs in nine control subjects, two of whom were ASO positive and two were both ASO and ADN positive.

The purely observational nature of this study regarding the data on initial antibiotic choices and subsequent changes should be kept in mind. The treatment decisions were solely made by the attending physician and the choice of initial antibiotic and the evaluation of inadequate response to initial treatment varied according to the individual experience and clinical judgement. The isolation of *S. aureus* from clinical specimens may have influenced the decision to switch antibiotic treatment, regardless of clinical response.

Most patients with serological evidence of BHS infection or adequate response to penicillin treatment had skin erythema with non-clear margins. Thus, most patients could be classified as having cellulitis. In clinical practice, the distinction between erysipelas and cellulitis is not of great importance, unless an abscess is present [9].

The role of *S. aureus* in erysipelas or cellulitis is controversial [2–5, 10, 12, 13]. There was a wide variation in the case definitions of cellulitis in previous studies [9, 14], which makes comparison of the results difficult. Moreover, bacterial cultures from intact skin, or even aspirates or punch biopsies, are frequently negative [13]. However, BHS are present in inter-digital spaces in cellulitis patients with athlete's foot [15]. *S. aureus* commonly colonises the skin, especially when it is broken, whereas BHS is considered a transient coloniser [15–17]. In our previous study, *S. aureus* was the only bacterial finding in ten acute cellulitis cases, but in 17 cases, it was isolated together with BHS [6]. Therefore, it is more likely that BHS, if found on skin swabs from cellulitis without pus formation, represent the true pathogen, in contrast to *S. aureus*.

ASTA serology has no value in acute bacterial non-necrotising cellulitis, underlined by the fact that the few ASTA-seropositive patients were also ASO seropositive. Furthermore, ASTA seropositivity was more common (although not significantly) among the population-derived control subjects than among the cellulitis patients. These findings are in line with a recent study assessing the value of staphylococcal serology in suspected deep-seated infection [18]. Although the diagnosis of acute cellulitis and the initial treatment decision must be made on clinical grounds, high ASO and ADN titres in the acute phase may give valuable support. In some cases, the antibody response may be rapid or the infection may have progressed over a prolonged time prior to admission.

Our clinical conclusion of the present study is that, if a patient is hospitalised with an acute cellulitis of suspected bacterial origin without pus formation, the infection is most likely of streptococcal origin and antibiotic therapy can be started with penicillin. However, it may be important to cover *S. aureus* in the initial antibiotic choice when cellulitis is associated with a chronic leg ulcer, as stated previously [5]. Also, one should bear in mind that this study did not include patients with diabetic foot, necrotising infections or those admitted to the intensive care unit.

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Conflict of interest The authors declare that they have no conflict of interest.

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Predictors of recurrent cellulitis in five years. Clinical risk factors and the role of PTX3 and CRP

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Summary *Objectives:* To identify risk factors for recurrence of cellulitis, and to assess the predictive value of pentraxin 3 (PTX3) and C-reactive protein (CRP) measured at baseline.
Methods: A follow up study of 90 hospitalised patients with acute non-necrotising cellulitis was conducted. Clinical risk factors were assessed and PTX3 and CRP values were measured at baseline. Patients were contacted by phone at a median of 4.6 years after the baseline episode and the medical records were reviewed.

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Results: Overall, 41% of the patients had a recurrence in the follow up. Of the patients with a history of a previous cellulitis in the baseline study 57% had a recurrence in five year follow up as compared to 26% of those without previous episodes ($p = 0.003$). In multivariate analysis, only the history of previous cellulitis was identified as an independent predicting factor for recurrence. The levels of pentraxin 3 (PTX3) or C-reactive protein (CRP) in the acute phase did not predict recurrence.

Conclusions: Risk of recurrence is considerably higher after a recurrent episode than after the first episode. Clinical risk factors predisposing to the first cellulitis episode plausibly predispose also to recurrences.

