

# CHAPTER 1

**General introduction**

## Balkenende openhartig over ziekbed

Premier Balkenende realiseerde zich pas achteraf dat zijn voetinfectie potentieel levensbedreigend was. Dat zei hij tijdens een openhartig interview in de actualiteitenrubriek Netwerk... De dokters ontdekten twee bacterietypen in Balkenendes bloed: stafylococci en streptococci. "Als het alleen

het eerste type was geweest, waren antibiotica waarschijnlijk wel afdoende geweest. Maar streptococci zijn zeer gevaarlijk, omdat het vleesetende bacteriën zijn. De combinatie van de twee kan fataal zijn", aldus Balkenende.

**Source:** NOS (<http://www.nos.nl/nieuws/artikelen/2004/10/31/balkenendeoverziekbed.html>)

## Introduction

During the Dutch presidency of the European Union in the second half of 2004, prime minister Balkenende was hospitalized on September 14<sup>th</sup>. He was operated on several times and unable to chair the European Council for six weeks due to a severe soft tissue infection of his right foot caused by *Streptococcus pyogenes*. *S. pyogenes*, or group A streptococcus (GAS) (73) is associated with a wide range of disease manifestations that have afflicted mankind before understandable accounts were recorded. Before the pathogen was identified, scarlet fever and puerperal sepsis were the most reliably diagnosed manifestations of GAS disease. The history of these diseases provides us with epidemiological clues to understand GAS disease today.

## History of group A streptococcal disease

### History of scarlet fever

Although probable case descriptions of scarlet fever have been attributed to Hippocrates (112), Sydenham coined the term febris scarlatina and its description as a separate clinical entity in 1676 (130). In 1778, Johnstone proposed a new classification system for scarlet fever (63). He distinguished three separate clinical entities: uncomplicated scarlet fever (scarlatina simplex); scarlet fever with pharyngitis (scarlatina anginosa) and scarlatina with gangrenous inflammation of the oropharynx (scarlatina maligna). Throughout the 18<sup>th</sup> and early 19<sup>th</sup> century, scarlet fever was a relatively benign childhood disease, although some fatal epidemics of scarlatina maligna did occur (68). In the early 19<sup>th</sup> century the situation changed dramatically. A lethal epidemic in Dublin in 1831 was followed by an equally lethal epidemic in Great Britain with case-fatality rates of more than 15% (49). An Irish clinician described the changing picture of scarlet fever as follows: “We now began to hear of cases which proved unexpectedly fatal...still it was not until the year 1834 that the disease spread far and wide, assuming the form of a destructive epidemic...the contagion seemed to act as a more deadly poison on the individuals of some families than upon those of others” (49). Geographic

clusters of severe cases were reported throughout the continent as well as in America with case-fatality rates that reached over 30% (141). During 1840-1883, scarlet fever became the most common deadly infectious childhood disease (135). While fatal cyclic epidemics of severe disease were raging in the large cities of Europe and America, epidemics were only rarely seen and of unpredictable severity in rural areas (87).

Scarlet fever continued to be a leading childhood killer until the mid-1880s, when severe cases became less frequent in Europe and America, with case-fatality rates dropping to ~1% or less in the next several decades. For instance, case-fatality rates from the London Fever Hospital decreased from 16% in 1863-1864 to 11% in 1883, 7.5% in 1887, 2.3% in 1890 and 0.9% in 1891 (141). This trend in decreased scarlet fever severity did not begin at the same time nor proceed at the same pace in all countries. In Russia for example, scarlet fever was associated with fatality in 22% of cases from 1892-1915 (68). Overall however, scarlet fever became a milder disease with epidemics gradually ceasing and fatal cases becoming increasingly rare.

