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To my family

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ABSTRACT

The human pathogen, *Streptococcus pyogenes* (Group A Streptococcus, GAS), may colonize the mucous membranes but can also cause non-severe infections, such as pharyngitis, and severe invasive infections, such as bacteremia. Often the portal of bacterial entry cannot be located. Penicillin is still the basis of the antimicrobial therapy of all GAS infections. In severe cases, adjunctive clindamycin may be used, but the evidence of its benefit is partly controversial.

The studies in this thesis included two retrospective studies and one prospective study. The aim of the first retrospective study was to assess the effect of consulting an infectious diseases specialist (IDSC) on the antimicrobial therapy among GAS bacteremia patients. The second retrospective study evaluated the incidence of acute kidney injury among GAS bacteremia patients. The incidence of GAS throat colonization and throat soreness as a presaging symptom of invasive GAS infection was studied in the prospective study.

IDSC significantly increased penicillin G treatment whereas patients not undergoing IDSC received more often broad-spectrum intravenous cephalosporin treatment. IDSC also increased the use of adjunctive clindamycin. Acute kidney injury was common among patients with GAS bacteremia, and its severity was related to the CRP-level on admission and to mortality. However, the renal recovery rate was also high. Throat colonization with GAS and/or prior throat soreness were common among especially younger patients with severe invasive infection.

In conclusion, bacterial seeding from the nasopharynx may be an important portal of entry for GAS bacteria, especially among younger adult patients with severe invasive GAS infection. Among patients with GAS bacteremia, IDSC led to a more responsible antibiotic use, but the indications for adjunctive clindamycin therapy should be evaluated carefully and further studies are needed on the role of adjunctive clindamycin treatment in this setting. The strong inflammatory reaction caused by GAS may explain the high incidence of AKI. The results indicate that the renal recovery rate is high among GAS bacteremia patients.

KEYWORDS: Group A Streptococcus, penicillin, clindamycin, infectious disease specialist consultation, acute kidney injury, throat carriage, portal of entry

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
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TIIVISTELMÄ

Streptococcus pyogenes, A-ryhmän streptokokki (Group A Streptococcus, GAS), voi kolonisoida limakalvoja, mutta aiheuttaa myös lieviä infektioita, kuten nielutulehdusta, sekä vakavia yleistyneitä infektioita, kuten veriviljelypositiivisia infektioita. Usein on epäselvää, mistä bakteeri pääsee tunkeutumaan elimistöön yleistyneissä infektioissa. Penisilliini on yhä GAS-infektioiden hoidon perusta. Vaikeissa tautitapauksissa hoitoon voidaan liittää klindamysiini, mutta tutkimusnäyttö sen hyödystä on osittain ristiriitainen.

Tämä tutkimus sisältää kaksi takautuvaa ja yhden etenevän tutkimuksen. Takautuvien tutkimusten aineisto koostui GAS:n aiheuttamaan veriviljelypositiiviseen infektioon sairastuneista potilaista. Ensimmäisessä osassa selvitimme, miten infektiolääkärin konsultaatio vaikuttaa hoidossa käytettäviin antibioottivalintoihin ja toisessa osassa tutkimme akuutin munuaisvaurion esiintymistä. Lisäksi selvitimme yleistyneeseen GAS-infektioon sairastuneiden potilaiden GAS-nielukantajuuden ja edeltävän kurkkukipuoireen esiintymistä etenevässä tutkimuksessa.

Infektiolääkärin konsultaatio johti merkittävästi useammin suonensisäisen penisilliinin käyttöön, kun taas ilman konsultaatiota potilaita hoidettiin useimmiten laajakirjoisella suonensisäisellä kefalosporiinilla. Lisäksi konsultaatio johti usein rinnakkaisen klindamysiinin käyttöön. Akuutti munuaisvaurio oli yleinen GAS:n aiheuttamissa veriviljelypositiivisissa infektioissa, mutta se myös korjaantui usein. Vaurioaste oli yhteydessä tulovaiheen CRP-tasoon ja kuolleisuuteen. Havaitsimme, että GAS:n nielukolonisaatio ja/tai edeltävä kurkkukipu oli yleistä erityisesti nuorilla vaikeaan infektioon sairastuneilla.

Yhteenvetona voidaan todeta, että bakteerikylvö nielusta saattaa olla merkittävä infektioportti vaikeaan GAS-infektioon sairastuneilla. GAS:n aiheuttamissa veriviljelypositiivisissa infektioissa infektiolääkärin konsultaatio parantaa vastuullista antibioottien käyttöä. Klindamysiinin käyttöindikaatioihin täytyy kuitenkin kiinnittää huomiota, ja aiheen jatkotutkimusta tarvitaan. GAS:n aiheuttama voimakas tulehdusvaste saattaa selittää akuutin munuaisvaurion yleisyyttä. Tässä tutkimuksessa munuaisvaurion korjaantumisennuste oli hyvä.

AVAINSANAT: A-ryhmän streptokokki, penisilliini, klindamysiini, infektiolääkärin konsultaatio, akuutti munuaisvaurio, nielukantajuus, infektioportti

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Abbreviations

AKI	acute kidney injury
AKIN	Acute Kidney Injury Network – classification
AUC	area under the receiver-operating characteristic curve
BMI	body mass index
BSI	blood stream infection
CAP	community-acquired pneumonia
CCI	Charlson comorbidity Index
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* infection
CFR	case fatality rate
CKD	chronic kidney disease
CRP	C-reactive protein
DAMP	damage-associated molecular pattern
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GAS	Group A Streptococcus
HBOT	hyperbaric oxygen therapy
HBP	heparin-binding protein
HDSWF	Hospital District of Southwest Finland
IAT	isothermal amplification test
ICU	intensive care unit
IgG	immunoglobulin G
IDSC	infectious diseases specialist consultation
IQR	interquartile range
IRR	incidence rate ratio
IMV	invasive mechanical ventilation
IV	intravenous
IVIG	intravenous immunoglobulin
iGAS	invasive Group A Streptococcus
KDIGO	Kidney Diseases: Improving Global Outcomes classification
MALDI-TOF MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry

MAP	mean arterial pressure
MDRD	Modification of Diet in Renal Disease equation
MIC	minimal inhibitory concentration
NADC	no available data on consultation
NIDR	National Infectious Diseases Register
NGAL	neutrophil gelatinase-associated lipocalin
NSTI	necrotizing soft-tissue infection
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
PAMP	pathogen-associated molecular pattern
PBP2x	penicillin-binding protein 2x
PCT	procalcitonin
PHD	Pirkanmaa Health District
PMN	polymorphonuclear neutrophils
PSGN	post-streptococcal glomerulonephritis
RBF	renal blood flow
RIFLE	Risk, Injury, Failure, Loss, End stage kidney disease classification
RR	risk ratio
RRT	renal replacement therapy
SA-AKI	sepsis-associated acute kidney injury
SCr	serum creatinine
SD	standard deviation
SOFA	Sequential Organ Failure Assessment score
SLO	streptolysin O
SLS	streptolysin S
Spe	streptococcal superantigens
SSTI	skin and soft-tissue infection
SD	standard deviation
SOI	severity of illness
STSS	streptococcal toxic shock syndrome
TEC	tubular epithelial cell
THL	The Finnish Institute of Health and Welfare (Terveyden ja hyvinvoinnin laitos)
TIMP-2*IGFBP7	tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein-7
TLR	toll-like receptor
TNFα	tumor necrosis factor alpha
TPE	throat as a possible portal of entry
UPE	unknown portal of entry

List of original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I Vilhonen J, Vuopio J, Vahlberg T, Gröndahl-Yli-Hannuksela K, Rantakokko-Jalava K, Oksi J. Group A streptococcal bacteremias in Southwest Finland 2007-2018: epidemiology and role of infectious diseases consultation in antibiotic treatment selection. Eur J Clin Microbiol Infect Dis. 2020 Jul;39 (7):1339-1348.

II Vilhonen J, Koivuviita N, Vahlberg T, Vuopio J, Oksi J. Acute kidney injury in group A streptococcal bacteremia: incidence, outcome and predictive value of C-reactive protein. Infect Dis. 2022 Sep; 1:1-9.

III Kailankangas V*, Vilhonen J*, Gröndahl-Yli-Hannuksela K, Rantakokko-Jalava K, Seiskari T, Auranen K, Lönnqvist E, Virolainen M, Hyyryläinen H-L, Oksi J, Syrjänen J, Vuopio J. Preceding throat carriage in invasive Group A Streptococcal disease: a prospective 2-year study in two health districts, Finland. Submitted manuscript.

* These authors contributed equally to this manuscript.

1 Introduction

Streptococcus pyogenes (Group A Streptococcus, GAS) is a well-recognized human pathogen that causes common non-invasive infections, such as pharyngitis, but can also cause invasive infections, such as bacteremia. The incidence of invasive GAS (iGAS) infections fluctuates, but an increasing overall incidence trend has been observed in the developed countries since 1990s (Siljander et al., 2006; Smit et al., 2015; Williamson et al., 2015). The most important virulence factor of GAS is the M protein encoded by the *emm* gene, the sequencing of which is used for classification of GAS strains (Metzgar & Zampolli, 2011).

To date, GAS has remained susceptible to penicillin, which is the basis of the antimicrobial therapy of all GAS infections (Bryant & Stevens, 2015; Johnson & LaRock, 2021). In severe cases, especially necrotizing soft-tissue infections (NSTI) and streptococcal toxic shock syndromes (STSS), adjunctive clindamycin may be used, but the evidence of its benefit is controversial (Babiker et al., 2021; Hamada et al., 2021; Linnér et al., 2014) and there is a lack of randomized controlled trials.

Acute kidney injury is a condition of prompt decline of renal function. Sepsis and septic shock are the most common etiologies for AKI and up to 68% of septic patients have AKI. AKI is related to a worse outcome among septic patients than non-septic patients (E. Peters et al., 2018; Uchino et al., 2005). However, only few studies have been published on investigated AKI among patients with GAS bacteremia (Björck et al., 2020; Bruun et al., 2021). Several serum biomarkers (e.g., proenkephalin and cystatin C) have been studied in detecting AKI in patients with sepsis (Poston & Koyner, 2019), but studies regarding serum C-reactive protein (CRP) and sepsis-associated AKI are relatively sparse.

In our retrospective studies (I and II), we study the incidence of GAS bacteremia in the Hospital District of Southwest Finland (HDSWF). We also assess the effect of an IDSC on the design of antimicrobial therapy and the incidence of acute kidney injury and its impact on outcome among GAS bacteremia patients. We also examine the serum CRP level as an early indicator of AKI.

The portal of bacterial entry in iGAS infections cannot often be located, but bacterial seeding from the nasopharynx has been assumed to occur (Stevens & Bryant, 2017), although the evidence for this assumption is scant. Only a few studies

have reported the incidence of prior or ongoing pharyngitis among iGAS infection patients (Gear et al., 2015; Zachariadou et al., 2014). A recent Scandinavian study found that streptococcal NSTI patients had more often had – in contrast to skin and soft-tissue infection (SSTI) patients - blunt trauma and no preexisting skin lesions. Thus, streptococcal NSTIs often occur without an obvious portal of entry (Bruun et al., 2021). In our prospective study, we test the hypothesis that the throat is one possible portal of entry for iGAS infections.

2 Review of the literature

2.1 General aspects of bacteremia, sepsis and septic shock

Bacteremia means the presence of viable bacteria in the bloodstream. Transient bacteremia occurs in ordinary daily activities (i.e., when conducting oral hygiene) but does not usually cause harm. However, sometimes immune response mechanisms fail, and bacteremia becomes a bloodstream infection (BSI). The portal of bacterial entry may be, e.g., in the oral cavity, the urinary or respiratory tract or related to medical procedures and instrumentation, such as surgery and catheterization. Determining the primary source of infection is critical for management of patients with bacteremia (Smith & Nehring, 2021), but sometimes the portal of bacterial entry cannot be located.

According to a recent retrospective population-based study, the incidence of BSI in Finland rose during 2004–2018 from 150 to 309 cases / 100,000 (Kontula et al., 2021). Rising incidences have also been reported from other Nordic countries (Mehl et al., 2017; Søgaard et al., 2011). The incidence in earlier studies has varied between 166 and 189 / 100,000 in Europe and between 174 and 204 / 100,000 in USA (Goto & Al-Hasan, 2013). The 30-day case-fatality rate (CFR) has been between 13 and 18% in Finland, Europe and the US. The most common pathogens in studies from high-income countries have been *Escherichia coli* and *Staphylococcus aureus* (Goto & Al-Hasan, 2013; Kontula et al., 2021; Uslan et al., 2007). In Finland, β-hemolytic streptococci caused 8% of BSI in 2004–2018 and the annual incidence more than doubled during the study period (Kontula et al., 2021). The proportion of community-acquired BSI of all BSI has varied from 44.5% in Minnesota (Uslan et al., 2007), to 47.4% in Denmark (Søgaard et al., 2011) and to 71% in Finland (Kontula et al., 2021).

Several studies have reported that the incidence and mortality is highest in the older age-groups, particularly among males (Kontula et al., 2021; Mehl et al., 2017; Uslan et al., 2007). In the study from mid-Norway, the incidence for males aged ≥ 80 years was 1,826 / 100,000 and for females 1,126 / 100,000. In the same study, the overall mortality rate due to BSI was 32 / 100,000 and was higher among males than females (36 vs. 28 / 100,000) (Mehl et al., 2017). Similarly, a Finnish study reported

higher CFRs among males than females (13.7% vs. 12.1%; RR 1.14, 95% CI 1.11–1.17; $p < 0.01$) (Kontula et al., 2021).

According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), sepsis is a life-threatening organ dysfunction caused by a patient's dysregulated response to infection. Organ dysfunction is defined as an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more associated with in-hospital mortality > 10%. Sepsis-3 defines septic shock as a subset of sepsis characterized by persisting hypotension requiring vasopressors to maintain mean arterial pressure (MAP) ≥ 65 mmHg and a serum lactate level > 2 mmol/ l despite adequate volume resuscitation. In-hospital mortality due to septic shock exceeds 40% (Singer et al., 2016).

An estimated 48.9 million cases of sepsis took place worldwide in 2017. Sepsis is associated with substantial mortality worldwide: 11 million deaths in 2017 despite a reduction in sepsis-related mortality by 52.8% from 1990 to 2017 (Rudd et al., 2020). According to a recent review and meta-analysis, the pooled incidence of hospital-treated sepsis was 189 cases per 100,000 person-years and an estimated mortality of 26.7% of sepsis patients during hospitalization. The estimated incidence of intensive care unit (ICU) -treated sepsis was 58 cases per 100,000 and the mortality rate was 41.9% (Fleischmann-Struzek et al., 2020). Kadri et al. showed in their retrospective study from the US that the incidence of septic shock rose but mortality fell from 2005 to 2014 (Kadri, Rhee, et al., 2017). A pan-European prospective study among ICU-treated patients demonstrated that 37% of patients had sepsis and that septic patients had higher hospital mortality (36%) compared to non-septic patients (17%) (Vincent et al., 2006). According to a Finnish retrospective study on blood-culture positive sepsis patients, the CFR by day 90 was 20%. The cause of death of patients who died by day 90 and who did not have any fatal underlying disease was sepsis-related in 16% of cases (Rannikko et al., 2017).

2.2 Group A Streptococci

The Group A Streptococcus, also known as *Streptococcus pyogenes,* is a facultatively anaerobic, gram-positive coccus that grows in chains (Bryant & Stevens, 2015). The only known reservoirs of GAS are the skin and mucous membranes of the human host. GAS can be spread by person-to-person contacts via nasal secretion or saliva droplets from infected individuals or, rarely, from carriers or by direct skin contact (Walker et al., 2014). Food-borne and waterborne outbreaks have also been documented (Bryant & Stevens, 2015).

2.2.1 Microbiological characteristics

2.2.1.1 Hemolytic pattern, Lancefield grouping and MALDI-TOF MS

Hemolysis is the breakdown of red blood cells. The ability of bacterial colonies to induce hemolysis of sheep blood cells on blood agar plates is used to classify microorganisms, particularly streptococcal species. The hemolysis pattern is classified as alpha, beta and gamma, corresponding to partial, complete or no lysis of red cells (Bryant & Stevens, 2015).

The different species of β-hemolytic streptococci were indistinguishable until the 1920s. Then Dr. Rebecca Lancefield discovered bacterial cell wall and surface antigens of β-hemolytic streptococci, which she originally named ”C-substance” group antigens. Later, these antigens were identified to be structurally carbohydrates (Lancefield, 1928). Antibody recognition of group antigens A, B, C and G was quickly adapted as a method for identification of clinically relevant pathogenic streptococci (Lue et al., 1978) and antibody-mediated agglutination is still used for rapid antigen detection and diagnostics of GAS and other β-hemolytic streptococci.

In recent years, many clinical microbiological laboratories have introduced Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). According to a systematic review by Fan et al, the MALDI-TOF MS correctly identified 99% of the species of *S. pyogenes*. The MALDI-TOF MS has the ability to identify species from pure bacterial colonies as well as directly from positive blood cultures. The method is fast and accurate for identification of *S. pyogenes* (Fan et al., 2017).

2.2.1.2 Virulence factors and *emm* typing

GAS is enveloped in a hyaluronic acid capsule, but the degree of encapsulation varies between strains. The capsule acts as an accessory virulence factor by rejecting phagocytosis by host polymorphonuclear neutrophils (PMNs) and macrophages. The capsular hyaluronate of GAS is chemically similar to what is found in human connective tissue and thus is a poor immunogen. The cell wall has a complex structure and contains many different substances, including the group-specific carbohydrate (Bryant & Stevens, 2015).

The well-known surface antigen, M protein, has a dimeric coiled-coil structure and is encoded by the *emm* gene. Strains not expressing M protein are avirulent (Bryant & Stevens, 2015). M protein has a hypervariable N-terminus, which determines the M type, conserved C-terminal region and between them, a variable central region. The N-terminal part of the M protein exhibits hypervariability, is the basis for serotyping and nucleotide-based *emm* typing and induces protective

antibodies in a type-specific manner and is therefore considered as a GAS vaccine candidate. The C-terminal region binds a number of host molecules and offers the opportunity to develop a broad-coverage vaccine (Smeesters et al., 2010). More than 200 different *emm* types and M proteins have been described (Gherardi et al., 2018). M protein inhibits complement deposition on the bacterial surface and thereby protects GAS from phagocytosis. M protein interacts with regulatory complement proteins, fibrinogen and albumin, which help GAS to evade complement-mediated killing (Smeesters et al., 2010).

M protein can be released from the bacterial surface to form complexes with fibrinogen. The complex triggers secretion of heparin binding protein (HBP), a potent inducer of vascular leakage, from neutrophils (Herwald et al., 2004). M protein interacts also with toll-like receptors 2 (TLR2) residing on monocytes which results in cytokine production, especially interleukin-6. The interaction among GAS, neutrophils, macrophages and HBP is demonstrated in biopsies from patients with severe streptococcal soft tissue infection (Påhlman et al., 2006).

The Centers for Disease Control and Prevention (CDC) in USA maintains *emm* type and subtype databases and instructions for assigning the types and subtypes of GAS strains. When a defined *emm* segment differs from known *emm* types in the database, a new *emm* type or subtype is assigned. The location and the number of alterations in the *emm* segment defines whether the strain is designated as a new type or subtype (CDC: Protocol for *emm* typing). Subtypes are separated from the types with decimals (e.g., *emm*1.25 is a subtype of *emm*1) and are genetically close to each other. The Finnish Institute of Health and Welfare (Terveyden ja hyvinvoinnin laitos, THL) reports *emm* types but the data on subtypes is also available.