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Introduction

Acute bacterial cellulitis is an infection of the skin and subcutaneous tissue. Mostly it has a relatively benign course.[1] However, recurrences are common and may be considered as the main complication of acute cellulitis.[2] Overall recurrence rates have varied between 22 and 47% within two to three years follow up.[3–5] Preventive measures, such as compression stockings to reduce chronic leg oedema, or careful skin care to avoid skin breaks, have considered to be essential in reducing the risk of recurrence.[3,6,7] Prophylactic antibiotics have been used in order to prevent further cellulitis episodes in patients suffering many recurrences, and recently, low-dose penicillin has been shown to be effective.[8] Yet, the optimal patient selection for prophylactic antibiotic use, antibiotic dosing regimen and actual effectiveness of other preventive measures remain to be proven.[2,8–11] It has been shown that the risk for a recurrence is greater for those patients who already have suffered recurrent cellulitis, as compared to those who have had only one episode.[3,4] Prior leg surgery,[12] dermatitis, cancer, and tibial localisation[5] have been associated with the risk of recurrence after the initial episode. Risk factors for acute and recurrent cellulitis have been investigated in several studies.[6,7,12–17]

In our previous case control study[15,18] assessing the clinical risk factors for acute non-necrotising cellulitis, we have shown that chronic oedema of the extremity, disruption of the cutaneous barrier and obesity are associated with acute cellulitis. Furthermore, in the baseline study[15] patients presenting with a recurrent cellulitis had a stronger inflammatory response, as measured by peak CRP level and leukocyte count and longer stay in hospital, than those with their first cellulitis episode. Based on these findings, we conducted a five year follow up study to investigate demographic and clinical risk factors for recurrent cellulitis. Also, we assessed the value of short and long pentraxins, i.e. CRP and pentraxin 3 (PTX3) as laboratory markers of inflammation in predicting recurrence of cellulitis in five years follow up.

Materials and methods

Patients and methods

Study population consisted of patients hospitalised due to acute cellulitis and participated in the baseline study.[15] The patient population is previously described in detail.[15] In short, adult (≥18 yr) patients with an acute onset of fever or chills and a localised erythema of the skin in one extremity or in the face were recruited in the baseline study (see figure legend, Fig. 1). Patients were contacted by phone during March and April 2009 and asked if they had had any new cellulitis episodes after the initial study period (from April 2004 to March 2005). Medical records concerning the recalled recurrent episodes were obtained. Also, the available electronic health records of all patients of the previous study were examined to detect possibly unrecalled episodes and collecting data concerning patients not reached by phone. One patient had declined to participate in the follow up study after the initial recruitment. Seventy-eight (88%) of 89 patients were alive at the follow up, and 67 patients could be reached by phone.

In the baseline study patients and matched controls were clinically examined and the possible clinical risk factors were recorded. The history of previous cellulitis episodes was recorded for the patients, i.e. whether the cellulitis episode at the baseline study was the first for the given patient (negative history of cellulitis, NH) or a recurrent episode (positive history of cellulitis, PH). Thus, for NH patients the recorded recurrence during the follow up of the present study was their first recurrence. The number of possible multiple recurrences during the follow up was not recorded. CRP levels were measured according to the clinical practice on the hospital days 1–5, where day 1 is the day of admission, as described earlier.[15] Serum and EDTA-plasma samples for subsequent analysis were obtained in the acute phase (on admission or on the next working day following admission) and convalescent phase and stored in aliquots at −20 °C. PTX3 levels were measured from the thawed EDTA-plasma samples by a commercially available immunoassay (Quantikine, R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. Acute phase sera were collected within less than three days after admission in 65 (75%) of the 87 cases as follows: day 1 (admission) in three cases, day 2 in 52 cases and day 3 in 10 cases. These 65 cases were included in acute phase PTX3 analyses. Convalescent phase sera were obtained from 73 patients one month after admission (median 31 days, range 12–67 days, except for one patient 118 days).

