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Aspects of Group B streptococcus (GBS) disease in the newborn

Epidemiology, characterisation of invasive strains and evaluation of intrapartum screening

Håkon Bergseng

Doctoral Thesis

NTNU
Norwegian University of
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Thesis for the degree of
philosophiae doctor
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CONTENTS

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Abbreviations

CC clonal complex

CPS capsular polysaccharide

DLV double locus variant

EOD early onset disease

GA gestational age

GBS group B streptococcus

IAP intrapartum antibiotic prophylaxis

LBW low birth weight

LOD late onset disease

MLST multilocus sequence typing

PCR polymerase chain reaction

PFGE pulsed field gel-electrophoresis

pPROM premature rupture of amniotic membranes

PROM prolonged rupture of amniotic membranes

QALY quality adjusted life year

SLV single locus variant

ST sequence type

List of papers

I Bergseng H, Rygg M, Bevanger L, Bergh K. Invasive Group B streptococcus (GBS) disease in Norway 1996-2006. Eur J Clin Microbiol Infect Dis Dec;27(12):1193-9. (Epub 2008 Jun 17)

II Bergseng H, Afset JE, Radtke A, Loeseth K, Valsoe Lyng R, Rygg M, Bergh K. Molecular and phenotypic characterisation of invasive group B streptococcus (GBS) strains from infants in Norway 2006 – 2007. Manuscript accepted Clin Microbiol Infect 30 October 2008

III Bergseng H, Bevanger L, Rygg M, Bergh K. Real-time PCR targeting the sip gene for detection of group B Streptococcus colonization in pregnant women at delivery. J Med Microbiol 2007; 56(Pt 2):223-228.

IV Bergseng H, Hallan S, Bevanger L, Bergh K, Rygg M. Impact of time on the feasibility of intrapartum PCR screening for Group B streptococci (GBS) Manuscript submitted

Background

Streptococcus agalactiae, Group B streptococcus or commonly GBS, became known as an agent infecting udders of cows and was therefore given the name *Streptococcus agalactiae contagiosae.* The name was later shortened to *Streptococcus agalactiae* (want of milk) [1]. GBS was first described as a cause of human infection in 1938, when three patients with fatal puerperal sepsis were described [2]. The bacteria remained unknown to most clinicians until the 1970s, when a dramatic increase of GBS septicaemia and meningitis in neonates was observed in different parts of the world [3]. GBS became the most prevalent agent of serious neonatal infections and was detected in more than 40% of invasive isolates from neonates [4]. In the same period, an increasing number of GBS infections was observed in pregnant women and non pregnant adults.

The clinical course of invasive GBS disease in infants is often dramatic, with high morbidity, and until the middle of 1980s the case fatality was more than 50%. Research on the epidemiology and pathogenesis of GBS was initiated, and preventive actions like antibiotic prophylaxis during labour to women at risk of having a child with GBS infection were introduced in USA and Europe in the 1990s. Two different strategies to identify women at risk were recommended; the risk-factor strategy and the screening strategy. Risk factors are prolonged rupture of membranes, premature birth, intrapartum fever, previous GBS infected infant or GBS bacteriuria detected during the current pregnancy, and GBS colonisation of the pregnant woman. In the USA, screening for GBS colonisation is at present recommended in week 35-37. However, rapid molecular methods like real-time PCR might replace the traditional culture screening, and make screening possible when the women are in labour.

Even if intrapartum antibiotics have reduced early onset GBS disease it will not be 100% effective and antibiotic prophylaxis during labour has no effect on late onset GBS disease. Vaccines based on GBS antigens have been developed, and maternal vaccination is expected to prevent GBS disease in neonates, but final trials and implementation still lie some years ahead. Variations of GBS characteristics have implications for the formulation of GBS vaccines. Thus, surveillance of GBS is of importance.

This study aimed to describe the epidemiology of GBS disease in Norway and characterise invasive GBS strains by phenotypic and genotypic methods. The performance of a molecular method for detection of GBS in pregnant women during labour, and a possible implementation of this or similar methods in intrapartum screening were studied.

Group B streptococci

Morphology and identification

Streptococcus agalactiae (Group B streptococcus; GBS) is the species designation for streptococci belonging to the Lancefield group B. GBS are facultative anaerobic gram positive cocci and form chains of variable length that grow on a variety of media. Colonies are 1-3 mm in diameter and greyish-white in colour when grown on sheep blood agar. The flat mucoid colonies are surrounded by a clear zone, caused by lysis of red blood cells in the agar medium, induced by bacterial haemolysins (β-haemolysis). Streptococci which generate β-haemolysis are also called β-*haemolytic streptococci.* 1-2 percent of the GBS strains are non-haemolytic [1].

GBS antigens

GBS has two distinct polysaccharide antigens; the group B specific antigen which is common to all strains, and the type specific capsular polysaccharides (CPS) which further divide GBS into serotypes. Other important antigens in GBS are the various strain variable or surface-anchored proteins.

Definitive identification of GBS requires detection of the group B specific antigen. Hyperimmune group B specific antisera or monoclonal antibodies have been developed to detect the GBS antigen. Latex agglutination is one of the most widely used methods.