### **History of puerperal sepsis**

Puerperal sepsis is a pelvic infection following delivery. Whereas postpartum infections have always been a risk (124), epidemics of puerperal fever developed from the mid-17<sup>th</sup> century onwards when women delivered in urban maternity hospitals rather than in their own homes. The first report of epidemic puerperal fever dates from 1646 on an epidemic in the Hôtel Dieu, Paris (124). The concept of puerperal fever as a consequence of institutional obstetric care, as well as its contagious nature, was articulated by Alexander Gordon in 1795 (47). He stated “the infection was as readily communicated as that of smallpox or measles, and operated more speedily than any other infection with which I am acquainted...It is a disagreeable declaration for me to mention that I myself was the means of carrying the infection to a great number of women”. Furthermore, he recognized an association between erysipelas and puerperal infections. The medical profession, with very few exceptions, rejected the concept of the contagiousness and microbial origin of the disease.

In 1843, Oliver Wendell Holmes, later dean of Harvard Medical School, read his paper “The contagiousness of Puerperal Fever” before the Boston

Society for Medical Improvement (60). At about the same time, Thomas Watson, professor of medicine at King's college in London, voiced his belief that contagious spread of puerperal fever was possible: "...the hand which is relied upon for succor in the painful and perilous hours of childbirth may literally become the innocent cause of the mother's destruction: innocent no longer, however, if after warning and knowledge of the risk, suitable means are not used to avert a catastrophe so shocking" (138).

Ignaz Semmelweis, a native of Hungary, was very keen to take all measures necessary to "avert a catastrophe so shocking". He was appointed assistant lecturer at Vienna's Allgemeines Krankenhaus in 1844, where he observed that the mortality rates in the two different divisions in his clinic differed dramatically (2). The first division, with mortality rates of 16%, was used to instruct medical students. In the second division, where midwives did deliveries, mortality rates were only 2%. Semmelweis also observed the negligible mortality among women who had given birth before reaching the hospital and did not undergo internal examinations during labor. He reasoned that the observed difference in mortality might be related to the medical students' habit of going to the obstetric ward directly after postmortem examination without hand washing. Midwives did not attend autopsies, practiced on models and examined fewer women in labor. It was clear to Semmelweis that medical students conveyed something to the women in labor which prompted him to institute a routine of hand washing with chlorinated lime in 1847. Within a few months, mortality from puerperal fever dropped from 18% during April and May 1847 to 2.5% during June-November 1847 (137). Nonetheless, Semmelweis met firm opposition by the medical establishment and his appointment in Vienna was not renewed. He returned to Pest, Hungary, where he was appointed obstetrician in Saint Rochus Hospital in 1849. Here too, the institution of strict hand hygiene again led to a great reduction in maternal mortality (118).

## **Discovery of the pathogen**

Those who seriously considered the work of Gordon, Holmes and Semmelweis speculated on the nature of the "harmful things" responsible for the transmission of puerperal sepsis. In the 1880s, Louis Pasteur was shifting from his studies on fermentation to infectious diseases. He came to believe that,

like fermentation, transmissible diseases were due to specific microorganisms infecting the body. In 1874, chain-forming bacteria had been seen in erysipelas lesions by the German surgeon Theodor Billroth who named them *Streptococcus* (from the Greek *streptos*=chain and *kokkus*=berry or seed) (12). In 1879, Pasteur found microbes “in rounded granules arranged in the form of chains or string of beads” in the lochia and blood of women suffering from puerperal sepsis (104). Initially, streptococci were classified according to the source from which they were recovered: *Streptococcus erysipelatosus*, *Streptococcus puerperalis*, *Streptococcus scarlatinae*. In 1884, another German surgeon, Friedrich Julius Rosenbach applied the designation *Streptococcus pyogenes* to the causative agent of wound infections (113). In 1889, Ferdinand Widal, working at Pasteur Institute Paris, argued that these different diseases were caused by one organism (142). In the subsequent 50 years, the streptococcus became well known as the causative agent of a remarkable range of clinical illnesses. In 1903, Schotmüller established the value of the blood agar plate for growing streptococci and Brown described the use of hemolytic reactions to distinguish the  $\alpha$  from  $\beta$  hemolytic streptococci (22,121).