Statistical analysis

For continuous variables, median, maximum and minimum values are given. Statistical analysis was performed with

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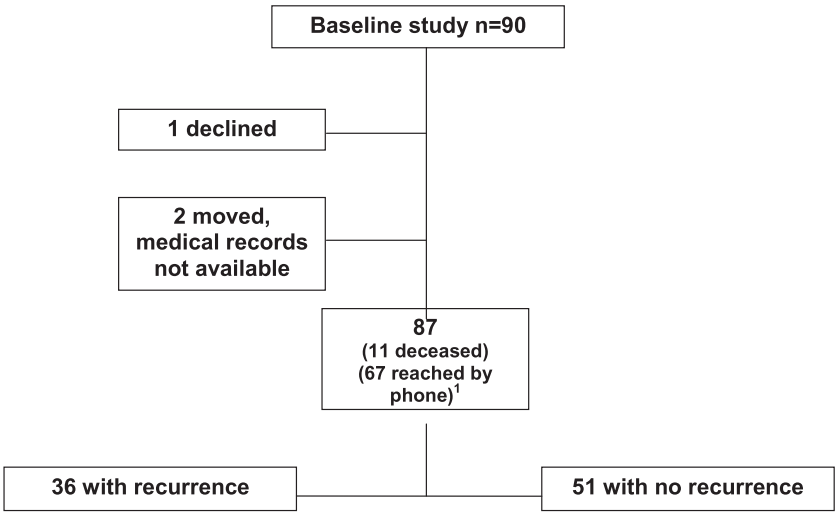

[1]Nine patients were not reached by phone. In these cases data were obtained from the medical records.

Figure 1 Five year follow up of 90 patients hospitalised for acute cellulitis in the baseline study. In the baseline study 104 patients were initially identified of which eight refused and further six were excluded due to obvious alternative diagnoses.

SPSS package version 14. Univariate analysis between categorical variables was performed by chi-squared test or Fisher's exact test, where appropriate, and between categorical and continuous variables by Mann–Whitney *U*-test. A logistic regression analysis (method Forward Stepwise) was performed to bring out independent risk factors for recurrence. This was performed separately for NH and PH cases and all patients, i.e. for the first and multiple recurrences. The value of CRP and PTX3 in predicting cellulitis recurrence was evaluated by ROC curves. PTX3 analysis was performed only for cases in which the acute phase sera were collected during hospital days 1 (admission) to 3. For CRP analysis the peak value during hospital days 1–5 was used.

The study was approved by the Ethical Review Board of Pirkanmaa Health District.

Results

The median follow up time was 4.6 years (range 4.1–5.5 years) for those alive at follow up ($n = 78$). For those deceased during follow up ($n = 11$) the follow up time ranged from three months to 5.1 years. During follow up, at least one recurrence of acute cellulitis could be verified in 36 (41%) patients and reliably excluded in 51 patients, thus the study comprised 87 patients (Fig. 1).

Seventeen (20%) of the 87 patients reported having only one recurrence during the follow up. There were two recurrences reported by 6 patients, three recurrences by 3, and four recurrences reported by 2 patients. All of these recurrences could be verified from medical records. In addition, eight patients reported more than one recurrence, and at least one but not all of the episodes could be reliably verified from medical records. No statistically significant associations were detected between the number of recurrences in the follow up and the risk factors identified in the baseline study, namely obesity (BMI $\geq$ 30), chronic oedema of the extremity, or disruption of the cutaneous barrier (data not shown). Six (7%) of the 87 patients had none of these risk factors. One of these six had a recurrence in the five year follow up and another had suffered 7 cellulitis episodes before the baseline study and had been on continuous penicillin prophylaxis since the last episode. She was diabetic and had received radiotherapy for vulvar carcinoma.

Of the 87 patients 43 were NH patients and 44 were PH patients as assessed by the baseline study. Eleven (26%) of the NH and 25 (57%) of the PH patients had a recurrence in 5 year follow up, respectively. Data of antibiotic prophylaxis was available in 70 cases. Twenty-two (31%) patients had received variable periods of prophylactic antibiotic treatment during the follow up, and 15 (68%) of those 22 reported a cellulitis recurrence during the follow up.

In the baseline study Group A (GAS) and group G (GGS) beta-haemolytic streptococci were recovered from skin swab specimen in 6 and 17 of the 87 cases, respectively.[18] One (17%) of the six GAS positive cases had a recurrence in 5 years as compared to 10 (59%) of the 17 GGS positive cases. However, this difference did not reach statistical significance ($p = 0.155$, two-tailed Fisher's exact test).