Surface-expressed virulence factors are connected to colonization, establishment of infection and development of disease, while the secreted virulence factors are probably the most important mediators of the tissue damage and toxicity of GAS infections. Streptolysin S (SLS) is an exotoxin, which is responsible for the characteristic β-hemolysis of *S. pyogenes* and is able to disrupt the function of multiple host cells, including erythrocytes, macrophages, neutrophils and keratinocytes. Streptolysin O (SLO) can form a large pore on eukaryotic membranes resulting in host cell apoptosis. SLO is related to invasive skin and soft-tissue infections. Streptococcal superantigens (Spe) are among the most potent known T-cell activators. They are also known as erythrogenic toxins or scarlet fever toxins and cause the red rash so typical of scarlet fever. In the most severe cases, the massive release of cytokines from both T-cells and antigen-presenting cells results in the potentially lethal condition known as streptococcal toxic shock syndrome (STSS). There are several different Spe types. SpeA and SpeC are often recognized as the most probable cause of STSS. Streptococcal pyrogenic exotoxin B is a protease and has the capacity to cleave many human chemokines (Shannon et al., 2019).

2.2.2 Epidemiology

2.2.2.1 Epidemiology of invasive infections

Unfortunately, there is no international definition for iGAS infections, but the Department of Health of the Australian Government and CDC in USA require only laboratory evidence for the criteria of a diagnose of iGAS infection to be fulfilled. Laboratory evidence includes isolation of GAS by culture from a normally sterile site (e.g., blood, cerebrospinal fluid, joint fluid, pleural fluid) or detection of GAS by nucleic acid testing from a normally sterile site (Australian Government; the Department of Health, 2021; CDC: ABC surveillance, 2019). In some previous studies, the definition of iGAS infection also included the option that GAS was isolated from a non-sterile site and that the patient had clinical signs of STSS, NSTI or puerperal sepsis (Lamagni et al., 2008; Leonard et al., 2019).

There are at least 663,000 new iGAS cases and 163,000 iGAS-related deaths worldwide each year (Carapetis et al., 2005). The incidence of iGAS does fluctuate, but there has been an overall increasing trend in the incidence of iGAS worldwide since the 1990s. In Finland, the incidence rose from 1.1 to 2.5 / 100,000 during 1995–2004 (Siljander et al., 2006). The average incidence in Finland was 2.5 / 100,000 during 1998–2007 and 3.6 / 100,000 during 2008–2013 (Siljander et al., 2010; Smit et al., 2015). Concordant observations of a rising incidence have been reported from New Zealand (Williamson et al., 2015), Sweden (Darenberg et al., 2013) and England (Lynskey et al., 2019; Rudman et al., 2021). However, according to a recent report from England, the rate of GAS bacteremia decreased by 38% from 3.8 / 100,000 in 2019 to 2.4 / 100,000 in 2020. The decline was larger than for other pyogenic group streptococci and was likely related to the COVID-19 pandemic (Rudman et al., 2021). Table 1 summarizes the incidences of iGAS infections in different countries worldwide. Invasive GAS infections have a seasonal variation with a peak incidence during the winter/spring and a low incidence in late summer/autumn (Lamagni et al., 2008; Smit et al., 2015).

GAS can also cause outbreaks of invasive infections in different populations. There are reports of outbreaks of iGAS infections in an eldercare facility (Worthing et al., 2020), among homeless adults (Mosites et al., 2018) and among intravenous drug abusers (Kwiatkowska et al., 2018). The strain causing an outbreak can be identified by antimicrobial susceptibility testing, *emm* typing and whole-genome sequencing.

2.2.2.2 Epidemiology of *emm* type distribution

During 2008–2013, the most common *emm* types in Finland were *emm*28 (26%), *emm*89 (12%) and *emm*1 (12%) (Smit et al., 2015). During 2003–2004, the most prevalent *emm* types in 11 countries across Europe were *emm*1 (19%), *emm*28 (12%), *emm*3 (10%), *emm*89 (8%) and *emm*87 (6%) (Luca-Harari et al., 2009). According to a study from the United States, the most common *emm* types in the US were *emm*1 (22%), *emm*12 (9%), *emm*28 (8%), *emm*89 (7%) and *emm*3 (7%) (Nelson et al., 2016). The seven major *emm* types identified in Europe and North America since 2000 have been *emm*1, *emm*28, *emm*89, *emm*3, *emm*12, *emm*4 and *emm*6 and these types account for approximately 50–70% of all isolates (Gherardi et al., 2018). However, it is known that the distribution of *emm* types varies by site and by year. The Finnish Institute of Health and Welfare (THL) reports annually the *emm* type distribution of iGAS isolates. According to the Annual report 2020, the most common *emm* types of iGAS isolates in Finland were *emm*84, *emm*1, *emm*28 and *emm*77. The proportion of *emm*1 decreased (2020: 15%, 2019: 32%, 2018: 40%) and the *emm*77 type which had been rare became more prevalent (2020: 13%, 2019: 2.6%, 2018: 0.6%). The proportion of *emm*89 has decreased since 2015 and accounted for less than 10% in 2020 (THL: NIDR: Annual reports).

Certain *emm* types and clades emerge occasionally and may cause outbreaks. Latronico et al reported that a new *emm*89 clone, clade 3, had emerged in Finland in 2009. Patients infected with certain subclades of clade 3 had a significantly higher mortality (Latronico et al., 2016). Lynskey et al reported a dominant new *emm*1 lineage that has emerged in England since 2015 (Lynskey et al., 2019). Similarly, there was an epidemic wave caused by *emm*1 in Finland during 2017–2019 (THL: NIDR: Annual reports).

Table 1. Studies describing iGAS infections worldwide.

Reference	Study set	Study years and countries	Study population iGAS definition	Incidence / 100 000 / year	Median age (range)	Most prevalent underlying conditions	Most prevalent infection foci	CFR (%)
Laupland et al 2019	Retro-spective	2011–2018 Canada	61 only bacteremic GAS cases	4.2	54	NA	ST (62%), Respiratory tract (16%), Primary/no focus (8%)	12 (30-day)
Nelson et al 2016	Retro-spective	2005–2012 USA	9557 *	3.8	52 (0–106)	Diabetes (21%), Acute skin break down (20%), Heart disease (15%)	SSTI (41 %), Isolated bacteremia (25 %), Pneumonia (16 %)	12 (in-hospital)
Naseer et al 2016	Retro-spective	2010–2014 Norway	756 **	3.0	59 (0–102)	NA	Sepsis (49 %), NF (11 %), Pneumonia (7 %)	19
Williamson et al 2015	Retro-spective	2002–2012 New Zealand	2861 ***	6.1	NA	NA	NA	10 (30-day)
Smith et al 2015	Retro-spective	2008–2013 Finland	1165 ****	3.6	52 (0–100)	NA	NA	5 (7-day)

Table 1. Continued

Reference	Study set	Study years and countries	Study population iGAS definition	Incidence / 100 000 / year	Median age (range)	Most prevalent underlying conditions	Most prevalent infection foci	CFR (%)
Gear et al 2015	Retro-spective	1998–2009 Australia	295 only bacteremic GAS cases	15.2 (overall) and 59.4 for Indigenous Australians	48	Hazardous alcohol use (35%), Diabetes (29%), Smoking (28%)	SSTI (35.6 %), Primary bacteremia (34.7 %), Pneumonia (6.6 %)	14 (in-hospital)
Plainvert et al 2012	Retro-spective	2006–2010 France	1542 *	NA	63 (18–103)	NA	SSTI (43.7%), Bacteremia without focus (25.4%), Gyneco-obstetrical sepsis (8.9%)	15 (in-hospital)
Lamagni et al 2008	Retro-spective	2003–2004 Europe	5522 *	2.8	NA	Skin lesions (25 %)	32 % cellulitis	19 (7-day)
Rantala et al 2009A and Rantala et al 2009B	Retro-spective	1995–2004 Finland	92 only bacteremic GAS cases	1.5	53	Alcoholism (26 %), Cardiovascular disease (19 %), Diabetes (12 %)	SSTI (71 %), Pneumonia (17 %), Deep abscess (11 %)	15 (30-day)
Darenberg et al 2007	Retro-spective	2002–2004 Sweden	746 **	3.0	68 (0–99)	Diabetes, Cardiovascular diseases	Skin (63%), Respiratory tract (13%), Urogenital tract (6%)	15

iGAS = invasive group A streptococcus; CFR = Case fatality rate; NA = Not available; ST = soft tissue; SSTI = skin and soft tissue infections; NF = necrotizing fasciitis. * Isolation of GAS from a normally sterile site or from a non-sterile site in a patient with NF or streptococcal toxic shock syndrome. ** Isolation of GAS from a normally sterile site. *** Isolation of GAS from a normally sterile site or from a non-sterile site in a patient with NF or streptococcal toxic shock syndrome or puerperal sepsis **** Isolation of GAS from blood or cerebrospinal fluid.

2.2.3 Antimicrobial susceptibility

To date, GAS has remained susceptible to penicillin. In 1998, Macris et al analyzed 133 GAS strains collected during the past 80 years and found that the minimal inhibitory concentration (MIC_{90}) for the oldest strains (0.032 µg/mL) did not significantly differ from those collected recently (0.032 µg/mL) (Macris et al., 1998). More recently, in a large study from the US, all iGAS isolates were susceptible to β-lactams (ampicillin, penicillin, cefotaxime) (Fay et al., 2021) and similarly, in a study from Spain, all isolates were susceptible to penicillin, and none showed reduced susceptibility (i.e., the MIC_{90} of penicillin was 0.032 mg/L in all isolates) (Villalón et al., 2021). However, there are rare reports of clinical isolates with elevated penicillin MIC values: three isolates from India (pediatric patients) (Berwal et al., 2019) and 10 strains from Mexican throat swabs with MIC 0.25–0.75 µg/mL (Amábile-Cuevas et al., 2001). According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the susceptibility of GAS to penicillin, ampicillin, cephalosporins and carbapenems is inferred from benzylpenicillin susceptibility. Benzylpenicillin MIC ≤ 0.25 mg/L is regarded as sensitive and > 0.25 mg/L as resistant (EUCAST 2022).

2.2.3.1 Resistance to erythromycin and clindamycin

Table 2 summarizes erythromycin and clindamycin resistance among bacteremic *S. pyogenes* strains in Finland during 2011–2020 according to the Annual Finres report 2020 of the THL. In 2020, erythromycin and clindamycin resistance of bacteremic GAS isolates were both 3.7%. Among all GAS isolates in Finland in 2020, erythromycin and clindamycin resistance rates were 6.1% and 5.2% (THL: Annual Finres reports). During 2008–2013 erythromycin and clindamycin resistance of iGAS isolates increased (from 1.9% to 8.7% and from 0.9% to 9.2%, respectively) and was related to the emergence of a novel clone *emm*33 in Finland (Smit et al., 2015). In a recent study, Fay et al analyzed 17,179 iGAS strains from the United States isolated during 2006–2017 and found 14.5% erythromycin resistance and 14.6% clindamycin resistance. 99.8% of the clindamycin resistant strains were also resistant to erythromycin. There were several different *emm* types which expressed clindamycin and erythromycin resistance. Both resistance rates increased during the period: erythromycin resistance from 12% (2006) to 23% (2017) and clindamycin resistance from 2% to 22% (Fay et al., 2021). In a recent Spanish iGAS cohort, resistance to erythromycin and clindamycin were 8.9% and 4.3%, respectively (Villalón et al., 2021) and a recent report on bacteremic GAS cases from England showed that resistance of clindamycin (2016: 6% and 2020: 9%) and erythromycin (2016: 7% and 2020: 8%) have remained quite stable while tetracycline resistance increased from 11% in 2016 to 30% in 2020 (Rudman et al., 2021). Similarly, a study

from Norway observed erythromycin and clindamycin resistance of iGAS isolates to be less than 5% during 2016–2018 (Oppegaard et al., 2020). However, higher clindamycin resistance rates were reported from GAS isolates of pediatric throat swabs from the United States (15%) (de Muri et al., 2017) and from pediatric oropharyngeal swabs from China (95.5%) (Peng, 2013).

Table 2. Erythromycin and clindamycin resistance among bacteremic *S. pyogenes* strains in Finland during 2011–2020 according to the Annual Finres report 2020 of the THL (THL: Annual Finres reports).

Antimicrobial agent	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Erythromycin resistance % (Proportion of all tested strains)	6.0	5.0	9.2	6.3	4.5	2.5	2.4	3.6	5.9	3.7
Clindamycin resistance % (Proportion of all tested strains)	3.4	4.5	9.6	4.1	4.1	2.2	2.4	4.2	4.6	3.7

2.2.3.2 Missense mutation in penicillin-binding protein PBP2x gene (*pbp2x*)

In 2020, Vannice et al reported for the first time of elevated ampicillin, amoxicillin and cefotaxime MIC-values found of two clinical GAS-isolates (*emm*43.4). This was due to a *pbp2x* mutation. Both isolates were collected during an outbreak of GAS from patients with invasive infection and who had a history of extensive prior β-lactam use. The MIC-values for ampicillin and amoxicillin were 8-fold and for cefotaxime 3-fold compared to the value of isogenic isolates without these mutations. The MIC for penicillin was unchanged (Vannice et al., 2019). The mutation may be a first step of *S. pyogenes* to evolve resistance to β-lactams, as has been seen for *S. pneumoniae* (Grebe & Hakenbeck, 1996). Later, Musser et al showed that amino acid-altering mutations in *pbp2x* were present in 137 out of 7,025 whole-genome sequenced *emm*1, *emm*28 and *emm*89 strains in their international collection (which also included strains from Finland and other Nordic countries). Some of the strains with the *pbp2x* mutation had decreased susceptibility to some β-lactam antibiotics, including penicillin G (Musser et al., 2020).

2.2.4 Factors predisposing for invasive infections

2.2.4.1 Clinical characteristics

There are numerous underlying conditions associated with iGAS infections. Table 1 summarizes the demographics and common underlying conditions of patients with iGAS infections according to the several studies worldwide.

In many studies, higher age and age below 5 years are related to an increased risk for iGAS infections (Nelson et al., 2016; Smit et al., 2015; Williamson et al., 2015). The risk increases gradually after age 65 years and incidences are high among patients aged > 85 (14.4 / 100,000) (Nelson et al., 2016) and especially among male patients > 75 years (around 28 / 100,000 (Williamson et al., 2015). Male gender often overrepresented, at least slightly, in many studies (Gear et al., 2015; Naseer et al., 2016; O'Loughlin et al., 2007).

Patients with iGAS infections are usually younger and have fewer underlying diseases compared to other invasive β-hemolytic streptococcal infections (Ekelund et al., 2005; Laupland et al., 2019; Oppegaard et al., 2015; Rantala et al., 2009a). The most common underlying conditions are heart disease, diabetes mellitus, malignancy, obesity, alcohol abuse and skin conditions (Langley et al., 2016; Laupland et al., 2019; Naseer et al., 2016; Nelson et al., 2016; Smit et al., 2015; Williamson et al., 2015). According to a large iGAS infection study by Nelson et al, a chronic skin condition was defined as the presence of a chronic dermatologic condition which compromises the integrity of the skin (e.g., psoriasis, eczema, decubitus ulcer or other chronic ulcers), while acute skin breakdown included burns, varicella, blunt or penetrating trauma and surgical wounds. The proportions of patients with these concurrent underlying conditions were 10.1% and 20.0%, respectively (Nelson et al., 2016).

Certain ethnic groups have a higher incidence of iGAS infections. In a study from the United States, the annual incidence was higher among non-white patients (4.5 / 100,000) than among white patients (3.6 / 100,000) (RR 1.2; 95% CI 1.2–1.3) (Nelson et al., 2016). In a study from Australia, the age-adjusted incidence was 59.4 / 100,000 among indigenous people and 9.9 / 100,000 among non-indigenous people (Gear et al., 2015). In New Zealand, ethnicity stratified incidence rates were highest among Pacific people and Māori (20.5 and 10.9 / 100,000, respectively) (Williamson et al., 2015).

GAS is a well-known pathogen causing puerperal sepsis and has resulted in significant maternal and infant mortality worldwide (Hamilton et al., 2013). The incidence of maternal iGAS infections is 89-fold higher compared to the background rate of iGAS infections in other females of the same age (15–44 years) (Leonard et al., 2019). The incidence of iGAS infections in age group 20–40 is often higher

among females than males and a most cases present as gyneco-obstetrical sepsis (Naseer et al., 2016; Plainvert et al., 2012).

2.2.4.2 Human carriage of GAS

S. pyogenes is not considered to be part of the normal human bacterial flora, but still a part of people carry GAS in their throat but have no symptoms (Strömberg et al., 1988). Human-to-human GAS carriage has been reported in sites like the anus, skin and vagina (Kolmos et al., 1997, Trell et al., 2019).

A recent large review focused on the role of GAS throat carriage and described the prevalence of i) GAS culture positive (GAS+ve) pharyngitis; ii) serologically confirmed GAS pharyngitis in symptomatic GAS+ve individuals; and iii) asymptomatic pharyngeal carriage in different settings and populations. The review included 285 articles of which 254 addressed GAS+ve pharyngitis, 21 studies serologically confirmed GAS pharyngitis and 56 studies asymptomatic GAS carriage. The study results were stratified by country income level (Organisation for Economic Co-operation and Development (OECD) or non-OECD member country). The overall all-age prevalences in the GAS+ve pharyngitis group, the serologically confirmed GAS pharyngitis group and the group of asymptomatic carriers were 22.7% (95% CI: 21.2–24.2%), 9.4% (95% CI: 5.6–15.5%) and 7.0% (95% CI: 5.6–8.8%), respectively. Around 37% of children aged 5–19 years in a passive recruitment setting (i.e., patients presented of their own accord to healthcare practitioners with manifestations of pharyngitis) had GAS+ve pharyngitis, both in OECD and non-OECD countries. The highest asymptomatic carriage rate was 11.2 % among children aged 5–19 years in OECD countries. The prevalence was 5.6% in the same age group in non-OECD countries. The rate of asymptomatic carriage among adults in OECD and non-OECD countries was 2.0% and 4.6%, respectively (Oliver et al., 2018). In a previous meta-analysis of streptococcal carriage in children, which included 29 studies, reported a 12% prevalence of asymptomatic GAS carriage and a 37% prevalence of GAS among children of all ages with sore throat (Shaikh et al., 2010).

2.2.4.3 Predisposing infections

Among children, there is evidence of an association between varicella zoster infection and iGAS infection (Laupland et al., 2000). Influenza is another known predisposing infection. Zakikhany et al. reported an increased incidence of iGAS infection during the influenza A (H1N1) season in England. Patients under 15 years and patients between 15 and 44 years had the highest likelihood of influenza and *S.*

pyogenes co-infection (14% and 13% of all iGAS infection cases in the age group, respectively) (Zakikhany et al., 2011).

There is a paucity of studies on the association between asymptomatic GAS carriage in the throat or prodromal sore throat in adults and iGAS infections. What studies have been published have largely been retrospective. The question was addressed in a review article on pregnancy-related *S. pyogenes* infections. The total number of patients was 67 which had been extracted from 43 reports published between 1974 and 2009. In that study, 55.6% of the patients in their third trimester of pregnancy who developed a severe GAS infection (i.e., probably an invasive infection although this was not defined in the article) had a prodrome of sore throat or upper respiratory tract infection and GAS was cultured from respiratory tract in 44.4% of cases. However, the number of patients in this group was only 9. In the same study, patients developing the GAS infection early after delivery had a lower frequency of prodromal sore throat or upper respiratory tract infection and GAS was more often isolated from the vagina, suggesting that the infection originated from prior or hospital-associated vaginal colonization (Hamilton et al., 2013).