In 1928, Rebecca Lancefield demonstrated that the  $\beta$  hemolytic streptococci could be subdivided in groups A-E according to the group specific polysaccharide or, in case of group D streptococci, lipoteichoic acid (LTA) (76). Most strains obtained from human infections belonged to group A. In 1935 she demonstrated that this also holds true for puerperal sepsis patients (79). Lancefield also showed that group A streptococci could be further subdivided, based on the differences in antigenic nature of their M-protein (77). At about the same time, Griffith differentiated group A streptococci with a slide agglutination test based on the T- rather than M-antigens (51).

## Strain typing

### T- and M-proteins

The T- and M-proteins are present at the surface of group A streptococci. In the laboratory, T-typing is performed by an agglutination test (51). Because certain T-types are associated with particular M-proteins, the testing for the M-type can be shortened by knowledge of the T-type (8).

Like the T-protein, the streptococcal M-protein extends from the cell membrane and is used for serotyping. The N- (amino) terminal portion of the protein constitutes the hypervariable region of the protein. Antigenic differences in this hypervariable region form the basis for the Lancefield serological classification scheme (45,78). Serotyping does not always yield unequivocal results, requires high-titered and well-standardized antisera with a limited life-span and does not allow the identification of new M-types (42). Therefore, a molecular biological technique has been developed using the *emm* gene, that encodes the M-protein, to replace M-protein based serotyping (9). A primer pair is used for the amplification of the N-terminal nucleotide residues encoding the hypervariable part of the M-protein. The amplicon can subsequently be identified using sequencing (9) or an *emm* type specific hybridization technique (69). There are currently 124 recognized M-genotypes (41). Functionally, the M-protein inhibits phagocytosis by interfering with opsonization (13). Absence of the *emm* gene allows rapid phagocytosis of the streptococcus (117). Immunity to the M-protein is protective against reinfection with that M-type and has led to the study of this protein for GAS vaccines (35).

### Other typing techniques

Other molecular biological techniques have been applied to allow typing of streptococci: pulsed-field gel electrophoresis (PFGE), ribotyping, random amplified polymorphic DNA analysis (RAPD), multilocus enzyme electrophoresis (MLEE) and multilocus sequence typing (MLST). PFGE involves digesting the bacterial chromosomal DNA with restriction enzymes that cleave infrequently. The resulting large restriction fragments are separated into a pattern of discrete bands by an alternating high-voltage field (85). Standardized criteria for analyzing the fragment patterns have been defined (133). PFGE patterns correlate with the M-type and are able to differentiate between strains of the same M-type (122). Multilocus sequence typing (MLST) is based on nucleotide sequencing the alleles of several housekeeping genes (39). These alleles are given numbers corresponding to specific mutations in the sequenced region. Analogous to a telephone number, the combination of all allele numbers defines the MLST type of a strain. Based on the nucleotide sequences of seven housekeeping genes, an MLST scheme for GAS has been developed that found a strong association between M-type and MLST type (40).

## Pathogenesis

### Adherence and invasion

Bacterial attachment to skin or mucosa is the first step leading to colonization and disease. Small wounds may disrupt the dermal or mucosal barrier and thus establish a portal of entry to the bacterium. The strategies by which GAS adhere, and later invade, the human host are multiple and complex, involving several different adhesins and invasins. Expression of specific adherence factors may differ from strain to strain depending on their genetic endowment and local environmental factors. Adhesion factors may confer a certain site- or tissue-specificity to the bacterium.

Courtney *et al.* hypothesized a two-step adhesion process (55). The first adhesion step is relatively weak and overcomes electrostatic repulsion. LTA has been proposed to serve as this “first-step” adhesin by hydrophobic interactions, bringing the organisms into close contact with host cells. Fibronectin was identified as the epithelial cell receptor binding LTA (101). Second-step adhesion may then involve M-protein (103) and a large number of other adhesins that promote high-affinity binding between GAS and host cells. The M-protein is important for attachment to keratinocytes in skin infections where membrane cofactor protein 46 (CD46) serves as the receptor for the M-protein (102). Other adhesion molecules that bind fibronectin, collagen and other extracellular matrix components are important in establishing adherence. Fibronectin binding proteins include PrtF1 (protein F1) (52), Sfb1 (streptococcal fibronectin binding protein 1) (131), and related proteins known as PrtF2 (61), PrtF15 (67), Sfb2 (75), Fbp54 (33), Fba (134), and Pfbp (111). Collagen binding proteins include: Cpa (collagen-binding protein) (105), Cpa1 (90), SclA (streptococcal collagen-like surface protein A) (110) and SclB (140).