The median PTX3 in acute phase (days 1–3) was 5.5 ng/ml (range 2.1–94.3 ng/ml) and in the convalescent phase 2.6 ng/ml (range 0.8–11.8 ng/ml). There was a significant correlation between the highest CRP in days 1–3 and PTX3 values ($r_s = 0.53$, $p = 0.01$). No difference was detected in acute phase PTX3 values between PH and NH patients. The ROC curves for analysing the value of acute phase PTX3 and CRP in relation to cellulitis recurrence are shown in Fig. 2. No cut-off value could be determined for either PTX3 or CRP for predicting recurrence [AUC(ROC) = 0.535 (CI 0.390–0.680), $p = 0.64$ and AUC(ROC) = 0.499 (CI 0.371–0.626), $p = 0.98$, respectively].

Univariate analysis of clinical risk factors is shown in Table 1. The patients with recurrence in follow up had been significantly younger at the time of their 1st cellulitis

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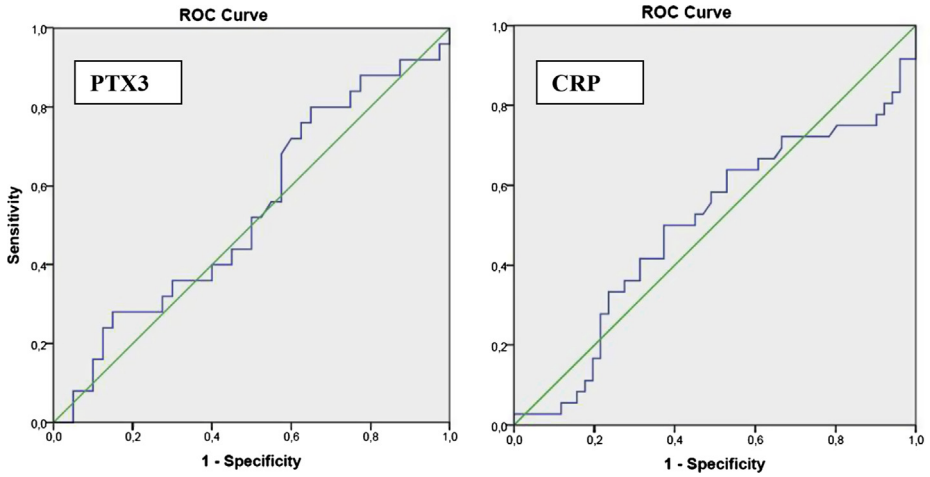

Figure 2 Receiver operating characteristic (ROC) curves for pentraxin 3 (PTX3) level measured in the baseline study on days 1–3 (1 = admission, $n = 65$), and the highest C-reactive protein (CRP) level measured on days 1–5 in relation to cellulitis recurrence in five year follow up ($n = 87$).

episode than those without recurrence [median 49 (12–78) and 58 (16–84) years (range), respectively; $p = 0.008$]. Furthermore, the patients with repeated cellulitis episodes (PH and/or recurrence in follow up) had had their 1st cellulitis episode younger than those with single episode (NH and no recurrence in the follow up) [median 49 (12–76) and 63 (28–84) years (range), respectively; $p = 0.002$], though the finding does not fit to our prospective study setting.

57% of the patients with a PH in the baseline study had a recurrence in five years follow up as compared to 26% in

Table 1 Univariate analysis of clinical risk factors for recurrence of cellulitis in 87 patients in 5 years follow up. The values are expressed as the number of patients [*n* (%)] if not otherwise stated.