Serotypes

Almost all clinical isolates of GBS carry a capsular polysaccharide (CPS) and can be classified into ten distinct serotypes or CPS types; Ia, Ib and II-IX [5;6]. The type specific polysaccharides are repeating units of five to seven monosaccharides (glucose, galactose, glucosamine, and N-acetylneuraminic acid, or sialic acid). The primary serologic method used for serotype determination was antigen extraction and precipitation reactions with adsorbed whole-cell antisera introduced by Lancefield in 1934 [7;8]. The fluorescent antibody test (FAT) described by Bevanger et al [9] is an alternative method of serotyping. A Latex agglutination test is also developed for serotyping of GBS [10]. Most GBS isolates can be classified into serotypes, but 4 to 7% do not react with hyperimmune sera to the characterised

capsular polysaccharides and are referred to as nontypeable [11]. Apart from technical reasons, nontypeability might be explained if the isolate is a nonencapsulated variant and if the isolate produces an uncharacterised polysaccharide for which antibodies not yet are available (a new serotype). Nontypeability can also be explained if the isolate has an insertion or a mutation in genes that are essential for capsule expression [12-14].

Recently, molecular typing of GBS based on detection of serotype-specific gene clusters of the capsular region has been developed [15]. In some studies, and also in routine characterisation of GBS, such molecular typing has now become more common [15;16].

Surface proteins

Most GBS strains express a variety of surface proteins. Many of the surface protein antigens induce protective immunity in animal models and are potential vaccine candidates [17]. The first surface protein identified in GBS was the c antigen [18]. The c antigen is composed of the trypsin resistant α-protein and the trypsin sensitive beta protein [19]. A GBS strain may express α c protein, β c protein, or both. The major surface-localised proteins include α c protein and β c protein, the R proteins R1, R3 and R4 (the last of these has been shown to be identical to Rib) [20;21], and the alpha like proteins Alp2 and Alp3 which may be variants of the R1 protein [22]. The epsilon (ε) protein has also been called Alp1. The alpha c protein, Rib, Alp2, Alp3 and the epsilon/Alp1 protein are, unlike the β c protein, characterised by similarity in primary structure, with up to 100% homology for some of the protein stretches [23], and by their generation of ladder-like patterns on Western blots. The patterns are probably due to large and identical repeat units which vary in number from strain to strain [23-25]. The proteins are encoded by stable mosaic genes, generated by a recombination of modules at the same chromosomal locus [26;27]. The *bca*, *ε/alp1*, *bac*, *rib*, *alp2* and *alp3* genes encode α c protein, epsilon/Alp1, β c protein, Rib, Alp2 and Alp3, respectively.

Subtypes

Subtyping is a more detailed categorisation of GBS, where GBS categories are based on the combination of capsular polysaccharide (CPS) and surface proteins, called serovariants. For instance, serotype III can be subdivided into the serovariants III/R4 or III/ c-αβ, type Ia into the variants Ia/c-α and Ia/c-αβ**.**

Table 1 shows the nomenclature of GBS c and R proteins and the most common capsular serotypes associated with the protein.

Table 1 Nomenclature and some characteristics of GBS c and R proteins (L.Bevanger, personal communication)

Designation	Alternate designation	Gene	Most common capsular serotype associated
c proteins			
beta		*bac*	Ib
alpha		*bca*	Ib
alpha, epsilon variant	Alp1	*epsilon/Alp1*	Ia
R proteins			
R1	Alp2*	*alp2*	III
R1	Alp3*, R28	*alp3*	V, VIII
R3		?	V
R4	Rib	*rib; r4*	III
R5	BPS	*sar5*	found in strain Compton 25/60

* Alp2 and Alp3 are considered variants of classical R1 protein

Genome sequence of GBS

The GBS genome consists of a circular chromosome. The complete genome sequences have been obtained for NEM316 [28], 2603V/R [29] and A909 [30] belonging to serotypes III, V and Ia respectively. Sequence analyses have revealed the composite organization with a stable backbone and 11–14 interspersed islands which are associated with virulence genes. Comparison of the genome sequences has defined a core genome of about 1800 genes shared by all isolates, accounting for ~80% of any single genome, plus a dispensable genome consisting of partially shared and strain-specific genes [30].

Epidemiological typing

Serologic or molecular typing of CPS and surface proteins distinguish between isolates of a microbial population, but may be insufficient in detecting virulent clones in a local outbreak and are also inadequate for evolutionary studies. Molecular typing methods may identify clones or closely related strains recovered from an outbreak, describe the population in an area and show the relatedness of isolates from different geographic areas to common ancestors. Several methods of molecular typing have been developed; the two most widely used methods in epidemiological studies of GBS at present are pulsed-field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST).

PFGE

The method may detect genetic variation that accumulates relatively rapid. Random genetic events, including point mutations, insertions and deletions of DNA may alter the PFGE pattern during an outbreak. With this method, genomic DNA of the bacteria is cut into large fragments by using infrequent-cutting restriction enzymes. Unlike small DNA fragments that can be resolved by conventional electrophoresis, these large fragments need to be separated in special electric fields with a pulsed current. Alternating electric fields run the DNA fragments through a gel matrix of agarose and organise the fragments in the gel according to their molecular size [31;32]. The PFGE profiles may be analysed by visual assessment of bands. Similarity of band patterns of different strains may also be assessed by similarity coefficients (Dice, Jaccard), which can be used for construction of dendrograms by methods like Unweighted Pair Group Method with Mathematical Averages (UWPMA or UPGMA) (Figure 1). The dendrogram provides a quantitative assessment of strain similarity [33]. PFGE is a frequently used method for studies of outbreaks and local epidemiology of different bacteria including GBS, but is considered to be too discriminatory for studies of evolutionary relationships in isolates. Another drawback of PFGE is that gel-based analyses are not easily comparable from one laboratory to another (low portability) [34-36].