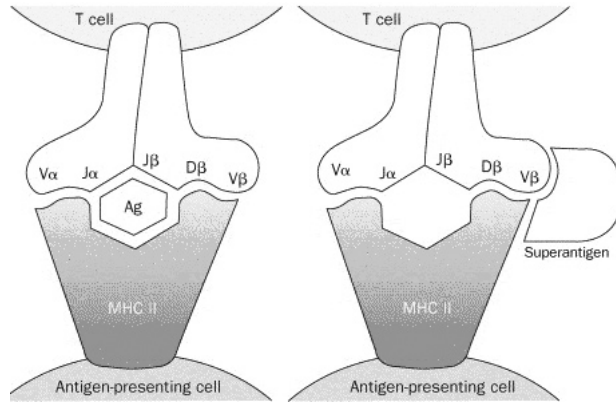
M-protein and Sfb1 have been implicated in intracellular entry of GAS (34,38). This process of cellular penetration may be the first step in the establishment of tissue invasion (80) but may equally provide an intraepithelial sanctuary where the microorganism is sheltered from immune mechanisms and certain classes of antibiotics (96). Spreading of streptococci through tissue is facilitated by the elaboration of a large number of hydrolytic enzymes.

## **Extracellular products contributing to virulence**

GAS produce several extracellular products that may facilitate the spreading of streptococci through tissue in characteristic streptococcal diseases such as cellulitis, myositis and necrotizing fasciitis. These include enzymes that participate in the degradation of DNA, so called streptodornases (streptococcal deoxyribonucleases): streptodornase B (SdaB, also known as mitogenic factor (MF)) (86), streptodornase D (SdaD) (106), Spd1 (or MF2) (20), Spd3 (or MF3) (54) and Sda1 (4). Other extracellular streptococcal virulence factors include a hyaluronidase, which degrades hyaluronic acid, an important component in the ground substance of connective tissue (15); streptokinase, which promotes the dissolution of fibrin clots by catalyzing the conversion of plasminogen to plasmin (82); streptococcal pyrogenic exotoxin B (SpeB), a potent protease (56,66); and C5a peptidase, which cleaves the human chemotaxin C5a (30,43). Streptococcal inhibitor of complement (Sic) is a secreted protein that inhibits complement-mediated lysis of the bacterium (3). Two distinct haemolysins, Streptolysin O (SLO) and Streptolysin S (SLS), damage the membranes of polymorphonuclear leucocytes, platelets, and other eukaryotic cells (26,83).

## **Pyrogenic exotoxins and the streptococcal toxic shock-like syndrome**

The streptococcal pyrogenic exotoxins (Spe's) are a family of proteins with particular structural features that result in the shared ability to bypass the mechanisms of conventional antigen processing (84). Microbial antigens are processed into peptide fragments within antigen-presenting cells (APCs) such as monocytes, B-cells and dendritic cells. These fragments are presented to T-cells in the peptide-binding groove of the MHC class II molecule at the cell surface of the APC. T-cells only respond if they recognize the class II molecule and the specific peptide being presented (81). In contrast, a superantigen can overcome this peptide dependent activation of T-cells by direct binding to the beta chain ( $V\beta$ ) of a characteristic set of T-cell receptors and to the MHC class II molecule expressed on APCs (Figure 1). Thereby, superantigens can activate up to 25% of an individual's T-cell repertoire (28). This massive T-cell activation results in the excessive release of proinflammatory cytokines, such as tumour necrosis factor alpha ( $TNF\alpha$ ), interleukin (IL) 6, interferon gamma ( $IFN\gamma$ ), and IL2 (27). The release of proinflammatory cytokines may



**Figure 1** Interaction of antigens (Ag) (left) and superantigens (right) with the antigen presenting cell and the T-lymphocyte. Superantigens trigger massive T-cell activation that results in the excessive release of proinflammatory cytokines (81).

activate the complement, coagulation, and fibrinolytic cascades, resulting in hypotension and multiorgan failure characteristic of many of the clinical features of toxic shock-like syndrome (TSS) (89).