In a retrospective study among 123 Australian iGAS infection patients, recent sore throat was reported in 4.9% and current sore throat in 2.4%. The proportion of recent and current sore throat was higher among non-indigenous patients than indigenous patients (Gear et al., 2015). In a prospective study from Greece, pharyngotonsillitis was reported in 2.2% of adult iGAS infection cases (n = 46) and 19.8% of pediatric iGAS infection cases (n = 96) (Zachariadou et al., 2014). In a prospective Finnish study that was carried out among patients with acute bacterial non-necrotizing cellulitis, only two out of 89 patients (2.2%) had culture-positive throat swabs for GAS (Siljander et al., 2008).

2.2.5 Clinical manifestations

The usual manifestations of GAS infections originate from noninvasive bacterial involvement, such as pharyngitis, scarlet fewer and pyoderma. It is estimated that there are more than 111 million prevalent cases of GAS pyoderma and over 616 million incident cases of GAS pharyngitis yearly. The prevalence of pyoderma is higher in less developed countries and among aboriginal Australians and Pacific nations (Carapetis et al., 2005).

Post-streptococcal diseases, i.e., rheumatic fever, post-streptococcal glomerulonephritis (PSGN) and arthritis, are nonsuppurative immunocomplex-mediated complications of GAS infections. Around 60% of rheumatic fever patients develop rheumatic heart disease, the prevalence of which is estimated to be at least 15.6 million cases, the annual incidence 282,000 and the annual mortality at least 233,000 cases. These complications occur mainly in developing countries (Carapetis

et al., 2005). PSGN is nowadays rare in industrialized countries, but the incidence ranges between 9.5 and 28.5 / 100,000 in developing countries (Rodriguez-Iturbe & Musser, 2008).

2.2.5.1 Clinical manifestations of invasive infections

Table 1 summarizes the most common clinical presentations of iGAS infections according to the several studies worldwide. The most common manifestations of iGAS infection are SSTI, which includes erysipelas, cellulitis, infected ulcers and wound infections (Gear et al., 2015; Laupland et al., 2019; Nelson et al., 2016; Plainvert et al., 2012). In the international literature, cellulitis includes often erysipelas and in clinical practice these two skin infections can rarely be differentiated. However, erysipelas is often thought to affect the upper dermis, including the superficial lymphatics, whereas cellulitis involves the deeper dermis and subcutaneous fat (Stevens et al., 2005). The proportion of SSTI of iGAS infections varies between 36 and 71% (Gear et al., 2015; Nelson et al., 2016; Rantala et al., 2009b).

Pneumonia and isolated bacteremia (bacteremia without an identified source) are also common manifestations. The most severe manifestations with a high CFR are septic shock, NSTI and STSS (Nelson et al., 2016). Necrotizing infections are called necrotizing soft-tissue infections in the recent literature, whereas prior literature used the term necrotizing fasciitis.

The diagnosis of NSTI is always surgical. The characteristics of NSTI are friability of the superficial fascia, dishwater-gray exudate and absence of pus. The clinical findings are soft-tissue edema, erythema, severe pain, fever and skin bullae or skin necrosis. The pain may be out of proportion to clinical signs. There are two distinct clinical presentations of NSTI caused by GAS: infection with a defined portal of bacterial entry and infection arising spontaneously in the deep tissues without a defined wound or lesion. The first is associated with chickenpox vesicles, lacerations, drug injections, surgical incisions and childbirth and the latter with infections that arise deep in the soft tissues, often at sites of nonpenetrating trauma. It has also been suggested that bacterial seeding to the deep tissues could originate from transient bacteremia from the nasopharynx in patients with current or antecedent pharyngitis (Stevens & Bryant, 2017). However, evidence in support of this argument is lacking.

STSS is usually defined according to the case definition of CDC and includes identification of GAS from a normally sterile site, hypotension (systolic blood pressure $\leq$ 90 mmHg) and at least two other organ failures (renal impairment, coagulopathy, liver involvement, acute respiratory distress syndrome, a generalized erythematous macular rash and soft-tissue necrosis) (CDC: STSS Definition 2010).

According to a large iGAS infection study from the United State, the occurrence of septic shock, NSTI and STSS was 13.7%, 6.7% and 4.0%, respectively. However, higher proportions have been reported with a distribution 26.9%, 7.0% and 10.3%, respectively (Ekelund et al., 2005). In an Australian study, 16.3% of patients with iGAS had STSS (Gear et al., 2015).

GAS can also cause meningitis, arthritis, osteomyelitis, endocarditis, abscesses, abdominal or peritoneal infections and epiglottitis. Puerperal sepsis is a well-known manifestation of GAS among women in childbearing age (Nelson et al., 2016). Of the patients with iGAS, around 25% require management in an ICU (Ekelund et al., 2005; Rantala et al., 2009a).

2.2.5.2 Association between *emm* types and clinal manifestations

There are significant associations between some *emm* types and certain disease manifestations. Well-known examples are the association between *emm* types 1, 3 and 28 with invasive disease and types 1, 3, 5, 6 and 19 with superficial disease (Walker et al., 2014). *Emm* types 1, 3 and 12 are related to more severe disease and worse patient outcomes (O'Loughlin et al., 2007; Luca-Harari et al., 2009), while *emm*28 is strongly associated with puerperal sepsis (Walker et al., 2014; Gröndahl-Yli-Hannuksela et al., 2021). Lynskey et al. reported the emergence of a new *emm*1 *S. pyogenes* lineage (designated $M1_{UK}$) and its association with an increased incidence of scarlet fewer and invasive infections (Lynskey et al., 2019).

2.2.6 Treatment strategies of invasive infections

2.2.6.1 Antimicrobial treatment – selection and duration

Universally, β-lactam antibiotics, and especially penicillin, are the basis for antimicrobial therapy of all GAS infections (Bryant & Stevens, 2015; Johnson & LaRock, 2021). GAS is not resistant to penicillin (see above), but treatment failures are reported (Gillespie, 1998; Orrling et al., 2001). Several mechanisms may explain treatment failures: bacterial and host factors, such as the Eagle effect (see below), intracellular GAS survival, biofilm formation and perfusion defects in the infected tissue (Johnson & LaRock, 2021; Sela & Barzilai, 1999). Since there is no vaccine against GAS, treatment relies on antimicrobial agents and symptom management.

Concurrent use of clindamycin has been widely recommended in severe iGAS infections despite controversial evidence on the benefit of such combined antimicrobial treatment. According to international guidelines, the first-line antimicrobial agents are penicillin (2–4 million units intravenously (IV) every 4–6 hours) plus clindamycin (600–900 mg every 8 hours IV) in NSTI caused by GAS, in

STSS and even in GAS bacteremia without organ failures. However, penicillin monotherapy is regarded as a reasonable alternative in GAS bacteremia without organ failure (Stevens et al., 2014; UptoDate: iGAS infections and STSS 2022). Of note, these recommendations are based on expert opinions and not on peer reviewed articles.

Clindamycin inhibits protein synthesis by binding to the 50S subunit of bacterial ribosomes and retains its bacteriostatic activity in a stationary growth phase in a mouse model. This contrasts to penicillin, whose efficacy against *S. pyogenes* myositis in a mouse model was lost, if the antibiotic was administered late in the course of the infection or after a high inoculum of GAS. This "Eagle effect" was first described in 1952 and later replicated by Stevens et al (Eagle, 1952; Stevens et al., 1988). The Eagle effect has been explained by bacteria entering a stationary-like phase of growth, during which the multiplication of bacteria is minimal. In vitro studies have demonstrated that certain penicillin-binding proteins expressed during the exponential growth phase are lost when the bacteria enter the stationary phase of growth (Stevens et al., 1993).

In a murine necrotizing fasciitis model clindamycin decreased the expression and production of GAS virulence factors and exotoxins and reduced lesion size, regardless of the susceptibility of the GAS strain (Andreoni et al., 2017). On the other hand, experimental animal model data and in vitro studies show that subinhibitory concentrations of clindamycin increase protein expression (e.g., virulence exotoxins) (Andreoni et al., 2017) and enhance adherence and biofilm production of GAS strains (Šmitran et al., 2018). Hamada et al suggested that impaired blood flow in infected necrotic tissue may cause subinhibitory concentrations of clindamycin and result in the clindamycin failure (Hamada et al., 2021).

GAS can form a biofilm and internalize into epithelial cells, and this may be of critical importance for GAS carriage, recurrent infection episodes and antibiotic treatment failure (Ogawa et al., 2011). Kaplan et al demonstrated in an in vitro study that cephalothin and clindamycin are more effective than penicillin in killing ingested GAS, but less active than erythromycin and azithromycin (Kaplan et al., 2006).

Observational clinical studies suggest a benefit of adjunctive clindamycin in the treatment of iGAS infections. However, the results are equivocal. In a recent large retrospective propensity-matched analysis, which included 1,079 patients with iGAS infection, mortality was significantly lower among patients on adjunctive clindamycin compared to patients who did not receive clindamycin (6.5% vs. 11%; odds ratio (OR) 0.44; 95% CI 0.23 - 0.81). The benefit was achieved even among iGAS infection patients without organ failures but not among patients with non-group A/B β-hemolytic streptococcal infections (Babiker et al., 2021). Table 3

summarizes the essential studies regarding the use of adjunctive clindamycin. There are observational studies suggesting a benefit of clindamycin for patients with NSTI (Mulla et al., 2003), with STSS (Linnér et al., 2014) and with iGAS infections (Couture-Cossette et al., 2018). In addition, there are observational studies without multivariable models (Kaul et al., 1997), without significant survival benefit (Carapetis et al., 2014; Mehta et al., 2006) and without separation of clindamycin from other protein-synthesis inhibitors (Zimbelman et al., 1999).

Clindamycin resistance varies by study site and by time of study publication and is not recommended as monotherapy unless sensitivity results indicate otherwise. Resistance profiles of GAS are discussed in a separate chapter above. In Finland, clindamycin resistance is uncommon, but globally resistance is increasing at an alarming rate. There are a few in vitro and animal studies demonstrating a benefit of linezolid in reducing streptococcal exotoxin release and improving outcome (Bryant et al., 2020; Coyle et al., 2003). However, there is a lack of clinical data.

According to guidelines, the recommended duration of antimicrobial therapy in iGAS infections is, in general, at least 10–14 days, but the final duration depends on individual patient circumstances, the source of infection and clinical response to treatment. Clindamycin in combination with penicillin should be discontinued after 48–72 hours if the patient is clinically and hemodynamically stable. In general, penicillin monotherapy is thereafter enough. However, these recommendations are based on expert opinions rather than on controlled trials (Stevens et al., 2014; UptoDate: iGAS infections and STSS 2022). In a large retrospective cohort study from the US, 32% of iGAS infection patients received adjunctive clindamycin therapy for a median of 4 days (IQR 2–7). Clindamycin therapy improved the outcome according to a propensity-matched analysis, but clindamycin treatment for more than 3 days did not increase this benefit (Babiker et al., 2021).

2.2.6.2 Intravenous immunoglobulin therapy

Intravenous immunoglobulin (IVIG) therapy was originally used as immunoglobulin G (IgG) replacement therapy for immunocompromised patients, but subsequently the indications for IVIG have broadened to cover autoimmune and inflammatory diseases as well. Case reports of the beneficial effect of IVIG in iGAS infections were published in the 1990s and this led to further investigations (Lamothe et al., 1995). IVIG preparations are pooled from thousands of healthy human donors and include mainly the IgG fraction which acts against diverse bacterial targets (Schwab & Nimmerjahn, 2013). Mortality in STSS and NSTI is high despite early recognition, antimicrobials (including clindamycin) and prompt surgical debridement and this has generated a need for additional therapies. Evidence of the benefit of IVIG in STSS is based on in vitro and animal data (Sriskandan et al.,

2006), on retrospective and observational case-control studies (Carapetis et al., 2014; Kaul et al., 1999; Linnér et al., 2014) and on one randomized controlled trial (Darenberg et al., 2003). One in vitro whole-blood assay study demonstrated that IVIG can neutralize superantigenicity of *S. pyogenes* and enhances bacterial killing. IVIG neutralizes circulating superantigens, reduces the systemic inflammatory response, enhances systemic clearance of bacteria and enhances neutrophil infiltration into the infected tissues when given to mice with iGAS infection. However, those benefits are lost, if IVIG is given late with antibiotics (Sriskandan et al., 2006). In another mouse model study, IVIG did not improve bacterial clearance from the thigh muscle in *S. pyogenes*-infected mice. In this study, systemic inflammatory responses were not studied, since the mice did not develop STSS (Patel et al., 2000).

Linner et al published a prospective comparative observational study of 75 STSS patients where both IVIG and clindamycin improved survival. After adjustment of the data for the Simplified Acute Physiology Score (SAPS), clindamycin and IVIG therapy improved survival at odds rations (OR) of 5.6 ($p = 0.030$) and 8.6 ($p = 0.007$), respectively. All except one patient received IVIG at a dose of 0.5g/kg, the duration of IVIG therapy was usually 1–3 days and IVIG therapy usually started during the first day of onset of illness (Linnér et al., 2014). In a large retrospective study with NSTI and vasopressor-dependent shock patients, in-hospital mortality did not improve despite IVIG treatment of 164 patients compared to 3,963 patients who did not receive IVIG. The data underwent propensity-matched and risk-adjusted analysis (mortality 27.3% vs. 23.6%; OR 1.00, $p = 0.99$). A lack of effect on mortality with IVIG was also seen in the subgroup of patients coded for toxic shock syndrome, GAS and/or *S. aureus* and in the subgroup of patients treated with clindamycin. IVIG use was generally quite unusual (4% of all patients) (Kadri, Swihart, et al., 2017). The only randomized and placebo-controlled trial among STSS patients was performed in Europe but was prematurely terminated because of slow patient recruitment. Altogether 21 patients were recruited, 10 received IVIG and 11 placebo. The IVIG dose was 1 g/kg bodyweight on day 1 followed by 0.5 g/kg for 1–3 days in most patients and the antimicrobial treatment clindamycin in combination with intravenous benzylpenicillin. The 28-day mortality was 3.6-fold higher in the placebo group than in the IVIG group, but this difference was not statistically significant (Darenberg et al., 2003).

A meta-analysis regarding the benefit of IVIG for clindamycin-treated patients with STSS demonstrated a significant reduction in mortality among the patients who got IVIG (33.7% vs. 15.7%; RR 0.46; $p = 0.01$). The analysis included 1 randomized and 4 nonrandomized studies. In none of these individual studies was IVIG significantly effective, but statistically significant superiority was reached in the pooled data. All in all, there were 70 patients who received IVIG and 95 who did

not. The study of Kadri et al, mentioned above, was not included in this meta-analysis, but, according to the authors, those results had a negligible effect on the results. The main limitations of the meta-analysis were small study population sizes and the risk that unadjusted confounding factors, such as baseline characteristic and selection of other antibiotics than clindamycin, may have affected the outcome (Parks et al., 2018).

A recent Scandinavian prospective observational study among patients with NSTI due to GAS found that not administrating IVIG was independently associated with 90-day mortality (Lasso regression OR 3.15). Most patients (122 / 126; 97%) were on β-lactam and clindamycin therapy and 75% got IVIG. The 30-day CFR was low (10%) (Bruun et al., 2021).

2.2.6.3 Surgery

In NSTIs caused by GAS or other microbes, prompt surgical exploration is extremely important to determine the extent of infection, assess the need for debridement or amputation and obtain specimens for Gram stain and culture. The importance of surgical debridement is universally agreed upon, although optimal timing has been problematic, especially in retrospective studies with varying definitions of delay (Stevens et al., 2021). According to a study from 1985, the mean time to appropriate surgical therapy was 1.9 days for NSTI patients. In the same study, it was demonstrated that mortality increased, if surgery was performed later than 24 hours after recognition of the infection (70% vs. 36%) (Freischlag et al., 1985). A recent review and meta-analysis showed that the mortality rates of patients with NSTI before and after the year 2000 have declined significantly (from 28.3% to 20.6%, $p = 0.004$). However, over the last 20 years, average mortality rates have remained constant at around 20%. Mortality has been shown to decrease from 32% to 19%, if surgery is performed within 6 hours after recognition of the infection (OR 0.43; 95% CI 0.26 – 0.70). Surgery within 12 hours reduced also mortality compared to surgery after 12 hours from presentation. Nevertheless, the time-related variables did not influence the amputation rate (Nawijn et al., 2020).

Table 3. Essential studies on the benefit of adjunctive clindamycin therapy in the treatment of iGAS infections.

Reference	Study set	Study years and countries	Study population	Results
Hamada et al 2021	Nationwide retrospective cohort study	2010–2018 Japan	404 patients with NSTI caused by GAS	In-hospital mortality was not decreased significantly among patients receiving clindamycin (19.2% vs. 17.5%; adjusted OR 1.11; 95% CI 0.59–2.09; p=0.74)
Babiker et al 2021	Retrospective propensity-matched cohort study	2000–2015 the United States	1079 inpatients with iGAS infection	In-hospital mortality decreased in patients receiving adjunctive clindamycin (6.5%) vs. (11.0%) (adjusted OR 0.44; 95% CI 0.23–0.81; p=0.011)
Couture-Cossette et al 2018	Retrospective cohort study	Jan 1996–Jun 2016 Canada	249 iGAS infection patients	In-hospital mortality decreased among patients receiving clindamycin 0–24 h post-admission: adjusted OR 0.04 (95% CI:0.003–0.55; p=0.02)
Carapetis et al 2014	Population-based prospective active surveillance	Mar 2002–Aug 2004 state of Victoria, Australia	84 patients with severe iGAS	30-day mortality among patients treated with clindamycin but not IVIG vs. patients untreated with clindamycin and IVIG: adjusted OR 0.39 (95% CI, 0.10–1.46; no statistical significance)
Linner et al 2014	Prospective surveillance study	Apr 2002–Dec 2004 Sweden	67 STSS patients	28-day survival benefit in patients receiving clindamycin: adjusted OR 8.6 (95% CI, 1.8–40.4; p = 0.007)
Mehta et al 2006	Prospective population-based surveillance	Jan 1992–Jun 2002 Canada	62 ICU treated iGAS infection patients	No association between the use of clindamycin and survival
Mulla et al 2003*	Population-based study	Florida	257 iGAS infection patients	Clindamycin reduced mortality in patients with NSTI (OR 0.11; 95%CI, 0.01–0.89) but not in patients without NSTI (OR 1.01; 95%, 0.31–3.33)
Zimbelman et al 1999	Retrospective unblinded chart review	1983–1997 the United States	19 Deep GAS infection patients	Favorable clinical improvement in patients receiving protein synthesis-inhibiting antibiotics vs. patients receiving only cell wall-inhibiting antibiotics (83% vs. 14%; p=0.006)
Kaul et al 1997	Propspective, observational, population-based study	Nov 1991–May 1995 Canada	77 NSTI caused by GAS	Lower CFR in patients receiving clindamycin 26% vs. 47%; RR 0.55; p=0.06

NSTI = Necrotizing soft tissue infection; GAS = Group A streptococcus; OR = Odds ratio; iGAS = Invasive GAS; IVIG = Intravenous immunoglobulin; CI = Confidence interval; STSS = Streptococcal toxic shock syndrome; ICU = Intensive care unit; CFR = Case fatality rate; RR = Risk ratio
* Only abstract available.

2.2.6.4 Hyperbaric oxygen therapy (HBOT)

The benefit of HBOT in NSTI has been and still is controversial. A review covering studies from 1997 to 2003 summarized that results regarding the survival rates in patients with NSTI were conflicting and that the overall quality of studies was poor (Wang et al., 2003). Later, a retrospective case-controlled study from Victoria with 341 NSTI patients (275 receiving HBOT and 66 not receiving) demonstrated that HBOT independently does reduce the mortality of patients with NSTI (OR 0.42; p = 0.01) (Devaney et al., 2015). Shaw et al published a retrospective multicenter study with 1,583 NSTI patients and found that HBOT improves survival significantly especially of patients whose severity of illness (SOI) score is high (Shaw et al., 2014). They also reported that surgical debridement should not be delayed despite HBOT.