Risk factors assessed in the baseline study	Recurrence in 5 yr follow up		*p*-Value	OR	95% CI
	Yes ($N = 36$) *n* (%)	No ($N = 51$) *n* (%)			
Previous cellulitis episode at baseline	25 (69)	19 (37)	0.003	3.8	1.5–9.5
Age at the baseline study, years [median (quartiles)]	56.7 (48.2–60.8)	63.3 (51.9–69.0)	0.079	0.98	0.95–1.01
Age at the 1st cellulitis episode, years [median (quartiles)]	48.9 (37.2–56.7)	58.3 (44.8–67.5)	0.008	0.96	0.93–0.99
Alcohol abuse	3 (8)	7 (14)	0.513	0.6	0.1–2.4
Obesity (BMI $\geq$ 30)	19 (53)	17 (34)	0.082	2.1	0.9–5.2
Current smoking	10 (29)	21 (41)	0.232	0.6	0.2–1.4
Malignant disease	8 (22)	5 (10)	0.110	2.6	0.8–8.8
Cardiovascular disease	4 (6)	12 (20)	0.141	0.4	0.1–1.4
Diabetes	6 (17)	6 (12)	0.542	1.5	0.4–5.1
Chronic oedema of the extremity[a]	13 (38)	10 (21)	0.095	2.3	0.9–6.1
Disruption of cutaneous barrier[b]	28 (93)	38 (86)	0.461	2.2	0.4–11.8
traumatic wound <1 mo	5 (14)	10 (20)	0.487	0.7	0.2–2.1
skin diseases	14 (39)	14 (28)	0.261	1.7	0.7–4.2
toe-web intertrigo[b]	20 (67)	29 (66)	0.946	1.0	0.3–2.8
chronic ulcer	4 (11)	2 (4)	0.226	3.1	0.5–17.7
Previous operation	19 (53)	19 (37)	0.151	1.9	0.8–4.5
Antibiotic treatment before admission	10 (28)	15 (29)	0.868	0.9	0.4–2.4
Peak CRP >218 mg/l[c]	10 (28)	12 (24)	0.653	1.3	0.5–3.3
Peak leucocyte count >16.9 $\times$ 10^9/l[c]	11 (31)	11 (22)	0.342	1.6	0.6–4.2
Duration of fever >3 days after admission to hospital	3 (8)	7 (14)	0.513	0.6	0.1–2.4
Length of stay in hospital >7 days	17 (47)	30 (59)	0.285	0.6	0.3–1.5

[a] Cellulitis of the face ($n = 6$) excluded.
[b] Cellulitis of the face ($n = 6$) and upper extremities ($n = 7$) excluded; disruption of cutaneous barrier comprises traumatic wounds <1 month, skin disease, toe-web intertrigo, and chronic ulcers. This combined variable was used in multivariate analysis.
[c] Seventy-fifth percentile of patients, corresponding to a CRP level of 218 mg/L and a leucocyte count of 16.9·109/L.

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those with NH ($p = 0.003$). In the multivariate analysis, only the history of previous cellulitis remained significantly associated with recurrence in the follow up (Table 2). The clinical risk factors for cellulitis identified in the baseline study were also analysed in the subgroups of the NH and PH patients. No statistically significant risk factors were found in either subgroup (data not shown).

Discussion

In the present study we found that the history of repeated cellulitis is the major risk factor for subsequent recurrence. The risk of a further recurrence in five years after a recurrent episode of acute cellulitis is more than twofold (26% vs. 57%) than that after the first episode.

An acute cellulitis attack may cause damage to the lymphatic vessels, leading to chronic oedema and thereby predisposing the patient to subsequent cellulitis episodes.[3] As it has been shown in previous studies,[6,12,15] chronic oedema, disruption of the cutaneous barrier and obesity are important risk factors for acute cellulitis, and — though not proved as risk factors for recurrence in this paper — it is not plausible that these factors wouldn't have any role in susceptibility to recurrent cellulitis, therefore it seems wise to focus attention to these in clinical practice.[2,19] The associations of the clinical risk factors with the risk of recurrence may not have reached statistical significance due to the relatively small number of patients in the present study. This applies especially to the subgroup of NH patients in which no risk factor was statistically significantly associated with the risk of first recurrence. Further studies are needed in order to identify the patient group at greatest risk for recurrence among those presenting with their first cellulitis episode. This would offer valuable information for the clinical decision making concerning antibiotic prophylaxis. Furthermore, it should be noted that nearly one third of the patients had received prophylactic antibiotic treatment during the follow up. As it was not possible to ascertain the duration of the prophylaxis in all cases, antibiotic prophylaxis was not included in the statistical analysis. Thus, the confounding effect of antibiotic prophylaxis can not be assessed. Also, other interventions during the follow up (e.g. treatment of skin breaks and relieving chronic oedema) may have had an effect to the risk of recurrence.