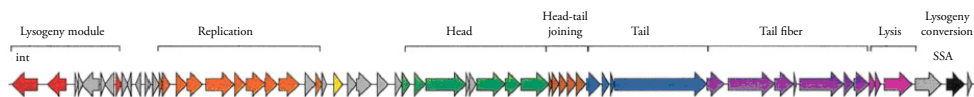
GAS possesses a large number of mitogenic exotoxins, which may function as superantigens. These include: SpeA (139), SpeC (48), SpeF (99), SpeG (107), SpeH (107), SpeJ (107), SpeK (6), SpeL (108), SpeM (108), SSA (91), SMEZ (64), and SMEZ-2 (107). Nucleotide sequencing of the streptococcal pyrogenic exotoxin A gene (*speA*) revealed four naturally occurring alleles of *speA*. The *speA1*, *speA2*, and *speA3* alleles encode toxins that differ in a single amino acid, while *speA4* encodes a toxin which is 9% divergent from the other three and has 26 amino acid substitutions (98). The streptococcal superantigens are roughly 25 kDa, and they contain distinct T-cell receptor and MHC class II binding sites.

Host susceptibility to TSS may depend on the affinity of GAS exotoxins for different HLA class II haplotypes. Recent research by Kotb *et al.* suggested that individuals with a certain class II haplotype are more susceptible to development of TSS (74).

## GAS genome-wide analysis

### Genome sequencing

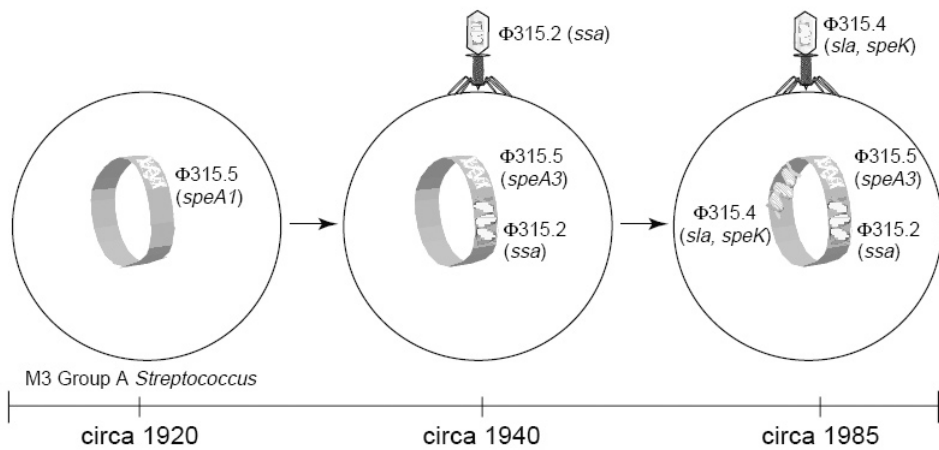
Currently, complete genome sequences from seven *S. pyogenes* strains have been published: two M1 isolates (SF370, isolated from a patient with a wound infection (44) and MGAS5005, associated with invasive GAS disease (128)); two M3 (MGAS315 (10) and SSI-1 (95), both recovered from patients with TSS); one M6 (MGAS10394, cultured from a child with pharyngitis (7)); one M18 (MGAS8232, associated with acute rheumatic fever (123)) and one M28 (MGAS6180, associated with puerperal sepsis (50)). The GAS genomes range in size from 1.84 to 1.90 Mb and have a G+C content of 38.5-38.7%. They encode for 1697 (SF370) to 1894 (MGAS6180) proteins. Per bacterial chromosome, three (MGAS5005) to eight (MGAS10394) prophages ( $\Phi$ ) were identified. With transformation and conjugation appearing to play no significant role, these prophages have been identified as the major source of variation in the gene content between GAS strains (5). For instance, MGAS315 and SF370 share 85.3% of their coding sequences (CDS). Of the remaining 14.7%, 75.3% is phage encoded, whereas prophages constitute less than 13% of the overall genomes. All GAS prophages have a conserved genetic structure between the left (*attL*) and right attachment sites (*attR*): lysogeny-DNA replication-transcriptional regulation-DNA packaging-head-joining-tail-tail fiber-lysis modules (Figure 2) (23). The tail fiber protein hyaluronidase is an enzyme that splits the hyaluronic acid-containing capsule surrounding the bacterial cell. This lytic enzyme allows the phage to reach the cell surface, where it injects its DNA into the bacterial cell. In addition to phage specific proteins, many GAS prophages encode virulence factors such as the pyrogenic exotoxins SpeA, SpeC, SpeH, SpeI, SpeK, SpeL, SpeM and SSA; streptodornases Spd (21), Sda (4) and Sdn (6) and Sla, a streptococcal