2.2.7 Outcome of invasive infections

The 30-day mortality of patients with iGAS infections is around 10–15% in the developed countries (Laupland et al., 2019; Nelson et al., 2016; Rantala et al., 2009a; Williamson et al., 2015). Mortality rates differ by clinical manifestation and age-group. The higher CFRs occur among iGAS infection patients with septic shock (45–57%), STSS (38–59%) and NSTI (29%) (Ekelund et al., 2005; Lamagni et al., 2008; Nelson et al., 2016). CFR is highest among iGAS patients aged ≥ 65 years with STSS (70%) (Nelson et al., 2016).

Several factors are independently associated with the mortality rates of patients with iGAS infections. These include advanced age, residence in a nursing home, recent surgery, septic shock, NSTI, meningitis, pneumonia, *emm* type 1, 3 or 12 and underlying chronic illnesses (Nelson et al., 2016; O'Loughlin et al., 2007).

2.2.8 Vaccine development

A GAS vaccine is needed to reduce the morbidity and mortality related to GAS infections. However, there have been several impediments to the development of safe and effective GAS vaccines. These impediments include a market uncertainty for GAS vaccines in high-income countries, a lack of commercial interest, concerns about efficacy and vaccine coverage due to the complexity of the epidemiology of GAS infections and concerns about the risk of autoimmune complications triggered by vaccines. Among several GAS vaccine candidates, the most recent M-protein based 30-valent vaccine contains M-types that are prevalent in the US, Canada and Europe with the potential to provide immunity against some 85% of all cases of pharyngitis and invasive infections in these geographic locations (Dale & Walker 2020).

2.3 Sepsis-associated AKI

2.3.1 Definition

Acute kidney injury (AKI) is a prompt decline in kidney function and has long been associated with critical illness (Brivet et al., 1996; de Mendonça et al., 2000). There have been several definitions to describe and stage AKI, including the RIFLE (risk, injury, failure, loss, end stage kidney disease) consensus classification and the Acute Kidney Injury Network (AKIN) classification (Poston & Koyner, 2019). The most recent and widely used clinical guidelines for AKI are the Kidney Disease Improving Global Outcomes (KDIGO) classification (Kellum et al., 2012). KDIGO defines AKI as one of the following: i) increase in serum creatinine (SCr) by ≥ 26.5 μmol/L within 48 hours, ii) increase in SCr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days or iii) urine volume < 0.5 mL/kg/h for 6 hours. KDIGO stages AKI into three classes based on SCr levels and urine output (Kellum et al., 2012). Table 4 presents these three AKI classifications (Bellomo et al., 2004; Kellum et al., 2012; R. L. Mehta et al., 2007; Poston & Koyner, 2019). AKI has several etiologies ranging from specific kidney diseases (e.g., acute interstitial nephritis and acute glomerular diseases) and non-specific conditions (e.g., ischemia and toxic injury) to extrarenal pathology (e.g., acute postrenal obstructive nephropathy) (Kellum et al., 2012). However, sepsis and septic shock are recognized as the most common etiology for AKI accounting for around 40–50% of cases with AKI in ICUs (Hoste et al., 2015; Uchino et al., 2005). According to a prospective international study of ICU-treated AKI-patients, the etiology of AKI was sepsis in 41%, hypovolemia in 34%, drug related in 14%, cardiogenic shock in 13%, hepatorenal syndrome in 3% and postrenal in 1.5% of cases (Hoste et al., 2015). The recommendation is that sepsis-associated AKI (SA-AKI) is described as fulfillment of the criteria for Sepsis-3 (discussed above) and the KDIGO criteria (Bellomo et al., 2017). Patients with SA-AKI differ in many ways from septic patients without AKI and AKI patients with some other etiology (Poston & Koyner, 2019).

Warnock et al categorized AKI based on the timing of the maximal and minimal SCr values during a single admission. The maximal SCr value occurred before the minimal SCr in resolving AKI and opposite in in-hospital AKI. In the same study, they showed that if AKI was defined with a “dynamic baseline SCr” (the lowest SCr value during a rolling 48-h window before each inpatient SCr), the discrimination of the risk for inpatient mortality and dialysis improved significantly compared to a “static baseline SCr” (a previous ambulatory SCr before the hospital period) (Warnock et al., 2021).

Table 4. The three AKI classifications published in the 20th century (Bellomo et al., 2004; Kellum et al., 2012; R. L. Mehta et al., 2007).

Stage	RIFLE	AKIN	KDIGO	Urine output (all)
Definition of AKI	SCr increase ≥ 50 % in 7 days	Increased SCr 1.5–2-fold from baseline or ≥ 26.4 µmol/L in 48h	Increased SCr 1.5–1.9-fold from baseline in 7 days or ≥ 26.5 µmol/L in 48h	
RIFLE: Risk **AKIN: stage 1** **KDIGO: stage 1**	Increased SCr 1.5-1.9-fold or GFR decrease > 25% in 7 days	Increased SCr 1.5–1.9-fold from baseline or ≥ 26.4 µmol/L in 48h	Increased SCr 1.5–1.9-fold form baseline in 7 days or ≥ 26.5 µmol/L in 48h	< 0.5 mL/kg/h for 6-12 hours
RIFLE: Injury **AKIN: stage 2** **KDIGO: stage 2**	Increased SCr 2-2.9-fold or GFR decrease > 50% in 7 days	Increased SCr > 2–2.9-fold from baseline	Increased SCr 2–2.9 times from baseline	< 0.5 mL/kg/h >12 hours
RIFLE: Failure **AKIN: stage 3** **KDIGO: stage 3**	Increased SCr ≥ 3-fold or GFR decrease 75% or SCr ≥ 354 µmol/L with an acute increase of at least 44 µmol/L	Increased SCr ≥ 3-fold from baseline or SCr ≥ 354 µmol/L with an acute increase of at least 44 µmol/L	Increased SCr ≥ 3.0 times from baseline OR increase in SCr to ≥ 353.6 µmol/L OR Initiation of renal replacement therapy	< 0.3 mL/kg/h for ≥24 h or anuria for ≥ 12 hours

RIFLE = the Risk, Injury, Failure, Loss of kidney function and End stage kidney disease consensus classification; AKIN = the Acute Kidney Injury Network classification; KDIGO = the Kidney Disease Improving Global Outcomes classification; AKI = acute kidney injury; SCr = serum creatinine; GFR = glomerular filtration rate

the RIFLE classification includes also: Loss of kidney function defined as complete loss of kidney function lasting for more than 4 weeks and End stage kidney disease defined as complete loss of kidney function lasting for more than 3 months.

2.3.2 Incidence

The global incidence of SA-AKI is estimated to be around 6 million cases annually or 1 per 1,000 population (Peerapornratana et al., 2019). Murugan et al prospectively investigated 1,836 patients hospitalized for community-acquired pneumonia (CAP). They used the RIFLE criteria to determine AKI and found that 34% of all patients and around 24% of subgroup patients with non-severe sepsis and CAP met the criteria for AKI. Furthermore, nearly two-thirds of patients with AKI had developed AKI at hospital admission (Murugan et al., 2010). According to a Dutch study, 10.8 % of patients presenting to the internal medicine emergency department with suspected infection had AKI defined by KDIGO criteria. Of the patients with AKI, 67% had AKI stage 1, 22.2% AKI stage 2 and 10.8% AKI stage 3 (Khairoun et al., 2021).

According to a prospective worldwide (most patients were from Europe, Asia and the Americas) study among ICU-treated septic patients, 68% had AKI based on

the AKIN criteria (E. Peters et al., 2018). AKI rates among ICU-treated septic patients in other studies have ranged from 47% (retrospective study in China, KDIGO criteria) (Xu et al., 2015), 51% (prospective cohort study in Europe, renal SOFA score > 2) (Vincent et al., 2006), 53 % (prospective multicenter study in Finland, KDIGO criteria) (Poukkanen, Vaara, et al., 2013) to 64% (retrospective cohort study in Canada, RIFLE criteria) (Bagshaw et al., 2009). In Finland, the population-based incidence of severe SA-AKI was 0.32/1000 adults per year in 2011–2012 (Poukkanen, Vaara, et al., 2013).

2.3.3 Pathophysiology

Although the most common cause of AKI in critically ill patients is sepsis, the pathophysiology of SA-AKI is not fully understood (Peerapornratana et al., 2019). Earlier, the etiology of SA-AKI was thought to be decreased global renal blood flow (RBF) caused by sepsis-related arterial vasodilatation, increased sympathetic tone, increased renal vasoconstriction and secondary tubular epithelial cell (TEC) death or acute tubular necrosis (Schrier & Wang, 2004). However, this theory has been challenged since AKI was common among hemodynamically stable CAP patients who did not need ICU treatment (Murugan et al., 2010). Also, in a small observational pilot study with ten ICU-treated AKI patients, RBF was measured by cine phase-contrast magnetic resonance imaging: reduced RBF was seen in several hemodynamically stable patients and there was no rank correlation between the RBF index and creatinine clearance (Prowle et al., 2012). Currently, the etiology of SA-AKI is recognized as being multifactorial with several concurrent mechanisms at play. SA-AKI occurs rather in the setting of renal vasodilatation and increased RBF (Fani et al., 2018) than the opposite. Three mechanisms are essential for organ injury during sepsis: inflammation, microcirculatory dysfunction and metabolic reprogramming (Peerapornratana et al., 2019).

The main defense mechanism against invading pathogens is the inflammatory response of the host. This response may, however, be dysregulated and result in organ dysfunction (Singer et al., 2016). The endotoxin of Gram-negative bacteria (a cell wall product consisting of lipopolysaccharides) and the exotoxins of Gram-positive bacteria (e.g., streptococcal superantigens) induce a generalized inflammatory response. The signaling of bacterial components, also called Pathogen Associated Molecular Patterns (PAMPs) and Damage Associated Molecular Patterns (DAMPs), is effectuated by endogenous molecules, like DNA, RNA and histones released from injured cells, which interact with pathogen recognition receptors, such as TLRs, which are expressed on the surface of leukocytes, endothelial cells and TECs. Signaling results in release of proinflammatory factors, such as the cytokine tumor necrosis factor alpha (TNFα) and interleukins, and increased synthesis of

reactive oxygen species in TECs. Endothelial activation enhances adhesion of leucocytes and platelets increasing the risk of thrombus formation and impaired flow continuity. Endothelial activation increases also vascular permeability and leads to leakage into the extracellular space (Peerapornratana et al., 2019; K. Peters et al., 2003; Umbro et al., 2016).

According to a review article on the histopathology of SA-AKI, acute tubular necrosis was relative uncommon while nonspecific morphologic changes were the most common histopathologic findings (Kosaka et al., 2016). Thus, the mechanisms of AKI could be, at least partly, reversible. After having been filtered through the glomeruli, DAMPs, PAMPs and pro-inflammatory cytokines enter the proximal tubules and can then directly activate TECs resulting in a change of the metabolic and functional state of these cells (Zarbock et al., 2014). The metabolic adaptations enable the cell to survive, which is prioritized at the expense of cell and organ function. An essential mechanism behind the metabolic reprogramming of TECs is a mitochondrial-mediated process, characterized by energy expenditure optimization, reprogramming of substrate utilization and counteraction of proapoptotic triggers (Peerapornratana et al., 2019; Umbro et al., 2016).

2.3.4 Risk factors

The risk factors for AKI can be divided into factors present before the acute illness and factors caused by the acute illness. Several factors are associated with the development of AKI, e.g., increasing age (Bagshaw et al., 2005; de Mendonça et al., 2000), chronic kidney disease (Bagshaw et al., 2005; Pannu et al., 2011), diabetes mellitus and heart disease (Bagshaw et al., 2005). The risk factors for AKI in the general population are thought to confer an equal or even greater risk if the patient has sepsis (Poston & Koyner, 2019).

According to a review and meta-analysis by Liu et al, the following comorbidities, medications and clinical features are significant predictors for SA-AKI: male gender (OR 1.22; 95% CI 1.06-1.40), hypertension (OR 1.43; 95% CI 1.20-1.70), diabetes mellitus (OR 1.59; 95% CI 1.47-1.71), chronic kidney disease (OR 3.49; 95% CI 2.36-5.15), cardiovascular disease (OR 1.31; 95% CI 1.24-1.40), vasopressor use (OR 3.15; 95% CI 2.00-4.96), mechanical ventilation (OR 1.64; 95% CI 1.24-2.16), septic shock (OR 1.40; 95% CI 1.13-1.72) and positive blood culture (OR 1.60; 95% CI 1.35-1.89) (Liu et al., 2020).

According to a prospective observational Finnish study among severely ill, septic patients with AKI (n = 488) were significantly older (66 vs. 63 years), had a higher body mass index (BMI) kg/m^2 (27.4 vs. 25.9), had more often diabetes mellitus and chronic renal failure, used more often regularly angiotensin convertase enzyme-inhibitors (ACEI) and angiotensin II receptor blockers (ATR), diuretics and warfarin

before their ICU-period, had higher SOFA scores at admission, needed more often vasoactive therapy (88% vs. 68%) and mechanical ventilation (75% vs. 66%) and required management at an ICU for a longer period of time (median 4.2 vs. 3.8 days) compared to severely ill, septic patients without AKI (n = 430) (Poukkanen, Vaara, et al., 2013).

Some studies have demonstrated that abdominal and genitourinary infections are associated with a higher risk of AKI among septic patients, but this is not the case for pulmonary infections (Liu et al., 2020; Poukkanen, Vaara, et al., 2013).

There is a little information about the impact on AKI development of the pathogen that causes the sepsis. Björck et al compared Swedish iGAS patients admitted to ICU to other septic ICU-patients and found that iGAS patients had statistically significantly higher levels of SCr at ICU admission compared to non-iGAS septic patients (median 173 vs. 133 µmol/L, respectively) and a higher maximal AKIN score during the first 10 days after admission (median 3 vs. 0, respectively). Furthermore, they found that iGAS infection caused by *emm* type 1 was more often associated with renal failure than iGAS infections caused by other *emm* types (Björck et al., 2020).

2.3.5 Biomarkers

Several biomarkers have been studied in association with AKI and they apply to SA-AKI, as well (Bellomo et al., 2017). Murray et al recommend in their report and summary of recommendations from the 10th Acute Dialysis Quality Initiative Consensus Conference the use of combinations of functional and damage markers to evaluate patients with AKI (Murray et al., 2014). Similarly, Bellomo et al claimed that, in the future, definitions of AKI may include novel biomarkers of renal injury. These biomarkers might also detect renal damage before functional change is evident (preclinical AKI) or even in the absence of functional change (subclinical AKI). On the other hand, low biomarker levels may help to distinguish physiologic and pathologic oliguria from each other (Bellomo et al., 2017). Prompt detection of SA-AKI is important for optimal treatment and for prevention of further kidney injury. Biomarkers can be divided into markers of tubular injury (e.g., neutrophil gelatinase-associated lipocalin (NGAL)), markers of cell cycle arrest (e.g., tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein-7 (TIMP-2*IGFBP7)), markers of endothelial injury (e.g., soluble thrombomodulin) and markers of inflammation (e.g., soluble triggering receptor expressed by myeloid cells 1 (sTREM-1)) (Peerapornratana et al., 2019). Some of these biomarkers are presented below.

NGAL is secreted by injured renal tubular cells and activated neutrophils. The molecular forms of NGAL secreted by TECs and neutrophils differ from each other

(Cai et al., 2010). Commercial assays only measure a mixture of the different forms which blurs specificity and diagnostic accuracy (Bellomo et al., 2017). According to a pooled analysis of data from over 2000 critically ill patients, increased NGAL but not increased SCr was associated with a significantly higher risk for starting renal replacement therapy (RRT) (OR 16.4; 95% CI 3.6–76.9), in-hospital-mortality (OR 2.8; 95% CI 1.9–4.1) and longer ICU stay (median difference 2.9 days; $p = 0.026$) compared to normal levels of NGAL and SCr (Haase et al., 2016). Plasma NGAL rises during sepsis, also when there is no AKI, but a higher cutoff provided a sensitivity of 72% and specificity of 74% for detection of AKI (Md Ralib et al., 2017).

TIMP2*IGFBP7 was evaluated as a predictor KDIGO AKI stage 2 or 3 (severe AKI) among critically ill patients with sepsis. A cutoff-value of 1.0 ng /mL^2 / 1,000 provided a sensitivity of 77.5% and a specificity of 75% for emergent severe AKI (Honore et al., 2016). A prospective observational international study of critically ill patients demonstrated that TIMP2*IGFBP7 predicted KDIGO AKI stage 2 and 3 with an area under the receiver-operating characteristic curve (AUC) of 0.8 (Kashani et al., 2013).

Studies on C-reactive protein (CRP) and SA-AKI are rather sparse. Cosentino et al showed that in acute myocardial infarction elevated levels of high-sensitivity CRP on admission is associated with AKI development and severity (Cosentino et al., 2019). Zhou et al reported slightly higher CRP levels in septic patients with AKI than without AKI but the difference was not statistically significant (Zhou et al., 2018). However, in another single-center retrospective observational study, CRP levels were statistically significantly higher among septic patients with AKI (137 mg/L) than without AKI (105 mg/L) ($p = 0.002$) (Katayama et al., 2017). Nie et al reported a correlation between, on the one hand, high serum procalcitonin (PCT) and CRP levels and, on the other hand, AKI among 1,361 patients with suspected infection. In the same study, PCT predicted more precisely than CRP emergence of AKI. (Nie et al., 2013).

Sensitive and specific biomarkers of AKI may direct the treatment options of SA-AKI and further studies are expected. Some of the biomarkers of AKI are commercially available (e.g., PCT and TIMP-2*IGFBP7).

2.3.6 Outcome

A prospective observational study among CAP patients showed that patients with AKI, compared to patients without AKI, developed severe sepsis more often (52% vs. 20%; $p < 0.001$), were more often admitted to the ICU (39% vs. 4%; $p < 0.001$), had a longer length of hospital stay (median 8 vs. 5 days; $p < 0.001$) and had higher 90-day (24% vs. 9.8%; $p < 0.001$) and 1-year (36% vs. 20%; $p < 0.001$) mortality

(Murugan et al., 2010). In another study among CAP patients, 15% of the 1,742 patients surviving to hospital discharge had KDIGO AKI stage 2–3 and 42.4% of them recovered when the definition SCr of returning to within 150% of baseline without dialysis was used. In the same study, 3-year mortality rates were 23% for patients without AKI, 28% with AKI but who recovered initially and 44.3% for those who did not recover by the time of hospital discharge. Thus, if AKI resolved by hospital discharge, long-term survival was similar to patients without AKI (Fiorentino et al., 2018). In a retrospective Chinese cohort study of 3,687 critically ill patients, non-recovery of renal function at 90 days was less frequent among patients with septic AKI compared to non-septic AKI (2.5% vs. 6.4%; $p < 0.001$). In the same study, ICU mortality, hospital mortality and 90-day mortality were similar in the septic and non-septic AKI patient groups. The KDIGO AKI classification was used (Shum et al., 2016).

In a large multinational prospective observational study, AKI recovery was studied among septic and non-septic patients. By the 7th day of follow-up, septic patients with AKIN stage 1 or 2 were more likely to have had complete recovery of AKI compared to non-septic patients for the same AKIN stage (36% vs. 26%; $p < 0.0001$ for AKIN stage 1 and 30% vs. 21%; $p = 0.005$ for AKIN stage 2). On the other hand, septic patients with AKIN stage 3 were less likely to have recovered to a lower AKIN stage than non-septic patients with AKIN stage 3 (21% vs. 32%; $p < 0.0001$). In the same study, in-hospital mortality was 25.9%, 29.3%, 35.6% and 51.7% in non-AKI group, AKI stage 1, AKI stage 2 and AKI stage 3, respectively. Mortality rates were higher in all AKI stages among septic patients than among non-septic patients (E. Peters et al., 2018).