If the patient is lacking any of the risk factors for acute cellulitis mentioned above, the risk for recurrence seems to be low. However these patients represent a minority among hospitalised patients, as 93% of the patients in the present study had at least one risk factor known to associate with acute cellulitis. On the other hand, there may be underlying dysfunction in the lymphatic vessels even prior to the first cellulitis attack.[20] Based on the present data we cannot determine whether an attack of acute cellulitis itself causes the susceptibility for subsequent recurrences, as there may be other hitherto unknown factors predisposing to recurrences. However, our finding that the patients with a recurrence in the 5 years follow up had had their first cellulitis episode at a younger age than those without recurrence refers also to some hereditary factors predisposing to recurrences.

In the baseline study acute phase CRP levels were higher in PH than in NH patients.[15] Therefore we tested the hypothesis that acute phase reaction measured by CRP or PTX3 levels at acute or convalescent phase of acute cellulitis could predict subsequent recurrence of cellulitis. CRP, a short pentraxin, is a pattern recognition molecule participating in systemic inflammatory response and innate immunity.[21] It has been shown to be of value in predicting the outcome of some serious acute infections such as community acquired pneumonia[22] and endocarditis.[23] Also, PTX3, a member of the long pentraxin family, plays an important role in humoral innate immunity and is one of the regulatory components of both local and systemic inflammation.[24] It has recently been shown to be associated with the severity of different inflammatory and infectious conditions, e.g. Puumala hantavirus infection,[25] bacteraemia,[26] ischaemic stroke,[27] and febrile neutropenia[28] as well as psoriasis.[29] In contrast to CRP, which is mainly produced by liver cells stimulated by interleukin 6, PTX3 is synthesised by various cell types including fibroblasts, polymorphonuclear leukocytes and dendritic cells existing also in the skin and stimulated by TNFα and IL1.[24] The hypothesis could not be confirmed for either parameter in either phase (data for convalescent phase not shown). For CRP the highest value in days 1—5 was used, similarly as in the baseline study. However, PTX3 was measured from one serum sample only, taken 1—3 days after admission to

Table 2 Multivariable analysis (logistic regression, forward stepwise method) of clinical risk factors for cellulitis recurrence in 5 years follow up. Patients with cellulitis of the face ($n = 6$) are excluded as chronic oedema of the extremity is not relevant in those cases.

Risk factors	*p*-Value	OR	95% CI
Variables in the equation			
Previous cellulitis episode at baseline	0.011	3.4	1.3—8.8
Variables offered but not entered in the equation			
Age at the 1st cellulitis episode	0.052		
Obesity (BMI $\geq$ 30)	0.560		
Malignant disease	0.240		
Cardiovascular disease	0.340		
Chronic oedema of the extremity	0.151		
Previous operation	0.250		

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hospital in 65 cases, most often on day 2 (52 cases). Thus, the highest PTX3 value for a given cellulitis episode could not be determined, which may have influenced the analysis regarding PTX3. Also, in contrast to acute phase CRP as reported in the baseline study, PTX3 values did not differ significantly between PH and NH patients (data not shown), which may be due to the aforementioned flaw in collecting the sera for PTX3 measurements. However, in the baseline study, the peak CRP value was recorded on days 1 or 2 in the majority (84%) of cases.[15] Thus, it is likely that the peak PTX3 levels had been reached in the majority of cases during days 1–3 as PTX3 levels increase even more rapidly than CRP levels in the acute phase of infection.[30] The inflammatory response measured by CRP or PTX3 as well as other variables reflecting the severity of cellulitis attack (peak leukocyte count, duration of fever and length of stay in hospital) do not predict further recurrence, hence, in clinical practice, predicting the risk of recurrent cellulitis and decision concerning antibiotic prophylaxis remain to be made on clinical grounds. The optimal timing of antibiotic prophylaxis is unclear.[8] If a bout of acute cellulitis itself makes one more prone to subsequent recurrences, it would probably be reasonable to institute antibiotic prophylaxis after the very first cellulitis attack.

In conclusion, the history of previous cellulitis episodes is highly predictive for a subsequent cellulitis recurrence. Overall, 41% of patients hospitalised due to acute cellulitis had a recurrence in five years follow up, and among those with a history of previous cellulitis the recurrence rate was as high as 57%. These figures highlight the need for understanding the risk factors for recurrence in order to find and appropriately target preventive measures. CRP or PTX3 values in the acute phase of acute cellulitis do not predict further recurrences.

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