**Figure 2** Organization and ORF map of the prophage 315.2 present in the genome of strain MGAS315 and encoding the superantigen SSA. Putative ORFs are indicated by arrows that show the direction of transcription. Groups of genes whose protein products are functionally related are color coded (23).

phospholipase (10). The vast majority of these virulence factors are encoded between the lysis cassette and the right phage attachment site (Figure 2). Even when they encode different virulence factors, some GAS prophages share a large degree of sequence homology. This suggests that phages are prone to recombination processes, which may generate chimeric genomes (18,37).

It has been postulated that the acquisition of multiple prophage-encoded virulence genes might influence the pathogenic potential of *S. pyogenes* (94). Beres *et al.* discovered that M3 strains isolated in the 1920s contain  $\Phi$ 315.5 (encoding the SpeA1 variant), whereas M3 strains causing disease in the 1940s have this prophage plus  $\Phi$ 315.2, encoding SSA (Figure 2) (11). In addition,  $\Phi$ 315.5 present in strains from the 1940s onwards, encodes SpeA3, an allelic variant that is more mitogenic (72). Furthermore, the majority of contemporary M3 strains contain another prophage:  $\Phi$ 315.4 (encoding SpeK and Sla) that was not present in serotype M3 strains until the mid-1980s (Figure 3) (10).



**Figure 3** Early M3 *S. pyogenes* contained prophage  $\Phi$ 315.5 (encoding SpeA1). This strain then acquired prophage  $\Phi$ 315.2 (encoding SSA, figure 2), and a single nucleotide mutation resulted in the SpeA3 variant in the early 1940s. This *ssa*- and *speA3*-containing strain acquired prophage  $\Phi$ 315.4, encoding Sla, a phospholipase and SpeK. This new, virulent M3 clone gained predominance in the USA and Europe. Adapted from (10).

## **Microarrays**

With the availability of whole genome sequences and the advent of sophisticated bioinformatics tools, genome-wide analysis has become available using hybridization techniques on microarrays. For microarray construction, several thousand gene probes that comprise the bacterial DNA are printed on glass slides using robotic arrayers. The probes are either PCR products or synthetic oligonucleotides. Fragmented target DNA to be hybridized to the array is tagged with fluorescent dyes. After hybridization to the array, presence or absence of fluorescence in each microarray spot is used to sensitively detect the genomic composition of the tested strain. The aim of bacterial comparative genomics is to investigate what genetic features determine clinical or epidemiological differences between different strains.

## **Clinical manifestations**

GAS infections can give rise to a wide range of clinical syndromes ranging from uncomplicated superficial infections to severe invasive infections.

### **Superficial infections**

GAS is the most common cause of bacterial tonsillopharyngitis (120). The disease occurs predominantly in children aged 5 to 15 years. Physical findings are redness and oedema of the pharynx, enlarged hyperemic tonsils with a grayish-white exudate and fever. In the absence of suppurative complications, fever abates in 3-5 days (14). Incidentally, the infection can spread locally giving rise to otitis media or sinusitis. Antibiotic treatment is indicated to prevent deep suppurative complications (peritonsillar/ retropharyngeal abscess) and acute rheumatic fever (97,120). Streptococci can also cause discrete purulent lesions of the skin known as streptococcal impetigo or pyoderma (14).