A Finnish study among septic ICU-patients reported slightly lower in-hospital mortality rates: 18.1%, 19.1%, 30.9% and 38.6% in non-AKI, AKI stage 1, AKI stage 2 and AKI stage 3, respectively. In the same study, KDIGO AKI stage 3 was independently associated with 90-day mortality, but stages 1 and 2 were not. Advanced age and the non-renal SOFA score on the first day of ICU admission were also associated with an increased risk for 90-day mortality (Poukkanen, Vaara, et al., 2013).

The only study comparing ICU-treated iGAS patients with non-iGAS septic ICU-patients concluded that the mortality of ICU-treated septic iGAS infection patients is lower (95% CI of HR 0.204–0.746; $p = 0.007$) despite a higher risk for renal failure by AKIN-criteria (95% CI of OR 1.266–4.034; $p = 0.006$). The same study reported a 90-day mortality rate of 44% among non-iGAS patients (n=968) and of 17% among iGAS patients (n=53) (Björck et al., 2020).

3 Aims of the study

1. To study the incidence and clinical picture of patients with GAS bacteremia in the Hospital District of Southwest Finland during 2007–2018.
2. To evaluate the impact of infectious diseases specialist consultations on the antimicrobial therapy of GAS bacteremia.
3. To report the incidence of acute kidney injury and to assess the impact of acute kidney injury on the clinical outcome of patients with GAS bacteremia.
4. To examine the serum CRP level as an early indicator of AKI among patients with GAS bacteremia.
5. To study throat carriage of GAS as a possible portal of entry for iGAS infections.

4 Patients, materials and methods

4.1 Retrospective study on GAS bacteremia (I and II)

4.1.1 Data collection

Invasive GAS infections (isolations from blood and cerebrospinal fluid) have been notifiable in Finland since 1995 according to the Communicable Diseases Act. The diagnosing laboratory reports GAS bacteremias and isolations from cerebrospinal fluid to the National Infectious Disease Register (NIDR) maintained by THL. Regardless of where the patient has been treated, the NIDR registers the information concerning the patients' place of residence as his/her hospital district. Data on incidence of bacteremic GAS cases were collected from NIDR (THL: NIDR: Statistical database).

The HDSWF is inhabited by a population of over 470,000 and harbors 5 hospitals (Turku University Hospital with its regional hospitals in Salo, Loimaa and Uusikaupunki and the Tyks Turunmaa Hospital). The Department of Hospital Hygiene and Infection Control in the HDSWF maintains a database on antimicrobial use and infections (Sairaalan antibiootti- ja infektiojärjestelmärekisteri, SAI-registry) in which all microbial findings in clinical samples, e.g., bacteremias, are recorded together with the national identity code of the patient and the date of the specimen. All blood culture positive (i.e., bacteremic) GAS cases from Jan 2007 until Dec 2018 were retrospectively identified from the SAI-register. The electronic patient records were then reviewed and the cases that met the inclusion criteria of study I and study II were included.

In study I, the inclusion criteria were at least one positive blood culture for GAS with concurrent clinical signs of infection, age $\geq$ 18 years and at least one contact to any of the units of the Turku University Hospital during the GAS bacteremia episode. Study II was a substudy to study I and the additional inclusion criterion was the documented SCr simultaneously with the positive blood culture for GAS. Patients on chronic dialysis were excluded.

An infectious diseases specialist (JVi) reviewed all the electronic patient records from the database of Turku University Hospital. Data on patient demographics,

underlying diseases, clinical course of bacteremia, infectious foci, laboratory results, antibiotic therapy, a record of IDSC, duration of hospital stay and outcome were entered into the electronic database.

Since 2005, THL has *emm* typed iGAS strains. Data on *emm* types were acquired from NIDR. In 10 cases the data on *emm* types were not available from NIDR. Eight of these, the isolate was obtained from the culture collection of the Turku University Hospital and were typed for *emm* at the University of Turku. *emm* typing was performed according to the guidelines of CDC (CDC: Protocol for *emm* typing).

4.1.2 Definitions

The Charlson Comorbidity Index (CCI) was used to classify the underlying diseases and their severity. It was further divided into four categories according to Charlson`s original study: 0 score is 0, 1–2 scores are 1, 3–4 scores are 2 and ≥ 5 scores are 3 (Charlson et al., 1987).

The definition of healthcare-acquired GAS bacteremia included three options: 1) bacteremia with GAS cultured in blood sample collected ≥ 48h after hospital admission or (2) GAS bacteremia associated with a healthcare-related procedure (including labor and previous hospital discharge) within 30 days before the GAS-positive blood cultures or (3) GAS bacteremia associated with foreign body surgery within 90 days before the GAS-positive blood cultures.

STSS was defined according to the CDC (CDC: STSS Definition 2010) and included identification of GAS from a physiologically sterile site, hypotension and failure of at least two other organs.

IDSC was defined as a written note of IDSC in the patient record done by the treating doctor or infectious disease specialist within five days after sampling of the GAS-positive blood cultures. No available data on consultation (NADC) was used for cases who died during the 48 h after the positive blood cultures for GAS or who were transferred to a health care unit outside of HDSWF before the blood culture results were available.

The first-line antibiotics and adjunctive antibiotics were defined as the antibiotics given to the patient at the time when the first positive GAS blood culture result was available until the fifth day after blood culture sampling. Beta-lactam antibiotics and vancomycin were regarded as first-line antibiotics and other antibiotics, if used, as adjunctive antibiotics.

The KDIGO classification system was used for staging of the AKI (Kellum et al., 2012). “Non-AKI” was used for the cases not meeting the criteria of KDIGO AKI. KDIGO AKI stages were based on SCr but not on urine output, since this data was not available in this retrospective study. All eligible patients had two SCr values: one on admission and one baseline value (defined below). According to the KDIGO,

AKI stage 1 and AKI stage 2 are defined as 1.5–1.9- and 2.0–2.9-fold SCr increases over the baseline, respectively. AKI stage 3 is defined as a more than 3-fold increase of SCr above baseline or as an increase of the SCr value to ≥ 353.6 μmol/L or as starting renal replacement therapy (RRT) (Kellum et al., 2012).

The baseline SCr value was the most recent value in the last 24 months prior to the positive GAS blood culture, as in the prior literature (Fiorentino et al., 2018; Mildh et al., 2016). If a baseline SCr was not available, we estimated it by using the Modification of Diet in renal Disease (MDRD) equation, as recommended by the Acute Disease Quality Initiative (Bellomo et al., 2004). According to the international consensus criteria, renal recovery was defined as SCr level < 1.5 times baseline without need for renal replacement therapy (Chawla et al., 2017).

The day of admission was defined as the day when the positive blood culture was taken.

4.2 Prospective observational study on iGAS infections

4.2.1 Study design

The study was conducted in two tertiary care Finnish hospitals: the Tampere University Hospital in the Pirkanmaa Health District (PHD) and the Turku University Hospital in HDSWF. The study was prospective and observational. All adult patients diagnosed with an iGAS infection in the study hospitals between June 2018 and July 2020 were invited to participate in the study. A patient was included if he/she consented to participation, was over 18 years of age and had a positive GAS culture finding in a sample taken from a physiologically sterile site (blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, synovial fluid, deep tissue sample). When GAS was isolated by the clinical microbiological laboratory in either of the study sites, the laboratory contacted the infectious diseases physician (Ville Kailankangas in Tampere or Johanna Vilhonen in Turku) who then approached the patient to obtain written consent, after which the patient was interviewed and clinical data was collected. For patients unable to give consent (e.g., sedated or intubated patients), consent was obtained from a close relative and confirmed, if possible, by the patient later. Immediately after recruitment, the infectious diseases physician obtained a throat swab. A follow up visit was arranged three to four months after discharge. All samples were marked with the study subjects' code numbers. The throat swabs were sent to the Turku University for culture and isothermal amplification testing.

All patients were interviewed carefully to collect information on any preceding tonsillitis-like symptoms, or possible GAS infections among the patients' social

contacts. Information on the patients' previous medical conditions was obtained by interview and from electronic patient records. All clinical and background data were compiled to a REDCap-database accessible only by the researchers. All further analyses were performed using codes without possibility to reveal personal identification.

Patients were classified into two groups. The first group was labeled "throat as a possible portal of entry" (TPE) and included patients with either preceding throat soreness or a positive GAS finding in the throat swab, or both. The second group was labeled UPE – "unknown portal of entry".

4.2.2 Microbiological tests

The GAS strains isolated from the study subjects originally identified by either of the clinical microbiology laboratories (Fimlab and Turku University Hospital clinical microbiology) were sent to the University of Turku for storage and further analysis. Antimicrobial susceptibility testing followed the EUCAST guidelines (European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 12.0, 2022). In addition to iGAS isolates, the throat swabs collected from the patients were sent to the University of Turku for further analysis.

Throat swabs (eSwab) were cultured on blood agar plates and inspected for β-hemolytic colonies. Any suspected GAS colonies were identified with a Lancefield agglutination test.

The throat swabs were tested for presence of GAS nucleic acids by two commercial isothermal amplification tests (IAT) (Solana GAS assay (Quidel, US) and ID NOW GAS assay (Abbot, US)) according to the instructions of the manufacturer.

4.3 Statistical analyses

Statistical analyses were performed with the IBM SPSS Statistics for Windows version 25 and 27 (IBM Corp., Armonk, NY). A two-sided $p < 0.05$ was considered statistically significant.

Categorical variables were expressed as counts and percentages. The normality of continuous variables was examined using histograms. In the cases of normally distributed variables, means and standard deviations (SD) were used, whereas non-normally distributed variables were characterized by medians and the interquartile range (IQR).

Comparisons of categorical data were made with Pearson's χ^2-square test or Fisher's exact test. The differences between groups in normally distributed

continuous variables were analyzed with one-way analysis of variance (ANOVA) with Tukey's method for pairwise comparisons or with the two-sample T-test. Non-normally distributed variables were analyzed with the Kruskal Wallis test with the Bonferroni method for pairwise comparisons.

Poisson regression analysis was used to examine the change in the incidence of GAS bacteremia in HDSWF during three consecutive 4-year follow-up periods (2007–2010, 2011–2014 and 2015–2018). The differences in antibiotic treatment selections between groups (IDSC+ and IDSC-) were analyzed with binary logistic regression.

The difference in mean CRP values between AKI stages was tested with analysis of covariance (ANCOVA) after adjustment for age and CCI, and the Bonferroni method was used in further pairwise comparisons. Binary logistic regression was used to compare mortality between AKI stages after adjustment for age, CCI index and ICU admission. The optimal cutoff value for CRP to identify the patients with AKI stage 2 or 3 was defined using a receiver-operating characteristics (ROC) curve and Youden's index.

The median time delay from onset of symptoms and fever until taking of the blood cultures was calculated and the 95% confidence intervals were estimated using bootstrapping.

4.4 Ethics

The retrospective registry study was approved by the HDSWF (decision numbers T05/014/17, T05/047/17 and T05/032/18). Ethics approval was not required due to the study set.

The prospective study protocol was approved by the ethics committee of the PHD, and local research permissions were obtained accordingly (permission numbers R18062, T05/026/18). The study was registered at ClinicalTrials.gov as ID NCT03507101 and was conducted in accordance with the Declaration of Helsinki.

5 Results

5.1 GAS bacteremia: retrospective study (I and II)

5.1.1 Incidence

The incidence and number of bacteremic GAS cases in the HDSWF according to the NIDR is presented in Figure 1. During study years 2007–2018, the overall incidence was 3.52 / 100,000 person-years, but the year-to-year variation was marked, and the highest incidence occurred in 2018, when it was 7.93 / 100,000 person-years. The annual incidence was also high in 2013 (6.78 / 100,000 person-years) and 2017 (6.05 / 100,000 person-years). The incidence was lowest in 2010 (1.29 / 100,000 person-years). After the epidemic years 2017 and 2018, the incidence decreased in 2019. The decrease continued in 2020 when the Covid-19 pandemic began.

The incidence of GAS bacteremia was evaluated over three 4-year follow-up periods: an increasing trend was observed: 2007–2010 (2.59 / 100,000 person-years), 2011–2014 (4.08 / 100,000 person-years) and 2015–2018 (5.34 / 100,000 person-years). Compared to 2007–2010 the increase was significant in 2011–2014 (incidence rate ratio (IRR) = 1.58; 95% CI 1.10–2.27; $p = 0.013$) and in 2015–2018 (IRR = 2.07; 95% CI 1.47–2.91; $p < 0.001$).

5.1.2 Study populations I and II

After analyzing the SAI data, a total of 212 adult GAS bacteremia patients met the inclusion criteria and were included in study I. The excluded patients were either under 18 years of age or had not been treated in the hospitals of the HDSWF. Study II was a substudy to study I with 195 patients. The excluded patients had missing SCr at admission or were on chronic dialysis (Figure 2).

The discrepancy between the number of cases recorded in the NIDR and SAI (n = 11) during the study period is probably due to the data being recorded in the NIDR according to patients` residential region, although treated in the HDSWF. Also, there had been some minor technical problems in data transition in the previous years.

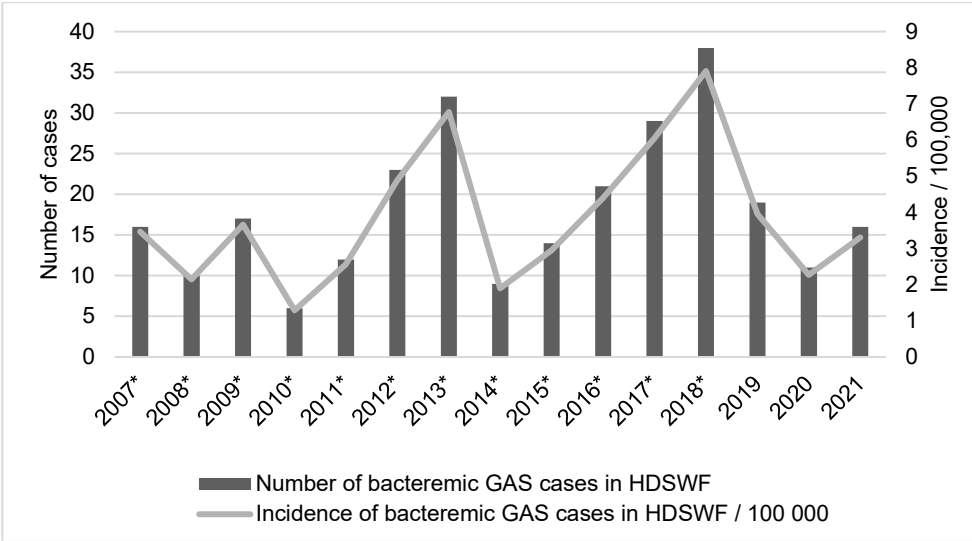

Figure 1. Annual number and incidence / 100,000 person-years of bacteremic GAS cases in the HDSWF 2007–2021 as reported in the NIDR. Study years 2007–2018 are marked with asterisks. Modified from Original publication I.

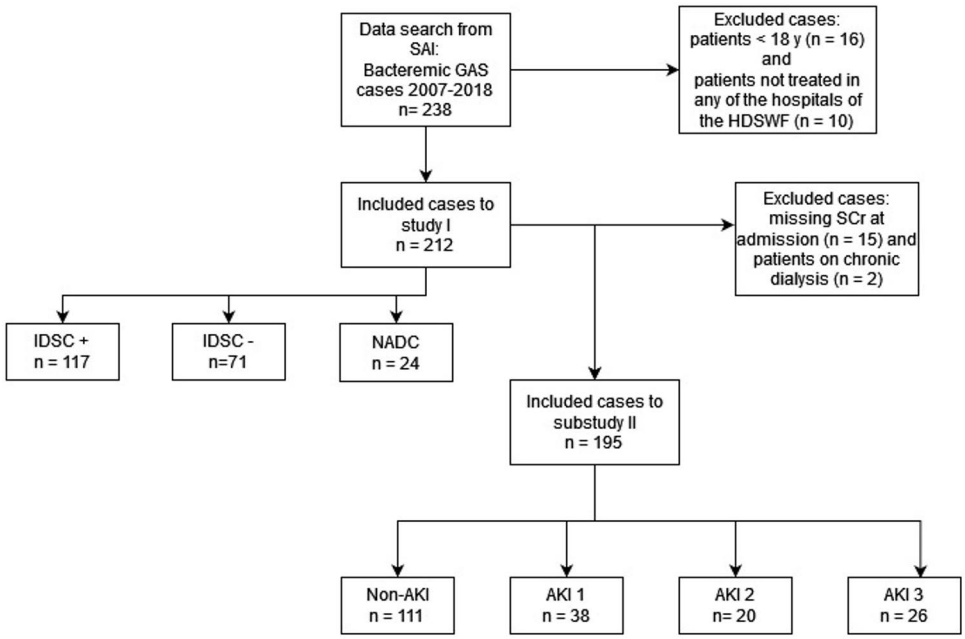

Figure 2. Flow chart of the study population of the retrospective studies I and II. HDSWF = Hospital District of Southwest Finland; IDSC = infectious diseases specialist consultation; NADC = No available data on consultation; AKI = Acute kidney injury (staged according to the KDIGO criteria).

5.1.3 Clinical characteristics and GAS *emm* type distribution (I)

Table 5 summarizes the clinical characteristics of the 212 bacteremic GAS patients included in study I. The mean age of all patients was 59.6 years. The most common underlying condition was any atherosclerotic disease (20.8%) while 22.2% of the cases had no underlying disease. SSTI and pneumonia were the most common clinical manifestations. 21.7% of the study population needed ICU admission and 15.6% met the STSS definition. 7-day and 90-day mortality were 6.6% and 13.2% among all patients and 24.2% and 27.3% among STSS patients, respectively. All GAS strains were susceptible to benzylpenicillin, while the resistance rate to clindamycin was 4.2% (9 cases) and 2.4% (5 cases) were intermediately sensitive to clindamycin. The erythromycin resistance rate was 1.4% (3 cases in 2018), but erythromycin sensitivity was not studied in 2011–2016.

A record of IDSC was found (+) in 117 (55.2%) cases, not found (−) in 71 (33.5%) cases and no data on consultation were available in 24 (11.3%) cases. Table 5 compares the IDSC+ and IDSC− groups. The patients with GAS bacteremia who received IDSC had more severe disease as judged by the need for ICU, by the prevalence of hypotension, STSS and the need for any surgical intervention. The patients who did not receive IDSC were more likely to have diabetes mellitus and malignancies. Antimicrobial susceptibility of the strains did not differ significantly between study groups. There was a slight trend toward lower mortality in IDSC+ group compared with the IDSC− group, but the difference was not statistically significant. Figure 3 presents the *emm* type distribution of the bacteremic GAS isolates during the study period. The most prevalent *emm* types were *emm*28 (a total of 58 cases, 27.4% of all), *emm*1 (42, 19.8%) and *emm*89 (40, 18.9%). During the peak incidence years 2013 and 2018, the most common *emm* types were *emm*89 (12, 40.0%) and *emm*1 (17, 42.5%), respectively. An increase in *emm*1 isolate numbers was observed already in 2016 when also the incidence of GAS bacteremia began to increase.