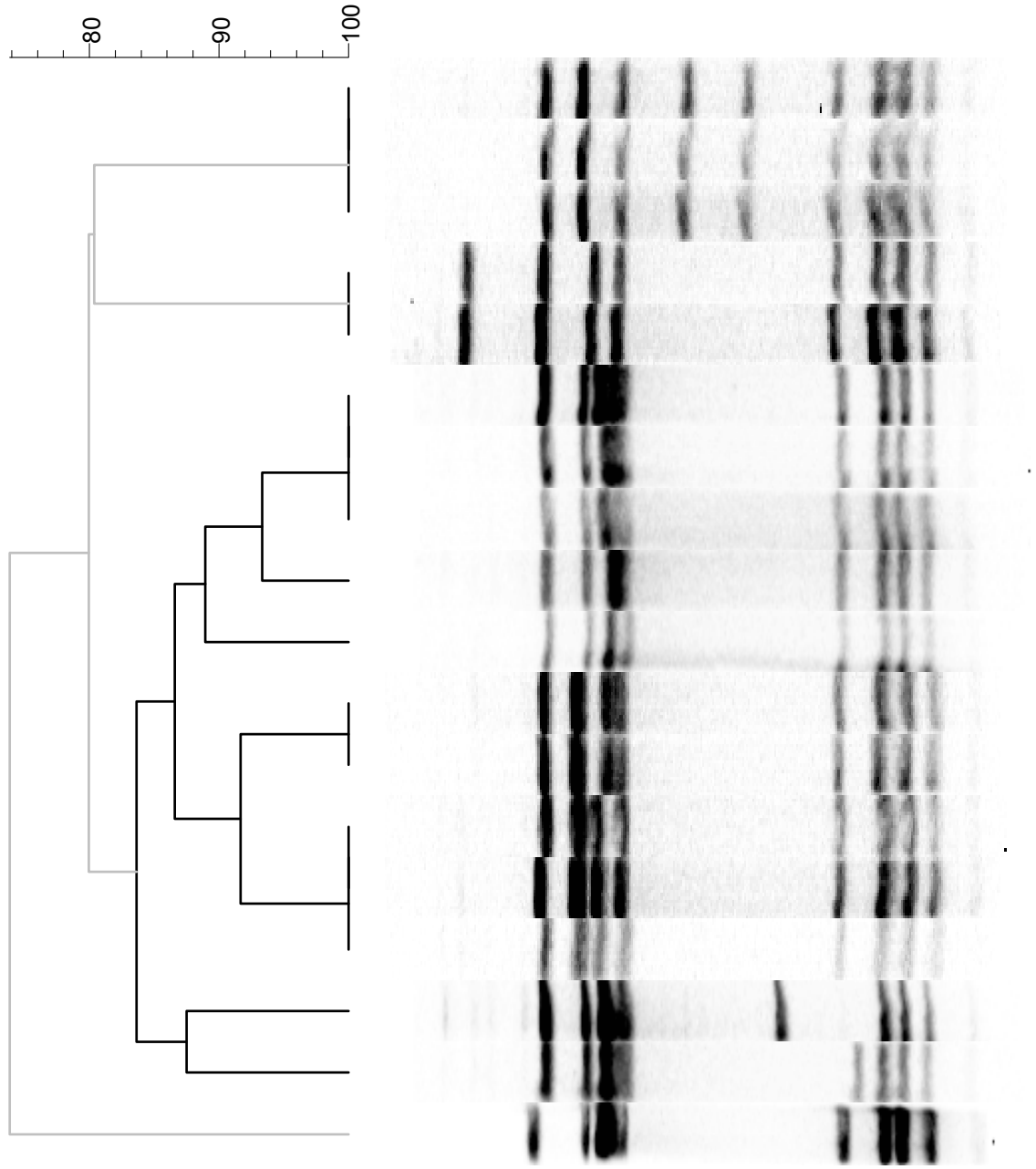

Figure1 Image of Pulsed-field gel electrophoresis (PFGE) of type V strains from 18 infants with invasive GBS disease in Norway 2006 and 2007 (Figure from Paper II). The dendrogram on the left side of the figure was constructed with the unweighted pair-group method with arithmetic mean (UPGMA) using Bionumerics (version 5.10, Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis of PFGE fragment patterns was done using the Dice coefficients.

MLST

Data from MLST can be used to investigate evolutionary relationships among bacteria. While PFGE is a gel-based analysis and dependent on visual interpretation, MLST is a more unambiguous genotyping method and sequence data are transferable between laboratories [31]. The method provides the sequences of 450-500 base pairs from fragments of seven "housekeeping genes" encoding central metabolic enzymes in the organism [37;38]. For each housekeeping gene, the nucleotide sequences may vary. The variants of the genes are assigned as alleles, and the combination of alleles at each of the seven loci represent an allelic profile which unambiguously defines the sequence type (ST) of each isolate. In MLST the number of nucleotide differences between alleles is ignored, and sequences are given different allele numbers whether they differ at a single nucleotide site or at many sites. The rationale for this is that a single genetic event resulting in a new allele can occur by a point mutation (altering only a single nucleotide site), or by a recombinational replacement that will often change multiple sites. Weighting according to the number of nucleotide differences between alleles would imply that the latter allele was more distantly related to the original allele than the former, which would be true if all nucleotide changes occurred by mutation, but not if the changes occurred by a recombinational replacement [38;39].

Nucleotide sequences of alleles and sequence types (STs) are available at http://mlst.net, and as of October 2008, 408 unique STs are registered in the GBS database (http://sagalactiae.mlst.net).