### **Invasive infections**

Erysipelas is an acute inflammation of the skin that affects the cutaneous lymphatic vessels. Often a predisposing condition is present such as impaired

lymphatic drainage or a surgical or traumatic wound (16). The erysipelas skin lesion has a raised border, sharply demarcated from normal skin. This feature allows it to be distinguished from GAS cellulitis: an acute spreading infection of the skin and subcutaneous tissue, often resulting from wounds or surgical incisions (16).

Necrotizing fasciitis is an infection of the deeper subcutaneous tissue and fascia characterized by rapidly spreading necrosis of skin and underlying tissues. The main portal of entry is the skin although an initial lesion or primary focus may be inapparent (16,24,114). Necrotizing fasciitis may be difficult to diagnose in its early stages: redness and bullae may be present but sometimes local erythema is the only presenting symptom. Fever, toxicity and severe pain, discrepant to the relatively benign aspect of the skin should prompt surgical inspection of the deep tissues (125). In case of necrotizing fasciitis, extensive tissue necrosis along the fascial planes will be revealed, possibly in combination with myonecrosis. Case-fatality of necrotizing fasciitis is about 30% but increases when TSS develops as a complication (125).

Puerperal sepsis follows delivery or abortion when GAS, colonizing the patient or transmitted from medical personnel, invade the endometrium. GAS pneumonia is frequently associated with preceding viral respiratory infections and chronic pulmonary disease. Empyema often complicates GAS pneumonia (30-40%) and it is accompanied by GAS bacteraemia in 10-15% of cases (17). GAS meningitis presents as other forms of bacterial pyogenic meningitis and is often preceded by GAS infection of the upper respiratory tract (29). Furthermore, GAS are the second cause of non-gonococcal arthritis after *Staphylococcus aureus* (36,46).

### **Streptococcal toxic shock-like syndrome**

In severe invasive GAS infections, hypotension and multiorgan failure may develop rapidly resulting in the development of TSS. Case definitions for TSS have been developed (Table 1) (1). In the majority of patients, bacteraemia and a focus of infection have been observed. Mortality rates are high (30-80%) and almost half of all TSS survivors have limbs amputated or require major debridements (16,17,36,115,125). Persons of all ages may develop TSS and most are not immunosuppressed, although an association has been observed with advanced age, alcoholism and underlying conditions such as

**Table 1** Case definition for streptococcal toxic shock-like syndrome (TSS)

Isolation of *S. pyogenes* from a normally sterile site (e.g., blood, cerebrospinal fluid, pleural fluid)<sup>a</sup>

*and*

Hypotension (5<sup>th</sup> percentile of systolic blood pressure for children or less than 90 mm Hg. for adolescents)

*and two or more of:*

1. Renal impairment (creatinine greater than two times the upper limit for age)
2. Coagulopathy (platelets less than  $100\,000 \times 10^6$  U/L or evidence of disseminated intravascular coagulopathy)
3. Liver involvement (transaminases or bilirubin greater than two times upper limit of normal)
4. Adult respiratory distress syndrome (pulmonary infiltrates and hypoxemia without heart failure or generalized edema)
5. Generalized erythematous rash that may desquamate
6. Soft tissue necrosis in the form of necrotizing fasciitis, myositis or gangrene

<sup>a</sup> If *S. pyogenes* is isolated from a nonsterile site (e.g., throat, sputum, vagina) but the patient has hypotension and two of the other criteria 1 to 6 above, it is considered a probable case if no other etiology for the illness is found.

diabetes mellitus, malignancies, and chickenpox (24,36,70,114,115,126). M1 and M3 have been particularly implicated in the pathogenesis of TSS (24, 57,59,70,93,114,132). T-cell activation by streptococcal pyrogenic exotoxins with subsequent excessive release of proinflammatory cytokines and activation of complement, coagulation, and fibrinolytic cascades is at the heart of TSS pathogenesis (88,89).