5.1.4 Infectious diseases specialist consultation and antibiotic treatment selection (I)

Figures 4 and 5 show the percentages of the selected first-line antibiotics and adjunctive antibiotics in the treatment of GAS bacteremia stratified by IDSC. The most frequently used first-line antibiotics were penicillin G and intravenous (iv) cephalosporins. The selection of first-line antibiotic differed significantly between the IDSC+ and IDSC- groups ($p < 0.001$). In the IDSC+ group, 57.3% received penicillin G treatment whereas in the group IDSC- only 22.5% did (OR = 4.61; 95% CI 2.37–8.97; $p < 0.001$). On the IDSC+ group, only 23.1% received iv-

cephalosporin treatment, but in the IDSC- group the figure was 50.7% (OR = 0.29; 95% CI 0.16–0.55; p < 0.001). The most frequently used adjunctive antibiotics were clindamycin, fluoroquinolones and metronidazole. The use of any adjunctive antibiotic was significantly less common in the IDSC- group (39.4%) than in the IDSC+ group (66.7%) (p < 0.001). The use of clindamycin as adjunctive antibiotic was more common among the IDSC+ (54.7%) than the IDSC- patients (21.1%) (OR = 4.51; 95% CI 2.29–8.87; p < 0.001).

The first-line antibiotic treatment selections were reviewed in two time periods, 2007–2012 and 2013–2018. In whole study groups (IDSC+ and IDSC-), the use of penicillin G as a first-line antibiotic increased during the study period from 25.4% (2007–2012) to 54.5% (2013–2018) (p < 0.001).

The antibiotic treatment selections were also compared between ICU-treated and non-ICU-treated patients with or without IDSC (Table 6). The ICU-treated patients were analyzed as one cohort, since 34 patients (81%) obtained IDSC. Patients with NADC were excluded from this analysis. Among ICU-treated patients, the use of clindamycin and carbapenems were more prevalent than among non-ICU-treated.

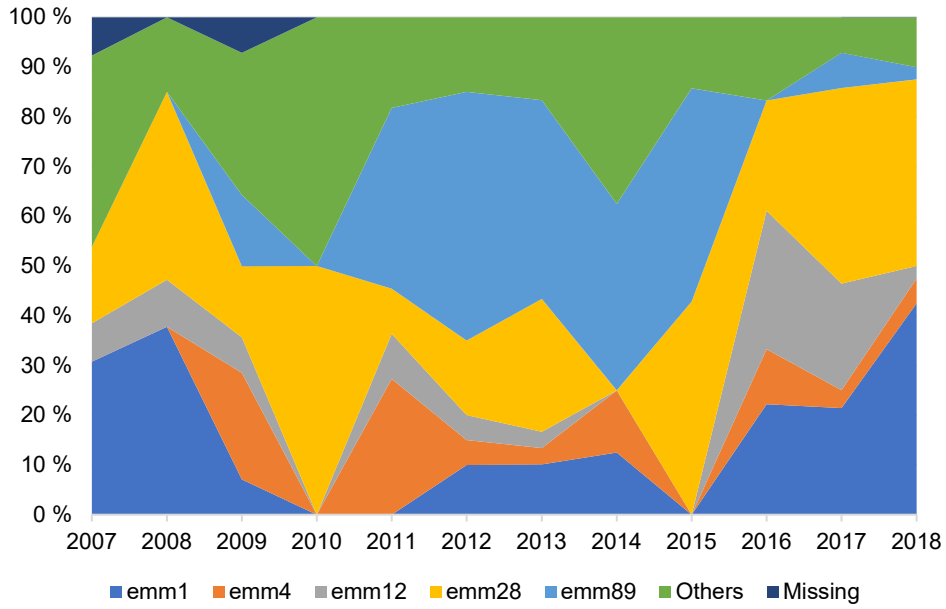

Figure 3. *emm* type distribution of bacteremic GAS isolates (n=212).

Table 5. Demographics and clinical characteristics of 212 patients with GAS bacteremia and comparison of patients with and without infectious diseases specialist consultation.

Variable	IDSC+ (n=117)	IDSC- (n=71)	p-value*	NADC (n=24)
Sex: male	56 (47.9)	38 (53.5)	0.452	10 (41.7)
Age mean (SD)	56.3 (19.2)	60.9 (19.3)	0.115	72.2 (17.0)
Healthcare-acquired	19 (16.2)	9 (12.7)	0.506	3 (12.5)
Charlson class			0.245	
0	36 (30.8)	14 (19.7)		1 (4.2)
1	25 (21.4)	15 (21.1)		6 (25.0)
2	20 (17.1)	11 (15.5)		3 (12.5)
3	36 (30.8)	31 (43.7)		14 (58.3)
Underlying conditions				
Diabetes mellitus	13 (11.1)	16 (22.5)	0.036	6 (25.0)
Any atherosclerotic disease	17 (14.5)	17 (23.9)	0.104	10 (41.7)
Any malignancy	11 (9.4)	15 (21.1)	0.024	2 (8.3)
No underlying disease	32 (27.4)	14 (19.7)	0.238	1 (4.2)
Allergy to penicillin	3 (2.6)	4 (5.6)	0.281	1 (4.2)
Clinical manifestation				
SSTI	67 (57.3)	41 (57.7)	0.948	15 (62.5)
Necrotizing fasciitis	5 (4.3)	1 (1.4)	0.279	0 (0)
Pneumonia	23 (19.7)	18 (25.4)	0.359	5 (20.8)
Puerperal sepsis	7 (6.0)	1 (1.4)	0.132	0 (0)
Any abscess	17 (14.5)	6 (8.5)	0.217	2 (8.3)
Other microbiological finding	20 (17.1)	14 (19.7)	0.650	4 (16.7)
Disease severity				
Hypotension	43 (36.8)	20 (28.2)	0.033	5 (20.8)
ICU admission	34 (29.1)	8(11.3)	0.005	4 (16.7)
STSS	22 (18.8)	5 (7.0)	0.026	6 (25.0)
Any surgical intervention	50 (42.7)	19 (26.8)	0.028	1 (4.2)
Cumulative mortality				
by 7 days	1 (0.9)	3 (4.2)	0.121	10 (41.7)
by 30 days	6 (5.1)	6 (8.5)	0.366	11 (45.8)
by 90 days	7 (6.0)	8 (11.3)	0.195	13 (54.2)

IDSC = infectious disease specialist consultation; NADC = no available data on consultation; SD= standard deviation; SSTI = skin and soft tissue infections; ICU = intensive care unit; STSS = streptococcal toxic shock syndrome

Data represent: No, (%) of the group

* p-value: Comparison of groups IDSC+ and IDSC- with the χ^2-square test expect with two-sample for age

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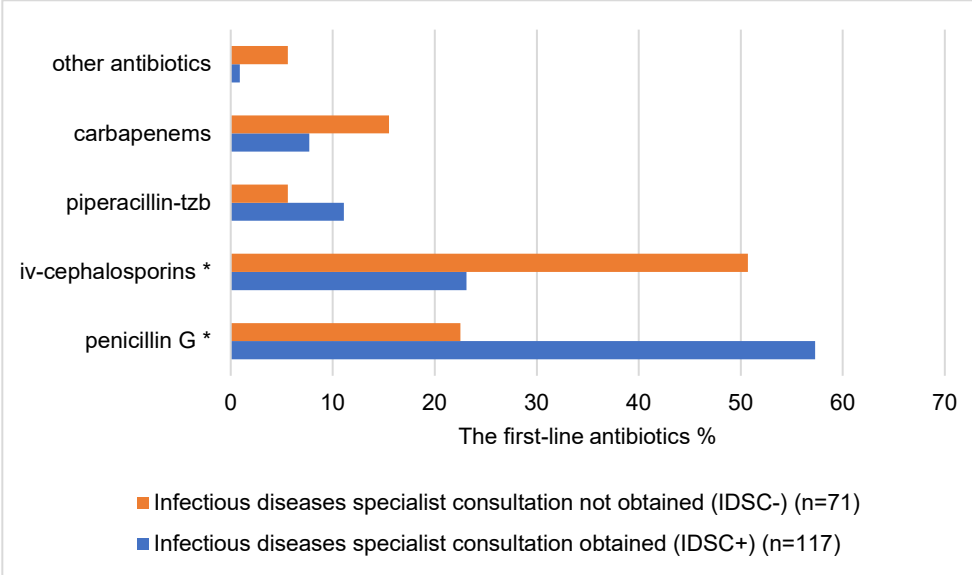

Figure 4. Frequency of selected first-line antibiotics in the treatment of GAS bacteremia stratified by IDSC. * $p < 0.001$ (binary logistic regression).

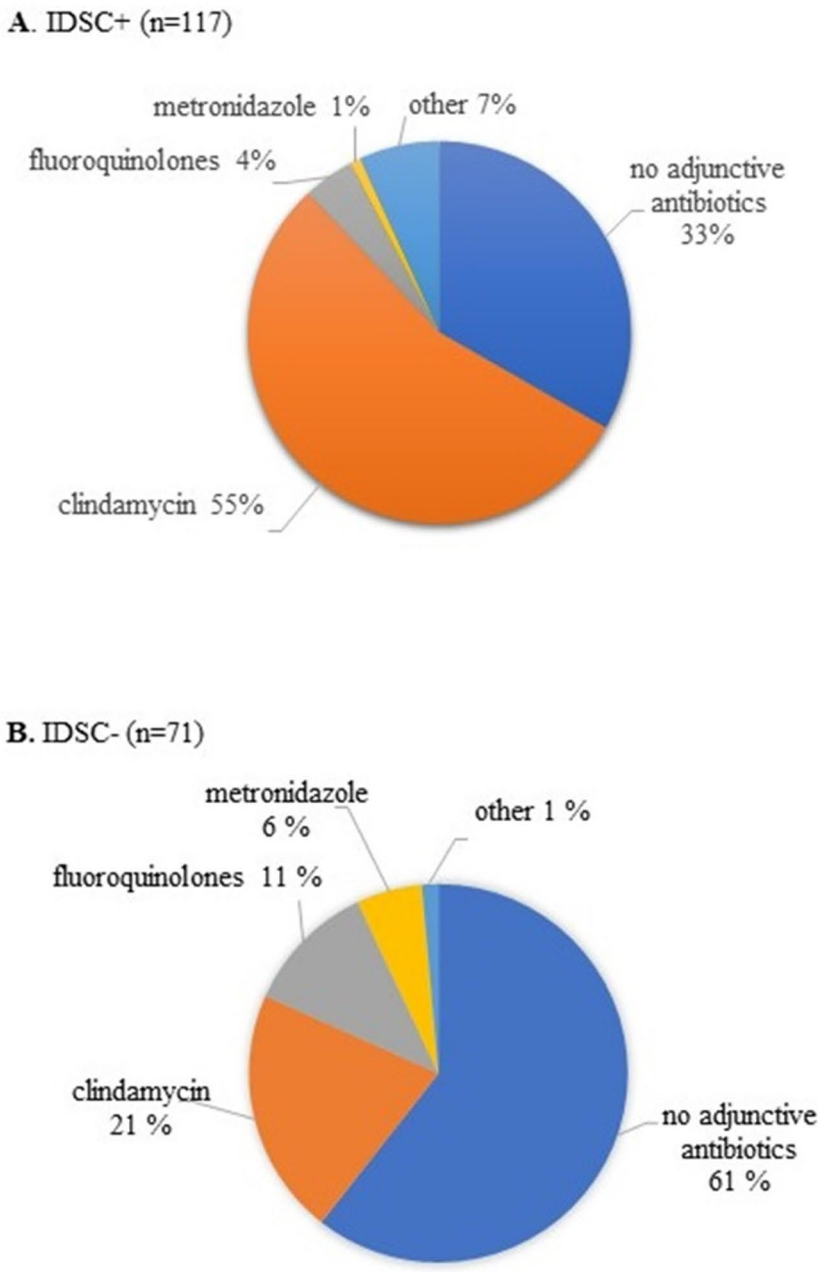

Figure 5. **A and B.** Frequencies of selected adjunctive antibiotics in the two study groups (IDSC+ and IDSC-).

Table 6. Comparison of the antibiotic treatment selections in ICU-treated and non-ICU-treated patients with or without IDSC.

Antibiotic selection	ICU treated (n=42)	Non-ICU-treated IDSC+ (n=83)	OR (95% CI)	p-value *	Non-ICU-treated IDSC- (n=63)	OR (95% CI)	p-value **
Penicillin G as FA	21 (50.0)	49 (59.0)	1.44 (0.68-3.04)	0.337	13 (20.6)	0.26 (0.11-0.61)	0.002
Iv-cephalosporins as FA	5 (11.9)	23 (27.7)	2.84 (0.99-8.11)	0.052	35 (55.6)	9.25 (3.21-26.64)	<0.001
Piperacillin-tzb as FA	6 (14.3)	7 (8.4)	0.55 (0.17-1.76)	0.316	4 (6.3)	0.41 (0.11-1.54)	0.185
Carbapenems as FA	10 (23.8)	3 (3.6)	0.12 (0.031-0.47)	0.002	7 (11.1)	0.40 (0.14-1.15)	0.090
Clindamycin use in all combinations ***	36 (85.7)	43 (51.8)	0.18 (0.07-0.47)	<0.001	8 (12.7)	0.02 (0.01-0.08)	<0.001

IDSC = infectious disease specialist consultation; ICU = intensive care unit; FA = first-line antibiotics
Data represents: No, (%) of the group
* p-value: comparison of the groups ICU-treated and non-ICU-treated IDSC+ with logistic regression
** p-value: comparison of the groups ICU-treated and non-ICU-treated IDSC- with logistic regression
*** Clindamycin in all combinations includes all reported patients with clindamycin use (even as third antibiotic)
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5.1.5 Clinical characteristics of study population II

A total of 195 adult GAS bacteremia patients met the inclusion criteria and were included into study II. The excluded patients were either on chronic dialysis or missing the SCr status concurrently with the positive blood culture for GAS (Figure 2). All included patients were hospitalized. Of the patient cases, 21 (10.8%) had healthcare-acquired GAS bacteremia. The premorbid SCr was estimated by the MDRD equation in 59 patients (30.3%) for whom a premorbid SCr value was not available.

Table 7 summarizes the clinical characteristics of the patients stratified by AKI stages. Of all cases, 43.1% had AKI according to the KDIGO AKI criteria. Most of the patients with AKI (84.6%) had the highest SCr during their hospital period on admission. The mean age of the whole study population was 61.2 years, but the age differed significantly among AKI stages and was lowest (51.6 years) in AKI stage 3. In AKI stage 3, 42.3% had the Charlson class 0, whereas in non-AKI, AKI stage 1 and 2 the proportions were 21.6%, 5.3%, 20.0%, respectively ($p = 0.012$). The higher AKI stages were associated with a more severe disease (regarding to prevalence of hypotension, ICU admission and need for invasive mechanical ventilation (IMV), STSS or RRT) and with a higher prevalence of abdominal abscess.

Of all 195 patients, 16 (8.2%) needed RRT during the GAS bacteremia episode. All patients with RRT were ICU-treated and 3 of them died during the hospital period. The mean age was 53.6 years (SD 14.33) and mean number of treatment days with RRT was 14.3 (SD 10.6). In all cases, the RRT was started within two days of admission. Anuria with or without acidosis was the most common indication for RRT. None of the patients needed RRT at discharge.

5.1.6 AKI and duration of hospital stay

The number of the hospital days increased in relation to AKI severity: the median duration of stay was 9 (IQR 8) in non-AKI, 15 (IQR 14) in AKI stage 1, 16 (IQR 29) in AKI stage 2 and 23 (IQR 28) in AKI stage 3. The difference was statistically significant between non-AKI vs. AKI stage 1 ($p = 0.020$), non-AKI vs. AKI stage 2 ($p = 0.040$) and non-AKI vs. AKI stage 3 ($p = 0.005$).

5.1.7 AKI and mortality

In-hospital mortality was 10.3% among all patients. Mortality rates are presented in Table 8. Mortality was higher in the more severe AKI stages. After adjustment for age, CCI and ICU admission, 7-day mortality was significantly higher for patients with AKI stage 2 than patients with non-AKI (adjusted OR = 5.87; 95% CI 1.03-33.43; $p = 0.046$). Patients with AKI stage 3 had higher 7-day mortality (adjusted

OR = 16.0; 95% CI 2.17–117.7; p = 0.006), 30-day mortality (adjusted OR = 10.77; 95% CI 2.03–57.05; p = 0.005), 90-day mortality (adjusted OR = 7.26; 95% CI 1.53–34.35; p = 0.012) and 1-year mortality (adjusted OR = 6.74; 95% CI 1.59–28.64; p = 0.010) compared to patients with non-AKI.

5.1.8 Renal recovery

Patients who died during the hospital period (n = 20) were excluded from this analysis. The SCr status was available at discharge of 167 patients and 160 patients of these (95.8%) met the renal recovery criteria. The renal recovery rate was significantly higher in the milder AKI stages: 93.8% in AKI stage 1, 100% in AKI stage 2 and 80.0% in AKI stage 3 (global p = 0.003). One year after discharge, the SCr status was available in 135 patients and only 1 patient of these did not meet the renal recovery criteria.

5.1.9 AKI and CRP

The higher the AKI stage, the higher was the mean and median serum CRP level (Figure 6). The odds of having AKI stage 2 or 3 increased 1.74-fold per every 50 mg/L increase in CRP values (95% CI 1.45–2.08). The optimal cutoff for CRP to identify patients with AKI stage 2 or 3 was ≥244 mg/L (sensitivity 82.6% and specificity 75.8%).

Table 7. Demographics and clinical characteristics of 195 patients with GAS bacteremia included in study II and comparison of patients of different KDIGO AKI stages.

Variables	Non-AKI (n = 111)	AKI 1 (n = 38)	AKI 2 (n = 20)	AKI 3 (n = 26)	p-value*	total 195
Sex: female	55 (49.5)	18 (47.4)	12 (60.0)	11 (42.3)	0.687	96 (49.2)
Age mean (SD)	60.2 (19.82)	71.0 (16.21)	60.8 (17.81)	51.6 (13.22)	0.001	61.2 (18.92)
Charlson class					0.012	
0	24 (21.6)	2 (5.3)	4 (20.0)	11 (42.3)		41 (21.0)
1	22 (19.8)	8 (21.1)	6 (30.0)	7 (26.9)		43 (22.1)
2	18 (16.2)	8 (21.1)	3 (15.0)	5 (19.2)		34 (17.4)
3	47 (42.3)	20 (52.6)	7 (35.0)	3 (11.5)		77 (39.5)
Underlying conditions						
Diabetes mellitus	16 (14.4)	9 (23.7)	5 (25.0)	3 (11.5)	0.333	33 (16.9)
Hypertension	45 (40.5)	15 (39.5)	9 (45.0)	11 (42.3)	0.981	80 (41.0)
Any atherosclerotic disease	25 (22.5)	9 (23.7)	4 (20.0)	5 (19.2)	0.985	43 (22.1)
Chronic kidney disease**					0.009	
CKD 1	31 (27.9)	4 (10.5)	2 (10.0)	11 (42.3)		48 (24.6)
CKD 2	64 (57.7)	21 (55.3)	15 (75.0)	11 (42.3)		111 (56.9)
CKD 3	14 (12.6)	12 (31.6)	3 (15.0)	2 (7.7)		31 (15.9)
CKD 4	2 (1.8)	1 (2.6)	0 (0.00)	1 (3.8)		4 (2.1)
CKD 5	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.8)		1 (0.5)
No underlying disease	21 (18.9)	8 (21.1)	5 (25.0)	7 (26.9)	0.732	41 (21.0)
Clinical manifestation						
SSTI	68 (61.3)	22 (57.9)	11 (55.0)	15 (57.7)	0.947	116 (59.5)
Pneumonia	25 (22.5)	6 (15.8)	5 (25.0)	8 (30.8)	0.555	44 (22.6)
Necrotizing fasciitis	2 (1.8)	1 (2.6)	1 (5.0)	2 (7.7)	0.253	6 (3.1)
Abdominal abscess	1 (0.9)	2 (5.3)	2 (10.0)	2 (7.7)	0.034	7 (3.6)
Any abscess	9 (8.1)	6 (15.8)	5 (25.0)	5 (19.2)	0.078	25 (12.8)
Disease severity						
Hypotension	16 (14.4)	14 (36.8)	14 (70.0)	22 (84.6)	< 0.001	66 (33.8)
IMV	5 (4.5)	4 (10.5)	4 (20.0)	18 (69.2)	< 0.001	31 (15.9)
ICU admission	10 (9.00)	7 (18.4)	10 (50.0)	19 (73.1)	< 0.001	46 (23.6)
RRT	1 (0.9)	2 (5.3)	4 (20.0)	9 (34.6)	< 0.001	16 (8.2)

AKI = Acute kidney injury 1-3 according to the KDIGO criteria and non-AKI refers to the cases not meeting the criteria of KDIGO AKI; CKD = chronic kidney disease; SSTI = skin and soft tissue infection; IMV = invasive mechanical ventilation; ICU = intensive care unit; RRT = renal replacement therapy
Data represent: No, (%) of the group
*p value: comparison of the groups AKI 1-3 and non-AKI with the χ^2-square test or Fisher's exact test except with one-way ANOVA for age
**Chronic kidney disease classification according to the National Kidney Foundation (Inker et al., 2014)
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Table 8. Mortality rates of GAS bacteremia patients (n=195) by KDIGO AKI stage.