Analysis of MLST data

There are several approaches that can be employed in analysing the genetic relationship between isolates analysed by MLST, two commonly used are:

- Approaches that determine relationships on the basis of allele designations and STs (e.g. eBURST, based upon related sequence types)
- Approaches that analyse nucleotide sequences directly (e.g. Neighbour joining method).

Distance methods

UPGMA and Neighbour Joining methods are methods for the construction of dendrograms (phylogenetic trees) by converting aligned sequences into a distance matrix of pairwise differences (distances) between the sequences. UPGMA constructs clusters by assuming that all sequences are equally distant from a root, which is unlikely. The Neighbour Joining method is based on a distance matrix similar to UPGMA, but it calculates distances directly to internal nodes [40]. Figure 2 shows a phylogenetic tree constructed by the Neighbour Joining method. The different STs at the ends of the branches are called external nodes. The lengths of the branches between the nodes illustrate the differences in nucleotide sequences between the STs. Thus, the tree illustrates phylogenetic relationships between the sequence types.

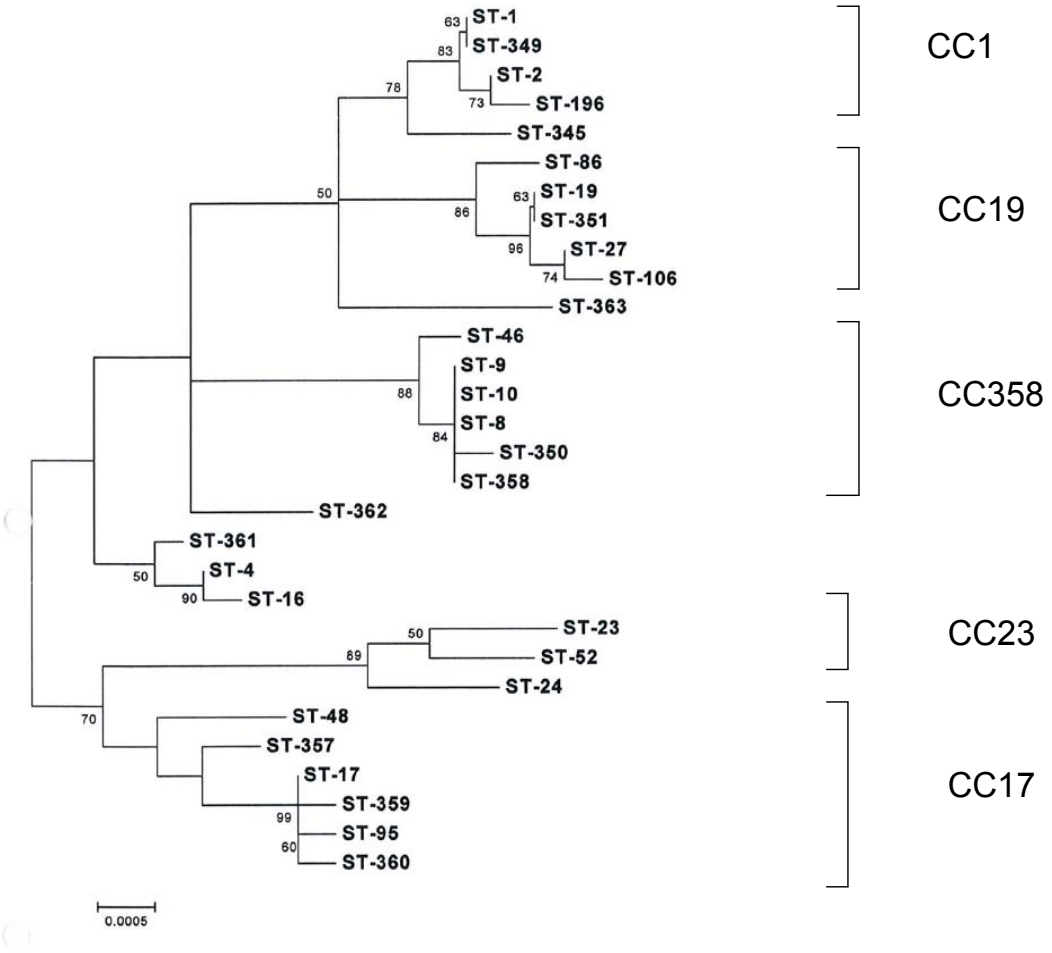

Figure 2 A phylogenetic tree (Neighbour Joining) of STs with corresponding clonal complexes (CC) (see Figure 3) constructed from MLST analyses of invasive GBS from infants in Norway 2006 and 2007 (Data from Paper II).

eBURST

eBURST analysis focuses on identifying groups of closely related isolates (clonal complex, CC) within a bacterial population which is assumed to share a recent common ancestor, and on exploring how these may have emerged and diversified [41]. eBURST divides a MLST data set of any size into groups of related isolates and clonal complexes, predicts the founding (ancestral) genotype of each clonal complex, and computes the bootstrap support for the assignment [41]. (Bootstrap is a way of estimating the reliability of the model by finding the sampling distribution, at least approximately, from just one sample) [40]. The most parsimonious patterns of descent of all isolates in each clonal complex from the predicted founder(s) are then displayed geographically (Figure 3).

Isolates that share identical alleles at six of seven loci, and only differ from each other at one allele, are designated single-locus variants (SLVs). Isolates with five out of seven shared alleles are called double-locus variants (DLVs). The diagrams in Figure 3 represent clonal complexes (CCs). CCs are composed of a "founder" which is defined as the ST that has the greatest number SLVs. If two STs have the same number of associated SLVs, the one with the greatest number of DLVs is selected as the founding ST.