## Epidemiology

As described earlier, the decline in morbidity and mortality due to invasive streptococcal infections began long before the introduction of antibiotics. Apart from the obvious effect of hygienic measures on the prevalence of puerperal sepsis, the reason for the decline in the severity of invasive GAS disease in general has been attributed to changes in host resistance, bacterial virulence and environmental factors, especially crowding in the urban setting. No particular change in either one of these factors has been identified as the sole explanation for the observed decrease in incidence and severity of invasive GAS disease (109). There is no indication to assume that streptococci in general became less prevalent: non-invasive GAS disease such as bacterial pharyngitis continued unabatedly throughout the 20<sup>th</sup> century (19,109). Nonetheless, there was optimism that serious GAS disease had almost been eradicated and notification for specific GAS diseases such as scarlet fever was abolished in many European countries (114). In the mid-1980s however, reports of a sudden increase of severe invasive GAS infections re-emerged worldwide (14,24,32,58,65,70,92,100,126). This resurgence resembled that of scarlet fever in the early 19<sup>th</sup> century. Initial reports came from hospital-based series and the first prospective population-based study came from Ontario, Canada, where the estimated incidence of invasive GAS disease was 1.5 per 100 000 person years (36). The overall mortality rate was 15% but much higher (81%) among those with TSS. This, and other population-based surveillance studies, have used microbiological and clinical criteria for the definition of invasive GAS disease (71,100,119,129). Other studies described isolation of GAS from normally sterile body sites as an indication of invasive GAS disease (31,62,116,127,136). Some of these studies relied on the voluntary cooperation of participating hospitals and laboratories whereas other surveillance systems were actively stimulated. In the Netherlands, a prospective population-based national surveillance program on invasive GAS disease was initiated in 1992. It is estimated that there are currently over 660 000 cases of invasive GAS disease worldwide each year, with over 160 000 case-fatalities (25).

## Outline of the thesis

The aim of this thesis was to gain understanding in the epidemiology and genetics of invasive GAS infections in the Netherlands. What are the epidemiological and clinical characteristics of these infections? Are there specific genetic profiles associated with these GAS disease characteristics and why did a resurgence of invasive GAS disease occur? To address these issues we obtained population-based data on invasive GAS infections in The Netherlands and investigated the relationship between the genetic composition of the bacterium and its clinical and epidemiological manifestations.

The first part of the thesis deals with the epidemiology of invasive GAS disease in the Netherlands. In **chapter 2**, clinical and microbiological characteristics of invasive GAS infections in the Netherlands are described. Furthermore, epidemiological differences between invasive and non-invasive GAS disease are evaluated. The national surveillance system that formed the basis for this study initially relied on both clinical and microbiological data. These data were obtained from regional public health laboratories (RPHLs) and individual hospitals on a voluntary basis. After two years, surveillance was formally organized with RPHLs. The effect of actively stimulating the surveillance system was evaluated in **chapter 3**. The dynamics in the incidence of invasive GAS disease and the relative contributions of different M-types over one decade in The Netherlands, as well as patient characteristics, are described in **chapter 4**.

The second part of the thesis aims at the identification of genetic profiles underlying invasive GAS disease. In **chapter 5**, isolates obtained from patients with clearly defined GAS infections affecting different tissues or organs are evaluated for the presence of superantigens and extracellular matrix binding factors. Thus, a possible relationship between genes encoding these virulence factors and the clinical manifestations of invasive GAS disease is scrutinized. Since GAS virulence is determined by a complex interplay between multiple genetic factors, we constructed a mixed-genome microarray, described in **chapter 6**. The aim was to detect the main genetic differences between GAS strains on a genome-wide level. GAS M1 and M3 types are overrepresented

in invasive GAS disease and TSS. Therefore, we assessed the main genetic differences between GAS of different M-types and sought after commonalities in gene profiles of M1 and M3 as compared to other M-types. Also, the existence of a common gene profile among TSS-associated GAS strains across all M-types was evaluated. Changes in bacterial virulence may have driven the resurgence of severe invasive GAS disease as witnessed since the mid-1980s. In **chapter 7**, we study temporal alterations in the genetic composition in a collection of GAS strains, spanning a period of more than four decades using the mixed-genome microarray.

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