Mortality		Non-AKI (n = 111)	AKI 1 (N = 38)	AKI 2 (N = 20)	AKI 3 (N = 26)
7-day	n (%)	4 (3.6)	1 (2.6)	3 (15.0)	5 (19.2)
	OR (95% CI)	1	0.76 (0.08–7.50)	5.87 (1.03–33.43)	16.00 (2.17–117.68)
	p-value		0.816	0.046	0.006
30-day	n (%)	7 (6.3)	4 (10.5)	4 (20.0)	6 (23.1)
	OR (95% CI)	1	1.33 (0.34–5.11)	4.28 (0.98–18.77)	10.77 (2.03–57.05)
	p-value		0.682	0.054	0.005
90-day	n (%)	11 (9.9)	5 (13.2)	4 (20.0)	6 (23.1)
	OR (95% CI)	1	0.94 (0.29–3.10)	2.67 (0.66–10.86)	7.26 (1.53–34.35)
	p-value		0.920	0.170	0.012
1-year	n (%)	16 (14.4)	8 (21.1)	4 (20.0)	7 (26.9)
	OR (95% CI)	1	1.03 (0.37–2.86)	1.71 (0.44–6.67)	6.74 (1.59–28.64)
	p-value		0.949	0.442	0.010

OR = Odds ratio; CI = Confidence interval

Binary logistic regression was used to compare mortality between AKI stages after adjustment for age, the Charlson comorbidity index and intensive care unit admission.

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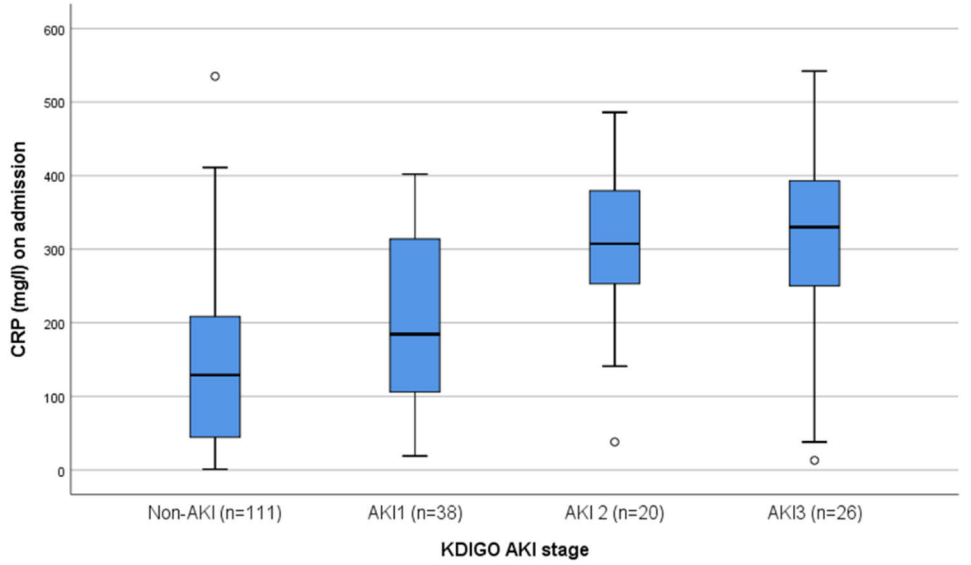

Figure 6. Box plot of CRP-values (mg/mL) of GAS bacteremia patients included in study (n=195) on admission stratified by KDIGO AKI stage. Shown are the median, lower and upper quartiles, and whiskers represent ±1.5-fold interquartile ranges. There was a significant difference between non-AKI vs. AKI stage 1 ($p = 0.0498$), non-AKI vs. AKI stage 2 ($p < 0.001$), non-AKI vs. AKI stage 3 ($p < 0.001$), AKI stage 1 vs. 2 ($p = 0.014$) and AKI stage 1 vs. 3 ($p = 0.001$) but not between AKI stage 2 vs.3 ($p = 1.000$). The comparison between AKI stages was done using ANCOVA after adjustment for age and the Charlson comorbidity index. Bonferroni's method was used for pairwise comparisons.

5.2 iGAS infections: prospective observational study (III)

5.2.1 Patient recruitment

The incidence of GAS bacteremia in HDSWF decreased promptly during the study period which slowed enrollment. However, in PHD the incidence was maintained at a higher level. Figure 7 shows the study population. During the recruitment period, 45 patients (44% of all iGAS cases) were enrolled, 30 from PHD and 15 from HDSWF.

5.2.2 Clinical characteristics and disease severity

44 of 45 patients had positive blood cultures for GAS. One patient had NSTI and GAS was isolated from deep tissue sample whereas blood cultures were negative. Tables 9 and 10 summarize the details of the clinical characteristics and disease severity of the 45 enrolled patients. Male gender was slightly overrepresented (60%).

The mean age of all patients was 55 years. The most common underlying diseases were obesity and hypertension, while 40% had no underlying chronic disease. The most common infection focus was SSTI with or without a wound (56%). Five patients had NSTI. Of all patients, 29% were admitted for ICU treatment; in-hospital mortality was 13%. The most common *emm* types among the enrolled patients were *emm*1 (19%), *emm*1.25 (14%) and *emm*89 (23%).

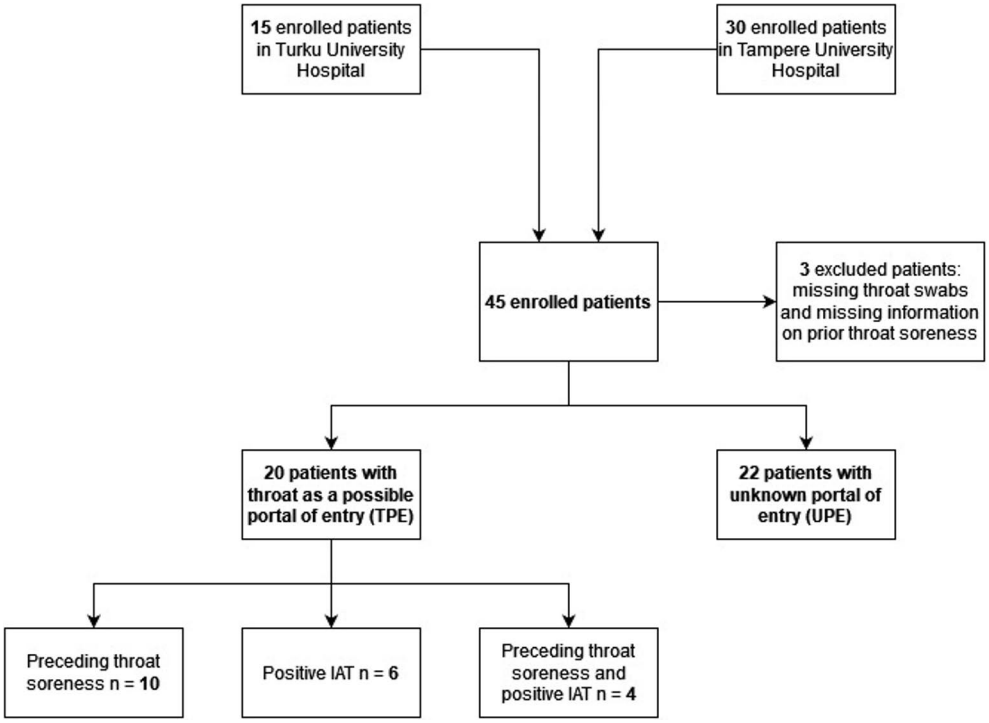

Figure 7. Flow chart of the study population of the prospective study (III). IAT = Isothermal amplification test.

Table 9. Demographics and clinical characteristics of the iGAS patients (n = 45) included in the prospective study (III).

Patient demographics and clinical characteristics	n (%)
Age mean (SD)	55.07 (20.31)
Sex male	27 (60.0)
Charlson class	
0	9 (20.0)
1	19 (42.2)
2	6 (13.3)
3	11 (24.4)
Healthcare-acquired	3 (6.7)
Underlying conditions	
Obesity (BMI ≥ 30 kg/m^2)	14 (31.1)
Hypertension	14 (31.1)
Diabetes mellitus	8 (17.8)
Atrial fibrillation	8 (17.8)
Congestive heart failure	5 (11.1)
Pulmonary disease	5 (11.1)
Coronary artery disease	4 (8.9)
Chronic kidney disease	
CKD1 or unknown status	37 (82.2)
CKD2	3 (6.7)
CKD3	4 (8.9)
CKD4	1(2.2)
CKD5	0 (0)
Any malignancy	6 (13.3)
Immunodeficiency	
HIV/AIDS	0 (0)
Immunosuppressive medication	1(2.2)
Alcohol abuse	7 (15.6)
Current smoking	13 (28.9)
No underlying disease	18 (40.0)

SD = standard deviation; CKD = chronic kidney disease
Chronic kidney disease classification according to the National Kidney Foundation (Inker et al., 2014)

Table 10. Infection foci and disease severity of iGAS patients included in study III (n = 45).

Infection foci and disease severity	n (%)
Infection focus	
SSTI with or without wound	25 (55.6)
Respiratory tract	10 (22.2)
Arthritis, spondylitis, epidural abscess	8 (17.8)
NSTI	5 (11.1)
Septicemia only	3 (6.7)
Other*	3 (6.7)
Disease severity	
ICU admission	13 (28.9)
Hypotension	14 (31.1)
Need for vasoactive drug	12 (26.7)
IMV requirement	6 (13.3)
RRT requirement	1 (2.2)
STSS	9 (20.0)
Outcome	
In-hospital mortality	6 (13.3)

SSTI = Skin and soft tissue infections; NSTI = Necrotizing soft tissue infections; IMV = Invasive mechanical ventilation; ICU = Intensive care unit; RRT = Renal replacement therapy; STSS = Streptococcal toxic shock syndrome
Data represent: No, (%) of the group
*Other: includes one endocarditis, one peritonitis and one gynecological infection.

5.2.3 Entry portal of infection

There were three patients with missing information on throat swabs and on prior throat soreness and they were excluded from this analysis. Thus, 42 patients were classified into either the TPE or the UPE group (Figure 7). The mean and median intervals between initiation of antibiotics and obtaining of the IAT sample were two days in both groups. All throat swabs were culture negative, which was expected due to prior intravenous antimicrobial therapy. However, five patients tested positive with both IAT tests (ID Now and Solana), and a further five with patients with either one of the IAT tests (ID Now, three patients and Solana, two patients). Table 11 compares the patients in the TPE and UPE groups. Patients in the TPE group were significantly younger and their Charlson class was lower compared to UPE group. Furthermore, patients in the TPE group were less likely to be obese but needed IMV more often. All three patients with bacteremia only and four out of the five patients with NSTI were in the TPE group. During hospitalization, 6 patients died. Three of them were in the TPE group, one in the UPE group and two uncategorizable.

Seventeen patients (38%) reported, before becoming ill, that they had had close contact with persons with confirmed or with a high clinical suspicion of GAS infection: tonsillitis (11/17), impetigo (3/17) or several mixed infections (3/17). Eight of these seventeen patients (47%) were in the TPE group.

Table 11. Comparison of the iGAS cases by TPE and UPE groups.

	TPE (n=20)	**UPE (n=22)**	**p-value**
Age mean (SD)	45.30 (18.85)	60.73 (17.98)	0.010
Sex male	15 (75.0)	10 (45.5)	0.051
Charlson class			0.033
0	7 (35.0)	2 (9.1)	
1	10 (50.0)	9 (40.9)	
2	0 (0)	5 (22.7)	
3	3 (15.0)	6 (27.3)	
Obesity			0.005
BMI ≥ 30 kg/m²	2 (10.0)	11 (50.0)	
No underlying disease	9 (47.4)	8 (36.4)	0.476
Infectious focus			0.069
Wound infection	0 (0)	4 (18.2)	
SSTI without wound	7 (35.0)	11 (50.0)	
NSTI	4 (20.0)	1 (4.5)	
Disease severity			
ICU admission	7 (35.0)	5 (22.7)	0.379
Vasoactive drug	3 (15.8)	7 (31.8)	0.292
IMV	5 (25.0)	0 (0)	0.018
STSS	5(25.0)	3 (13.6)	0.445
In-hospital mortality	3 (15.0)	1 (4.5)	0.174

TPE = Throat as a possible portal of entry: patients with sore throat and/or positive IAT; UPE = Unknown portal of entry: patients with no sore throat nor positive IAT test; IAT = Isothermal amplification test; BMI = Body mass index kg/m2; ICU = Intensive care unit; NIV = Non-invasive ventilation; IMV = Invasive mechanical ventilation; CI = Confidence interval
Data represent: No, (%) of the group

6 Discussion

6.1 Incidence of GAS bacteremia

We observed an increasing trend in incidence of GAS bacteremia during the study period 2007–2018 in HDSWF. This is in line with other reports from New Zealand (Williamson et al., 2015), Sweden (Darenberg et al., 2013) and England (Rudman et al., 2021). The overall incidence of GAS bacteremia in our study is comparable with earlier studies from Finland on the incidence of iGAS infections which ranged from 2.46/100,000 to 3.6/100,000 (Lamagni et al., 2008; Siljander et al., 2010; Smit et al., 2015) and with studies on iGAS conducted in other western countries (Laupland et al., 2019; Naseer et al., 2016; Nelson et al., 2016). Since there is no common international definition on iGAS and since the surveillance system differs between countries, exact comparisons of iGAS rates between countries and studies must be done with caution. With a broader case definition (including also positive GAS culture cases from any sterile site and cases with clinical signs of iGAS infection and a positive culture from a nonsterile site), we would have recorded even more cases. However, the peak incidence is higher than ever before reported from Finland (Lamagni et al., 2008; Siljander et al., 2010; Smit et al., 2015). In 2018, the incidence of iGAS infections in whole Finland increased to the highest level ever since 1995, when NIDR was established of (6.74 / 100,000). However, after the study period, the incidence of GAS bacteremia in HDSWF and in whole Finland decreased in 2019 and decreased even more in 2020 when the COVID19-pandemic began (THL: NIDR: Statistical database). A similar decrease in the incidence of GAS bacteremia during the first COVID-19 pandemic year was reported from England where the incidence decreased from 3.8 / 100 00 in 2019 to 2.4 / 100 000 in 2020 (Rudman et al., 2021). This decrease in incidence probably reflects the reduced physical contact between individuals during the pandemic. The criteria for reporting iGAS to NIDR in Finland have, nevertheless, remained unchanged throughout the years.

We did not study incidence changes by age groups. However, according to a recent Finnish study, there has been an increase in the incidence of BSI, most notably in the oldest age groups, i.e., persons over 90 years of age (Kontula et al., 2021). Several earlier studies have shown that advancing age is a risk factor for iGAS infections (Nelson et al., 2016; Smit et al., 2015; Williamson et al., 2015). The aging

of the population and the rising life expectancy in industrialized countries may explain, at least partly, the increase in the incidence of iGAS infections. On the other hand, pathogen-associated factors are also at play. It has been demonstrated how horizontal gene transfer events in *S. pyogenes* type *emm*89 and *emm*1 creates new pathogenic genotypes with an increased ability to cause infection and global epidemic waves (Beres et al., 2016; Zhu et al., 2015). However, the exact mechanisms and the totality of factors affecting to beginning and ending of an epidemic are unknown and not fully understood.

6.2 *emm* type distribution

The most common *emm* types in the retrospective study (I) on GAS bacteremia in HDSWF were *emm*28, *emm*1, *emm*89 and *emm*12. The distribution is in line with earlier studies from Finland, Europe and the US (Gherardi et al., 2018; Smit et al., 2015). The proportion of *emm*1 started to increase in 2016, and in 2018 it was the most prevalent *emm* type in HDSWF as well as in the whole country and was the most probable cause for the epidemic wave (THL: NIDR: Annual reports). Similarly, Lynskey et al reported a dominant new *emm*1 *S. pyogenes* lineage emerging in England from the year 2015 onwards (Lynskey et al., 2019).

In the prospective study (III) covering the period from June 2018 to July 2020 in HDSWF and PHD, the most common *emm* types were *emm*1, *emm*1.25, *emm*28 and *emm*89. The distribution varied by study site and the most conspicuous differences occurred especially in the incidences of *emm*1.25, *emm*28 and *emm*89. In both retrospective and prospective studies (I and III), the infection caused by the *emm*28 type appeared to be related to a more invasive course since it was the causative pathogen in the most cases with NSTI and STSS. This differs from a previous large European study, in which only 9% of patients with STSS and 5% with NSTI were caused by *emm*28 (Luca-Harari et al., 2009). However, the small sample size, especially of prospective study (III) patients, limits the generalizability of our findings.

6.3 Role of infectious diseases specialist consultation on selection of antibiotic treatment

We observed that IDSC had a significant impact on the antibiotic treatment selection for iGAS infection patients. IDSC increased the use of penicillin G as the first-line antibiotic, whereas no IDSC increased the use of broad-spectrum β-lactam antibiotics. The use of penicillin G in both study groups (IDSC+ and IDSC-) increased significantly during the later study period (2013–2018). The first generally available antimicrobial guide used within the HDSWF was published in the end of

2012 and the number of infectious disease specialists increased during the later study period. The antimicrobial guide of the HDSWF has recommended penicillin for the treatment of GAS bacteremia since 2012 with iv-cephalosporin listed as an option to penicillin. Clindamycin has been recommended to be combined to treat severe cases (i.e., STSS or NSTI).

This may explain, at least partly, the increased use of penicillin and the improved adherence to the rational use of antibiotics among both infectious disease specialists and other physicians who consult the antimicrobial guide. This is important with respect to the global concern of antimicrobial resistance. Nevertheless, excess use of clindamycin among infectious disease specialists is not explained by the antimicrobial guide.

A recent large European randomized trial studied the impact of the availability of expert teleconsultations on the quality of treatment of patients in intensive care and of patients with infectious diseases. The primary outcome measure was adherence to the ten Choosing Wisely recommendations for infectious diseases provided by the German Society for Infectious Diseases and it turned out that patients in the intervention group were significantly more likely to be treated according to the recommendations for *S. aureus* BSIs (OR 4.00; 95% CI 1.83–9.20; $p < 0.01$). Also, extension of treatment with prophylactic antibiotics after surgery was significantly less likely (OR 9.31; 95% CI 3.79–25.94; $p < 0.01$). Interestingly, the quality improvements for several outcomes reached not only those patients who were treated as suggested by direct expert teleconsultations, but also other patients treated by the same physicians. Thus, it seems as if there is a positive effect of initial training courses and/or an indirect effect of expert teleconsultations (Marx et al., 2021). A Norwegian study explored the factors affecting the antibiotic prescribing practices of hospital physicians. An important theme was “decision support”, i.e., the availability and use of microbiological test results and consultations as facilitators of rational prescribing practices. These facilitators were often underused due to capacity constraints (Christensen et al., 2022).