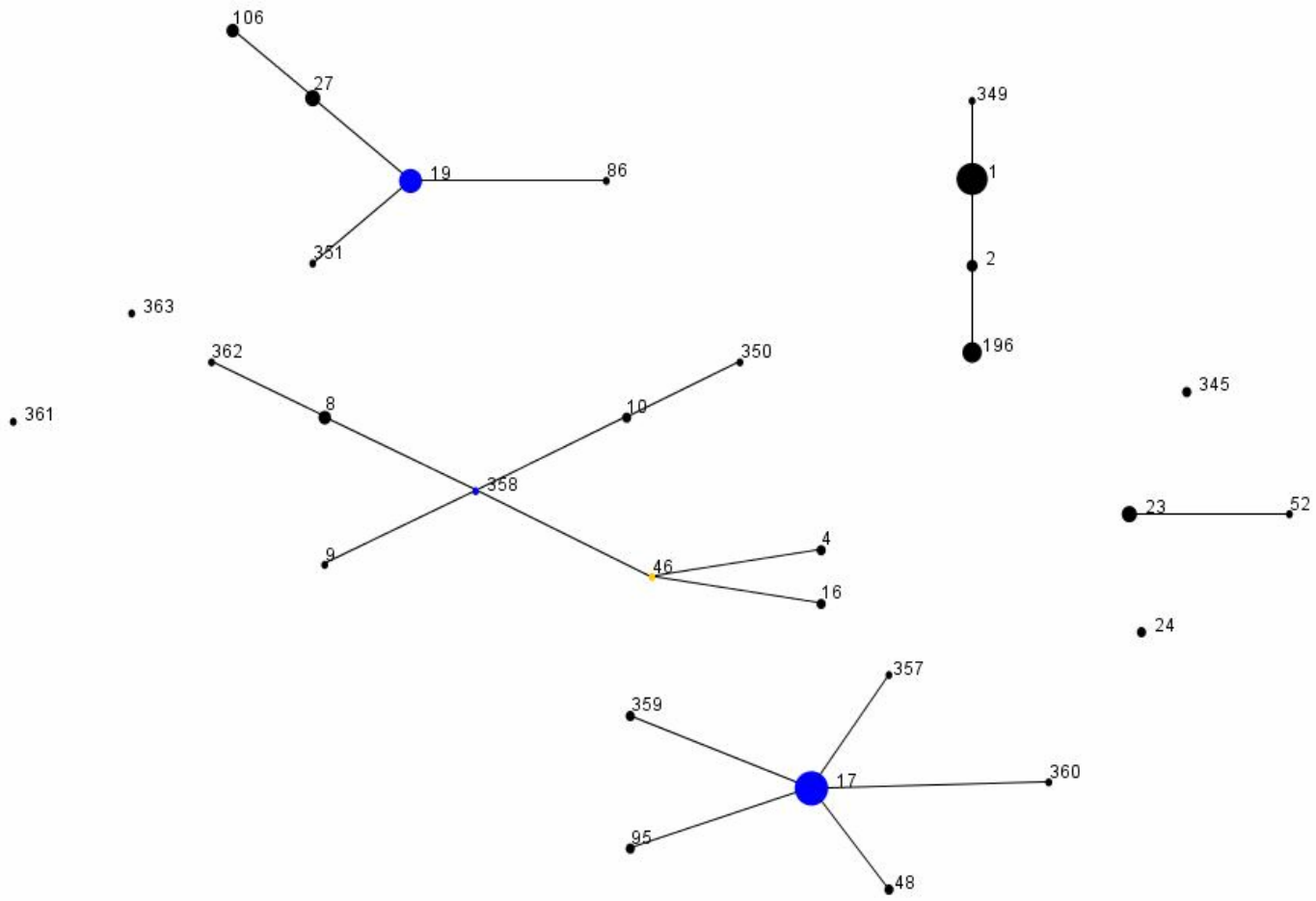

Figure 3 eBURST diagrams of groups of closely related isolates (clonal complexes, CCs) of invasive GBS strains from infants in Norway. The blue circles represent the founders or ancestral types which have given rise to descendent sequence type strains (black and yellow circles). The STs that are directly connected to the founders differ from the founder in only one single locus or allele and are called single locus variants (SLVs). STs that do not cluster into a clonal complex are called singleton STs (e.g. 361, 363, 24, and 345). The diameter of the founder circles is just illustrating the number of strains with that particular ST in our sample (Data from Paper II).

Invasive neonatal infections

Definition of neonatal sepsis

Neonatal sepsis refers traditionally to sepsis in newborn babies during the first month of life. However, increased survival of immature and premature babies has resulted in a large group of infants with a high susceptibility to infections for a long time after birth, and the inclusion period for neonatal sepsis and meningitis often covers the whole hospital period [42].
Neonatal sepsis may be classified according to the time of onset of the disease; early onset disease (EOD) and late onset disease (LOD). This distinction has clinical relevance as EOD is mainly due to bacteria acquired before and during delivery, and LOD to bacteria acquired after delivery (from nosocomial or community sources). Unfortunately, there is no consensus as to what age limits apply, making it difficult to compare studies where cases are grouped into EOD and LOD without further details. In most literature on GBS, EOD is 0-6 days and LOD 7-90 days after birth.

Sepsis, SIRS

The terms “sepsis” or “septicaemia” are traditionally used for isolation of bacteria in blood in combination with clinical symptoms. The term SIRS (systemic inflammatory response syndrome) was originally proposed to describe the non-specific inflammatory process occurring in adults after trauma, infection, burns, pancreatitis and other diseases. The criteria for use in adults have later been modified for use in children and infants, and include a core temperature >38.5 ºC or <36 ºC, tachycardia, increased respiratory rate and an elevated or depressed leukocyte count [43]. Sepsis may be defined as SIRS in the presence of or as a result of suspected or proven infection. Severe sepsis is defined as sepsis plus one of the following; cardiovascular organ dysfunction or acute respiratory distress syndrome or two or more other organ dysfunctions. Septic shock is defined as sepsis and cardiovascular organ dysfunction [43].

Impact of neonatal sepsis

In developing countries the neonatal mortality rate ranges from 17 to 68 per 1000 live births in the first 28 days, and one third of these deaths are caused by infections [44]. Sepsis and

meningitis are responsible for most of these deaths. In developed countries, neonatal mortality is 2-5/1000 live births and 10% is caused by infection [45]. According to WHO, there was an estimated number of neonatal deaths, caused by infection, of 382000 (3.4% of all deaths) in Africa in 2004. In Europe the estimated number was 27000 (0.3% of all deaths). However, it is generally assumed that neonatal mortality in developing countries is under-reported by at least 20% (WHO 1996).

Neonatal infections represent a threat to neonatal health and take large health resources even in developed countries. In Norway, approximately five percent of neonates are transferred from the maternity wards to neonatal intensive care units for observation, diagnostics and treatment of possible infections (H.Døllner, personal communication).

Aetiology and predominant pathogens of neonatal sepsis

In developing countries, it appears to be a wide variety of bacteria causing EOD and LOD. In most studies, Gram-negative organisms are predominant. Among Gram-negative organisms *Klebsiella spp., Escherichia coli, Pseudomonas spp.* and *Salmonella spp.* are the most reported. Among Gram-positive bacteria *Staphylococcus aureus,* Coagulase negative staphylococci (CoNS)*, Streptococcus pneumonia and Streptococcus pyogenes* are the most reported species [44]. This variation may be true, but important confounders may include different definitions of EOD and LOD, different inclusion criteria for studies (including population sampled), inability to culture certain organisms, small numbers, and/or short periods of surveillance. The latter may be particularly important, as surveillance may be occurring during, or indeed may have been initiated because of, an outbreak of a specific pathogen and may not therefore be representative.

Organisms responsible for neonatal infections in developed countries have changed the last decades (Table 2). While *S. pyogenes* and *S. pneumoniae* constituted half of the cases at Yale from 1933 to 1943, no cases caused by these bacteria were detected in the period 1989-2003 [46]. Following the introduction of sulfonamides and penicillin, Gram-negative bacteria, and in particular *E. coli,* became predominant in neonatal infections. From the 1970s GBS emerged as the predominant microbe, and especially the first 24 hours after birth [4;46-49] (Figure 4). In the last twenty years Gram-positive organisms have dominated both EOD and LOD in term infants, while *E. coli* have been more common in premature infants [48-51].

Some recent studies have shown a declining incidence of EOD in infants born after 37 weeks gestational age and also a declining incidence of invasive GBS disease [4;52]. Infections caused by CoNS have increased, and especially in LOD.

The decline of EOD may be related to improved perinatal care of infants at risk and increased use of antibiotic prophylaxis to pregnant women and in neonates. On the other side, the improved neonatal care has also led to increased survival of immunocompromised immature and premature neonates susceptible to late onset infections like CoNS. CoNS are considered as opportunistic pathogens with increased virulence in immunocompromised patients, and they are also associated with the use of central venous catheters for patients with severe underlying conditions [48].

Table 2 Different studies on organisms isolated from blood cultures of neonates with sepsis

Period	Predominant pathogens	references
1928-1958	*S. aureus, E. coli, S. pneumoniae, S.pyogenes*	[46]
1959-1965	*E. coli, Klebsiella spp., Pseudomonas spp.*	[46]
1966-1988	GBS, *E. coli*	[46]
1989-1995	CoNS*, GBS, *E. coli, Enterococcus spp., S. aureus*	[46;53]
1996-2006	CoNS*, GBS, *E. coli,* viridans *streptococci*, *Enterococcus spp., S. aureus*	[4;46;48]