There are several previous studies demonstrating the benefits of IDSC with respect to the outcome of *S. aureus* infections (Forsblom et al., 2018; Honda et al., 2010; Rieg et al., 2009). However, to our knowledge, there are no earlier studies regarding the role of IDSC for selecting the antibiotic to treat GAS bacteremia. In this study (I), we did not evaluate the form of IDSC. The consultation might have been done by phone or bedside, but it had to be documented in patient records. Our results add evidence to the contention that the availability of IDSC facilitates significantly a more rational antibiotic use. Furthermore, the benefit of having access to infectious diseases specialist consultations availability may be also indirect or exponential as was seen in the fact that the use of penicillin G in both study groups (IDSC+ and IDSC-) increased significantly during the later study period when an

updated antimicrobial guide was available and infectious diseases specialist resources were better. Marx et al mentioned above made the same observation (Marx et al., 2021). Furthermore, it may also have been easier for infectious diseases specialists to follow the recommendations on antimicrobial use with an in-house written guide available.

6.4 Clindamycin treatment in iGAS infections

The third main observation in study I was that the use of clindamycin as an adjunctive antibiotic was more common than the prevalence of STSS or NSTI, and it occurred mainly in the IDSC group (55% of patients in IDSC+ group received clindamycin). In a Canadian retrospective study among 249 iGAS infection patients, clindamycin was used by 48.6% of the patients (Couture-Cossette et al., 2018). Prior literature shows that the question of the benefit of clindamycin as adjunctive therapy for iGAS infections is unsettled. However, after publication of our study, Babiker et al demonstrated in their study, which was retrospective but propensity-matched and statistically convincing that mortality was significantly lower among iGAS infection patients who received adjunctive clindamycin than among those who did not (6.5% vs. 11%; OR 0.44; 95% CI 0.23–0.81). The benefit was achieved also among iGAS infection patients without organ failures. Clindamycin had been used by 31.7% of iGAS infection patients. Regrettably, neither the incidence of *Clostridioides difficile* infection (CDI) nor the long-term outcome of patients were reported (Babiker et al., 2021). Brindle et al showed that the addition of clindamycin to flucloxacillin did not improve clinical outcome of patients with limb cellulitis but doubled the likelihood of diarrhea (Brindle et al., 2017).

Clindamycin use, like the use of broad spectrum penicillins, cephalosporins and fluoroquinolones, carries a higher risk for CDI than the use of other antibiotics. Age over 65 years is a significant CDI risk factor and related to a poor clinical outcome with respect to CDI severity and overall mortality. Other well-known risk factors for CDI are inflammatory bowel disease, gastrointestinal surgeries, immunological incompetence caused by malignant neoplasms, transplantations, chronic kidney diseases or immunosuppressant use (Czepiel et al., 2019).

Considering the well-known disadvantages of clindamycin and the lack of data from randomized controlled trials of adjunctive clindamycin therapy in iGAS infections, the use of adjunctive clindamycin therapy may be plausible on an individual patient basis. Among young patients without comorbidities, addition of clindamycin to β-lactams may be reasonable for treating severe iGAS infections (e.g., when there is at least one organ failure), even without STSS or NSTI. On the other hand, addition of clindamycin to the treatment of elderly patients with comorbidities should be evaluated carefully and reserved to the most severely ill

patients. Of note: Babiker et al found that clindamycin treatment for over 3 days does not increase the likelihood of a beneficial outcome (Babiker et al., 2021). Discontinuation of the adjunctive therapy as soon as the patient becomes hemodynamically stabile is stated in a guideline recommendation (UptoDate: iGAS infections and STSS 2022). However, this recommendation is based on expert opinion and not on controlled trials.

According to a retrospective study among iGAS patients clindamycin treatment started 0–24 hours post-admission decreased mortality with an adjusted OR of 0.04 (95% CI 0.003–0.55; p = 0.02). Mortality was also lower in the group of patients who received clindamycin 24–72 hours after admission than among patients who had not received clindamycin, but not statistically significantly so (Couture-Cossette et al., 2018). Thus, clindamycin might be considered for use as adjunctive treatment of patients with community-acquired septic infection with a suspected GAS etiology already before the streptococcal etiology is known and if clindamycin is not contraindicated due to allergy or major risk factors for CDI. If a GAS etiology is not confirmed and in any case after a few days, continued adjunctive clindamycin therapy should be evaluated carefully.

6.5 Incidence and outcome of AKI among patients with GAS bacteremia

We observed a high (43%) overall incidence of AKI among bacteremic GAS patients, and 85% of the patients with AKI had their highest SCr level at the time of hospital admission. The incidence of AKI was even higher (78%) among ICU-treated patients. The study population was heterogenic and ranged from patients with mild bacteremic infections to patients with severe sepsis, which makes the comparison of AKI incidence and mortality with prior literature complex. Also, the definition of AKI differs between studies. However, our study population (II) and results have similarities with a large prospective observational CAP study from the US. In that study, 34% of CAP patients developed AKI according to the RIFLE criteria and nearly 63% of them had AKI already at hospital admission. One-third of the study population developed severe sepsis (i.e., the SOFA score was ≥ 3 in at least one organ system) and AKI was more common among them (57.5%) (Murugan et al., 2010). Fiorentino et al used the KDIGO criteria and found that 15% of the patients with CAP who survived to hospital discharge had stage 2–3 AKI (Fiorentino et al., 2018). Furthermore, Chertow et al reported that 18% of hospitalized patients with infectious diseases had AKI (Chertow et al., 2005). In earlier studies, the prevalence of AKI among septic patients has varied between 47 and 68% (Bagshaw et al., 2009; E. Peters et al., 2018; Poukkanen, Vaara et al., 2013; Vincent et al., 2006; Xu et al., 2015).

A recent retrospective study from the Netherlands investigated AKI among *S. aureus* bacteremia (SAB) patients. AKI was defined as a 1.5-fold increase in baseline creatinine; it was found that 37% of all patients developed AKI. Of those with AKI, 39% had the maximum creatinine value on the day when the first blood culture was drawn. AKI during SAB was associated with 30-day mortality (OR 3.9; 95% CI 2.2–6.9; $p < 0.01$) and renal recovery occurred in 59% of patients (Westgeest et al., 2022). Comparing the study of Westgeest et al. and our GAS bacteremia patients (II), the overall incidence of AKI is quite similar but even a higher proportion of our study population with AKI had the peak SCr on admission and our patients had a much higher renal recovery rate (96%). In other studies, 42.4% of patients hospitalized with CAP and AKI stage 2–3 recovered (Fiorentino et al., 2018) and half of the patients with septic shock and AKI recovered renal function by hospital discharge (Kellum et al., 2016).

There are only a few earlier studies regarding SCr levels or AKI among iGAS patients. In a Swedish study, Björck et al compared iGAS patients admitted to ICU to other septic ICU-patients and found that iGAS patients had statistically significantly higher levels of SCr at ICU admission compared to non-iGAS septic patients (median 173 vs. 133 µmol/L) and higher maximal AKIN classification scores during the first 10 days after admission (median 3 vs. 0) despite the fact that the iGAS group was statistically significantly younger. After correction for severity of illness and age, iGAS patients still had a higher risk for AKIN-creatinine class 1 or worse (CI 95% 1.246–4.968; $p \leq 0.05$). Furthermore, they found that iGAS patients with *emm*1 had more often renal failure than patients with other iGAS serotypes (Björck et al., 2020). Similarly, we found that the mean age of AKI stage 3 was significantly lower than in the whole study population and in the non-AKI group. Furthermore, in our study (II), most patients with AKI stage 3 had CKD 1 or 2 and a Charlson class of 0 or 1. This, however, is in contrast with earlier studies, in which higher age, a higher number of comorbidities, CKD, hypertension, cardiovascular disease and diabetes mellitus have been associated with SA-AKI (Bagshaw et al., 2009; Liu et al., 2020; Poukkanen, Vaara et al., 2013). On the other hand, these studies have not stratified their results by pathogen. Instead, Bruun et al compared patients with NSTI caused by GAS and by *S. dysgalactiae* (SD) and found that the GAS-group had statistically significantly higher preoperative SCr values than the SD group (178 vs. 103 µmol/L) (Bruun et al., 2021).

The etiology of SA-AKI is currently recognized as being multifactorial; several mechanisms are at play. Three mechanisms are considered fundamental for septic organ injury: inflammation, microcirculatory dysfunction and metabolic reprogramming (Peerapornratana et al., 2019). Circulating inflammatory cytokines activate the endothelium, which amplifies the inflammatory response. Furthermore, an adaptive response of TECs (i.e., downregulation of the cell function in order to

ensure cell survival) to the changes of the local environment is an important pathophysiological mechanism (Umbro et al., 2016; Zarbock et al., 2014). GAS can produce toxins which can act as superantigens and as PAMPs stimulating immune-cells and TECs. This would result in induction of cytokine expression, tissue damage and, ultimately, organ dysfunction (Lappin & Ferguson, 2009; Shannon et al., 2019). The causing pathogen, or even the strain, may consequently impact on the development of AKI of septic patients. We also propose that GAS, or at least some GAS strains, could be strong cytokine expression inducers, cause a powerful inflammatory reaction and result in a higher prevalence of AKI. Fortunately, GAS-induced AKI seems often to be reversible, which may be related to patient characteristics like younger age and fewer comorbidities, and to the pathophysiological process.

Mortality has been related to severity of AKI, and AKI stage 3 is independently associated with 90-day mortality (Kellum et al., 2016; E. Peters et al., 2018; Poukkanen, Vaara et al., 2013). In previous studies, mortality among septic AKI patients has ranged from 27.7% (Kellum et al., 2016), 38.1% (Poukkanen Vaara et al., 2013) to 51.7% (E. Peters et al., 2018). In our study (II), the 7-day mortality was higher in AKI stage 2 and 3 than in the non-AKI group and 30-day mortality was higher in AKI stage 3 than in the non-AKI group. However, the 7-day and 30-day mortality rates in AKI stage 3 (19.2% and 23.1%) and AKI stage 2 (15.0% and 20.2%) were lower than in previous studies. Our results are better in line with the Swedish study, in which 28-day mortality among ICU-treated iGAS patients was 13% and was significantly lower than among non-iGAS septic patients (37%) although the iGAS group had a higher prevalence of renal failure (Björck et al., 2020).

6.6 Inflammation in iGAS infections and AKI

We observed that a high CRP level on admission associated positively and significantly with the incidence of higher severity stages of AKI. Earlier studies have shown that AKI is associated with high circulating levels of inflammatory mediators and that these mediators are related to the prognosis of AKI (Karabağ et al., 2019; Payen et al., 2012; J. Wang et al., 2021). Among acute myocardial infarction patients, elevated levels of high-sensitivity CRP on admission was associated with AKI development and severity (Cosentino et al., 2019). There are also some study results on septic patients in line with our results and show that patients with AKI have higher CRP levels than patients without AKI (Katayama et al., 2017; Zhou et al., 2018). Nie et al. published a prospective study with 1,361 patients where they had found that median CRP levels were higher among patients with suspected infection and AKI (93.5 mg/L) than among patients with suspected infection without

AKI (50.0 mg/L) ($p < 0.0001$). In the same study, CRP predicted AKI with an AUC of 0.647 (95% CI 0.605–0.689, $p < 0.0001$ against the AUC 0.5 line), but procalcitonin predicted AKI even more accurately (Nie et al., 2013). In the study of Katayama et al., median CRP levels were statistically significantly higher among septic patients with AKI (137 mg/L) than without AKI (105 mg/L) ($p = 0.002$) (Katayama et al., 2017). In our study (II), the median CRP levels were even higher and increased with more severe AKI stages (129, 184, 307 and 330 mg/L in non-AKI, AKI 1, AKI 2 and AKI 3 groups, respectively). The differences in the mean CRP values between AKI stages were significant after adjustment for age and CCI. Thus, our study results suggest that the CRP level may be considered as a marker of severity of the infection, which correlates with the severity of AKI. The high CRP level, especially among patients with severe AKI, implies that GAS is a strong stimulator of the immune system and inducer of cytokine expression, which nowadays are known to be an important factor in the pathogenesis of AKI.

6.7 Portal of bacterial entry in iGAS infections

The main finding in our prospective study (III) on iGAS infection was that 48% of the iGAS patients had confirmed or probable prior throat colonization with GAS (TPE group). The TPE group had a more severe disease course regarding the rate of ICU admissions and occurrence of STSS and NSTI. However, the TPE group was significantly younger, healthier as measured by Charlson class, more often non-obese but needed more often IMV. This may suggest that hematogenous seeding from the nasopharynx is a possible portal of entry for GAS, especially among younger and healthier adult patients who develop severe disease.

The clinical characteristics and disease course of our study population (III) is similar to earlier reports (Langley et al., 2016; Nelson et al., 2016; Rantala et al., 2009a). However, the proportion of patients without any underlying disease (40%) was higher than in previous reports (20–26%) (Gear et al., 2015; Langley et al., 2016; Nelson et al., 2016; Rantala et al., 2009a). The in-hospital mortality rate (13%) of the patients in study III is in line with previous reports from Europe, Australia and the US (Gear et al., 2015; Lamagni et al., 2008; Naseer et al., 2016; Nelson et al., 2016), but slightly higher than in study I and in another report from Finland (8–10%) (Siljander et al., 2010). This discrepancy is most probably due to a small sample size but may also reflect selection bias, since both recruiting centers were tertiary hospitals.

Several host-related factors, such as heart disease, diabetes, malignancy, obesity and conditions affecting the skin, have been associated with iGAS infections (Langley et al., 2016; Nelson et al., 2016; Rantala et al., 2009a). However, the portal of bacterial entry cannot often be identified. It has been proposed that hematogenous

seeding from the nasopharynx occurs, especially in the absence of wound colonization, but there is no strong evidence in favor of this assumption (Stevens & Bryant, 2017). A recent Scandinavian study observed that, compared to patients with streptococcal SSTI, patients with severe streptococcal NSTI had more often had a blunt trauma and no preexisting skin lesions. They also found that NSTI patients had lower BMI than SSTI patients, which is in line with our results (Bruun et al., 2021). The unknown portal of bacterial entry may, in fact, be pharyngeal seeding of GAS, since all three patients with bacteremia alone and no infection foci in study II and four out of the five patients with NSTI were in the TPE group.

A few earlier studies have investigated colonization of the pharynx by GAS. In a small prospective study from Greece, where the clinical data were collected prospectively from patients' charts and the attending physician, only one out of 46 (2.2%) adult iGAS patients and 19 out of 96 (19.8%) pediatric iGAS patients had recently had pharyngotonsillitis (Zachariadou et al., 2014). An Australian retrospective study reported that 4.9% of the iGAS infection patients had recently had and 2.4% had ongoing sore throat (Gear et al., 2015). However, our study (III) found a much higher prevalence of sore throat among adults (14/37, 38%), with a further three cases without information on throat soreness but having a positive IAT. Of note is that only one patient without a throat-related infectious focus or presenting symptom had preceding throat soreness noted in his/her patient record. This exemplifies the inability of retrospective registry-based studies to identify evidence of the throat as a portal of bacterial entry in iGAS infections.

The isothermal amplification test (IAT, a rapid nucleic acid test) has high sensitivity and specificity for GAS in throat swabs specimens and prior antimicrobial therapy does not invalidate the test (Arbefeville et al., 2018). GAS specific IATs for pharyngitis have an overall sensitivity of 97.5% and specificity of 95.1% (Dubois et al., 2021). In our study, all throat swabs were culture negative due to preceding antimicrobial therapy, which had lasted on average for of two days.

National treatment guidelines for GAS pharyngitis are controversial. Guidelines in North America, Finland and France recommend microbiologic testing in suspected cases and prescribing antibiotics in confirmed GAS cases to prevent suppurative complications and rheumatic fever. In contrast, guidelines in countries like the United Kingdom, Holland and Belgium do not routinely recommend testing and restrict antibiotic treatment to selected cases only (Chiappini et al., 2011). In our study (III), only 15% of the patients in the TPE group had sought medical advice for sore throat, which implies that at least 85% of these iGAS infections would have occurred regardless of the microbial treatment guidelines on GAS pharyngitis. Maybe only a vaccine effective in eradicating GAS carriage could prevent, at least a part of, the iGAS infections.

6.8 Strengths and limitations

The strength of the retrospective studies was that the clinical data were collected by an ID specialist who read all electronic patient records. Similarly, in the prospective study, all the enrolled patients were interviewed by an ID specialist, which provided accurate information on prior symptoms and gave strength to the study.

There are also some limitations in our studies. Firstly, the retrospective study set in studies I and II is a natural limitation. The study population represents patients from only one hospital district in Finland. Regarding antimicrobial therapy and its side-effects, we did not study the duration of clindamycin use or the prevalence of adverse events like diarrhea. There is a possibility that undocumented IDSC took also place although consultations regarding antibiotic selections in bacteremic patients, even over phone, are generally well documented in HDSW. An ID specialist visits regularly in ICU and participates in the rounds but these consultations are not necessarily always, although routinely very often, documented and the proportion of IDSC in the ICU may thus be even higher than reported in this study. In study II, the premorbid SCr value was estimated by the MDRD equation in 33% of cases. This may overestimate the AKI stage in these patients. However, the proportion of patients who did not have a premorbid SCr value in our study was markedly lower than, for example, in the FINNAKI study (33% vs. 69%) (Poukkanen, Wilkman, et al., 2013). The sample size was small in our prospective study (III) and the COVID-19 pandemic caused a premature halt to patient enrollment in March 2020. As all patients had had preceding antimicrobial exposure, it is possible that the UPE group had false negative IAT results. The UPE group included also five cases who were unable to provide information on prior throat soreness, and who might thus also have been miscategorized.

6.9 Future aspects

To date, there is no vaccine available against GAS. However, two GAS vaccine candidates have completed human trials and several vaccine candidates are currently being evaluated in pre-clinical trials (Castro & Dorfmueller, 2021). The need for an effective GAS vaccine is highlighted by the increasing concerns related to GAS resistance patterns. A vaccine effective in eradicating GAS throat carriage might be an important tool for preventing also severe iGAS infections, since hematogenous seeding from the nasopharynx may play an important pathophysiological role for iGAS infection.

The availability of ID specialists and of updated antimicrobial guidelines together with general access to stewardship for selecting of antimicrobials will be even more important in future. The aim is toward a more responsible use of antimicrobial pharmaceuticals and to reduce antimicrobial resistance.

A large randomized controlled trial would be needed to solve the question of the benefit, if any, of adjunctive clindamycin treatment in iGAS infections. Such plans would have to consider the low incidence especially of STSS and NSTI, which would impede the recruitment rate of such a trial.

7 Conclusions

I. Infectious diseases specialist consultations increase the use of narrow-spectrum β-lactam antibiotics and adjunctive clindamycin treatment. Further research is needed to identify the precise indications for adjunctive therapies.

II. AKI is common among patients with GAS bacteremia. The severity of AKI correlates with the CRP level measured on admission. The mortality of patients with GAS bacteremia and AKI is significantly higher than of patients with GAS bacteremia but without AKI. Among survivors, renal recovery is excellent. The inflammatory reaction caused by GAS may play an important role in the development of and recovery from AKI.

III. Prior throat soreness or GAS throat colonization is frequently observed among patients with iGAS infection. Hematogenous seeding from the nasopharynx may be an important portal of GAS entry, especially among younger and healthier adult patients who develop severe disease.

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