*Coagulase-negative staphylococcus

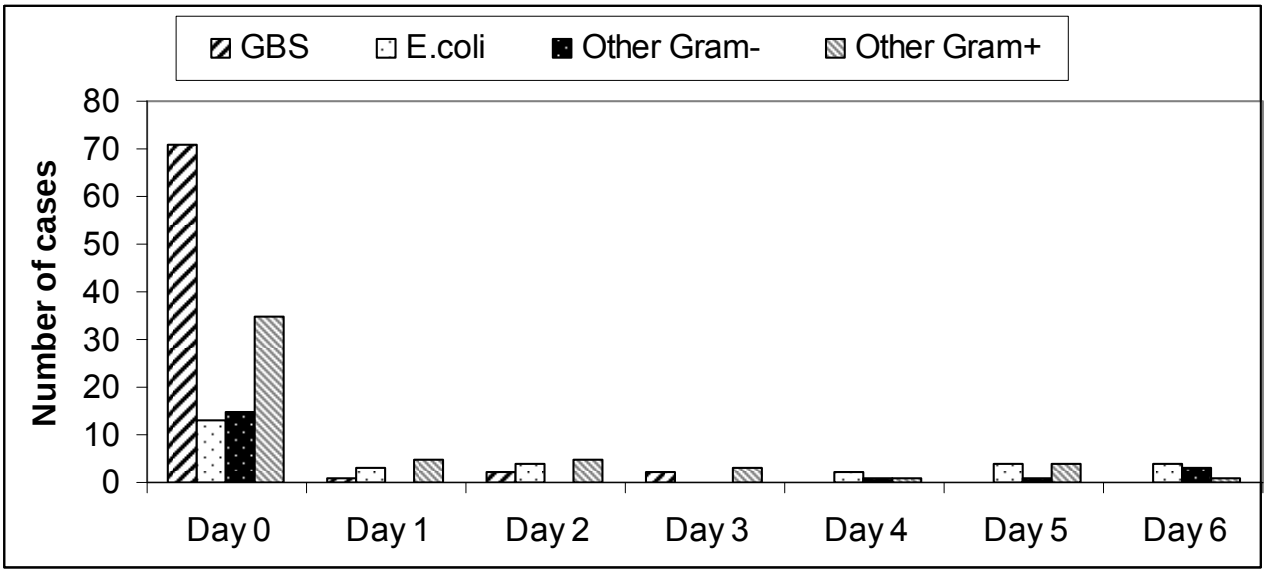

Figure 4 Cases of early-onset disease by pathogen and day of onset. (Results from a multicenter surveillance in USA during 1995 to 1996 for culture-confirmed, early-onset sepsis in an aggregate of 52 406 births; matched case-control study of risk factors for GBS and other sepsis) [54].

Invasive GBS disease in infants

Early onset GBS disease (EOD)

EOD (age at onset 0-6 days) almost always manifests itself within 24 hours of birth (median age 8 hours in 90% of cases, 5% appears during 24-48 hours) [55]. In premature infants, onset of symptoms is often within 6 hours of birth. GBS colonisation in pregnant women is the single most important risk factor for early onset newborn disease due to vertical transmission and colonisation of the infant during delivery.

The most common manifestations of EOD are septicaemia, pneumonia and meningitis. Irrespective of site of involvement, respiratory signs (apnoea, grunting respirations, tachypnea or cyanosis) are the clinical findings in more than 80% of neonates, and they can be difficult to oxygenate [55;56]. A differential diagnosis of GBS sepsis is RDS (Respiratory distress syndrome). Also radiographically, features consistent with and indistinguishable from those of hyaline membrane disease are present in more than one half of neonates with GBS and pulmonary infection. Treatment with surfactant improves gas exchange in a majority of these infants, although the response is slower than in non-infected infants [57].

Other associated signs include lethargy, poor feeding, hypothermia or fever, abdominal distension, pallor, tachycardia and jaundice. Hypotension is an initial finding in approximately 25%. Infant with foetal asphyxia related to GBS infection in uteri may have shock and respiratory failure at delivery. Meningitis is seen in 5-10% of neonates with EOD, most of them present with the same symptoms as those without meningitis.

Late onset disease (LOD)

LOD affects the infant from 7 days to 90 days of age. Nosocomial infection of premature infants in neonatal intensive care units (NICU) and transmission of virulent GBS strains from mother to infant via skin or breast milk might explain some of the cases. However, most infants with LOD have no known risk factors and an uneventful early neonatal history, and in most of these infants the mechanisms of infection are not revealed [55].

LOD often presents with hypothermia or hyperthermia, hyperglycaemia or irritability. Grunting respiration and apnoea are less frequent initial findings than in EOD [46].

Meningitis is a frequent clinical manifestation, occurring in estimatedly 35-50% of cases [55;58].

Antimicrobial therapy of GBS disease in neonates

Most invasive GBS strains have been, and still are susceptible to penicillin G [59;60]. Most strains are also susceptible to ampicillin, semisynthetic penicillins, vancomycin, linezolid, trimetoprim sulfametoxazol and first, second and third generation cephalosporins [60]. Resistance to erythromycin and clindamycin has increased during the last decades [61]. In several studies, most GBS strains show resistance to tetracyclines, metronidazole and aminoglycosides [55;60]. However, if aminoglycosides are combined with penicillin, an in vitro and also an in vivo synergy effect, often is observed [62]. Despite their uniform susceptibility to penicillin G, GBS require higher concentrations for growth inhibition in vitro than are required for strains belonging to group A streptococci [63]. Although some studies indicate that 6-7 days therapy might be sufficient for uncomplicated bacteraemia [64], recommended duration of treatment of GBS infections has been 10-14 days for bacteraemia without focus or with soft tissue infection, 2 to 3 weeks for meningitis or bacterial arthritis and 3 to 4 weeks for osteomyelitis.

Epidemiology of invasive GBS disease

Most reports on the incidence of invasive GBS disease in infants are based on blood or cerebrospinal fluid culture proven cases. Reports may be active laboratory based surveillance in certain areas during months or years, nationwide prospective studies or continuous surveillance of GBS disease where laboratories and or clinicians report to central registers. Retrospective search in local databases and registers of clinical diagnoses from hospitals (ICD-9, ICD-10) have also been published as well as questionnaires to clinicians and laboratories.

Incidence of early onset disease (EOD)

In most reports, EOD constitutes 60-80% of total invasive GBS disease in infants. Published data from USA and Australia from the late 1970s to the early 1990s show incidences of EOD of 1-3/1000 live births (Table 3). After 1996 the incidence seemed to drop, and after 1998-2000 the average incidence has been around 0.5/1000 live births. The incidence of invasive GBS disease among newborns in USA has been higher in infants of African-American women than in infants of white and Hispanic women [65]. The reported incidences of EOD in Scandinavia were mainly lower than in USA and Australia until the late 1990s. After the middle of 1990s the incidences dropped in both USA and Australia and have been similar or lower than in Scandinavia and Europe after 2000 (Table 3 and Figure 5). In Europe outside Scandinavia, most reports are from late 1990s and after 2000 and the incidences of EOD are similar to what have been reported from Scandinavia [16;53;66-69].

Table 3 Reported incidences of early onset GBS disease in neonates (cases per 1000 live births per year) in different geographic areas in the period from 1975 to 2007

Year	1975 - 1980 - 1985 - 1989 - 1991 - 1993 - 1995 - 2000 - 2007	References
USA	1.09 1.5 - 3.21 1.4 -1.7 1.3 -1.95 0.23 - 0.72 0.33 - 0.47	[56;65;70-76]
Australia	1.45 0.84 0.3	[77;78]
Sweden	0.1-0.5-1.24 0.78 0.40	[53;67;79;80]
UK	0,48	[68]
Norway	0.2 0.37 0.54 0.46	[16;81]
New Zealand	0.5	[82]
The Netherlands	0.9 0.43	[58;83]
Canada	0,9	[84]
Germany	0,28	[66]
Finland	0.63 0.65	[69;85]
S Africa	2.06	[86]
Denmark	1.5 0.73	[87]

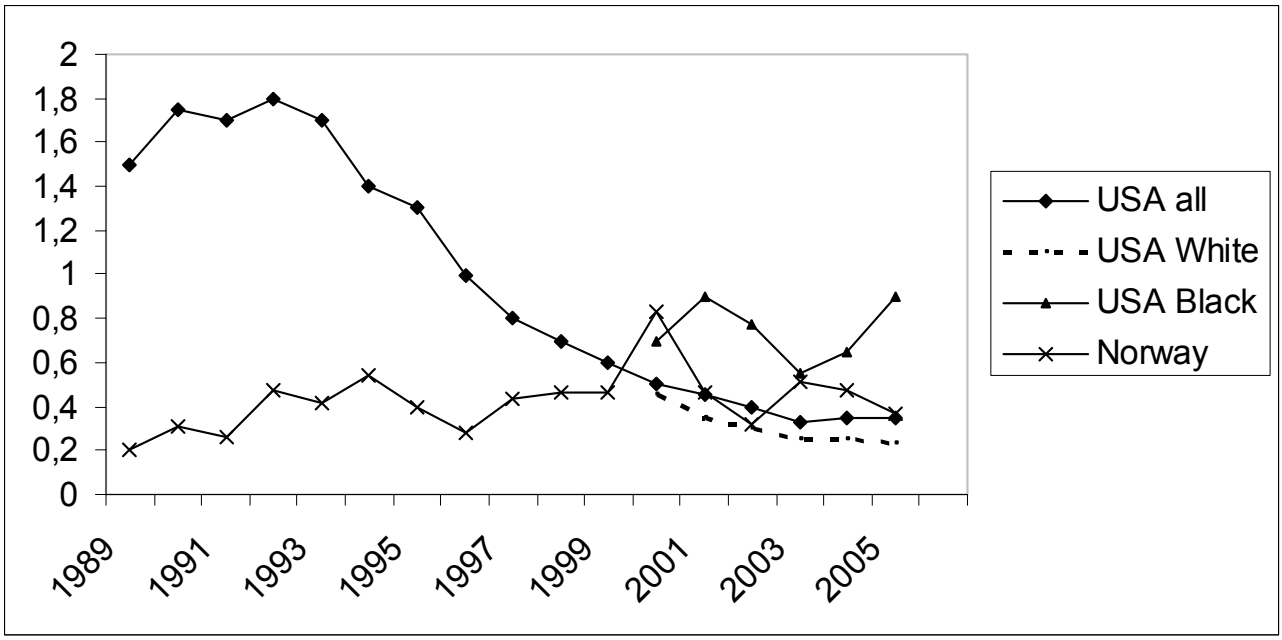

Figure 5 Incidence (cases per 1000 live births) of early onset disease in USA and Norway by year, and from 2000 also by race in USA [16;65;72;81].

Incidence of late onset disease

Even if incidence of EOD has changed through the last decades, the incidence of LOD has been quite stable. A common interpretation of this is that the different strategies to prevent GBS disease in infants have affected EOD only.

GBS meningitis

Two studies from England and Wales in the periods 1985-87 and 1996-97 showed an overall incidence of neonatal meningitis of 0.22 cases per 1000 live births. GBS was the leading pathogen responsible for meningitis (39% of cases in 1985-87 and 48% in 1996-97) followed by *E. coli* [88]. No significant change of incidence was observed between the two periods. Data from these studies were consistent with other published studies from the same period [89]. Later, a study from Australia suggested a decrease of neonatal meningitis in general and especially GBS meningitis, in the period from 1992 to 2002 [90].

Case fatality of invasive GBS disease in infants

The case fatality rate of invasive GBS disease was almost 50% in 1960s and early 1970s (Figure). After 1990 the rate has stabilised and has been reported from 4% to 7.5% [16;58;66-69;72;91-94]. The case fatality is reported to be significantly higher in preterm infants [91], while LOD has a lower fatality rate (2-6%) than EOD.

There are few studies on case fatality in developing countries, but a study from South Africa reported an incidence of EOD and LOD of 2.06 and 1 per 1000 live births, while the case fatality was 19.8% and 13.6% for infants with EOD and LOD, respectively [86].

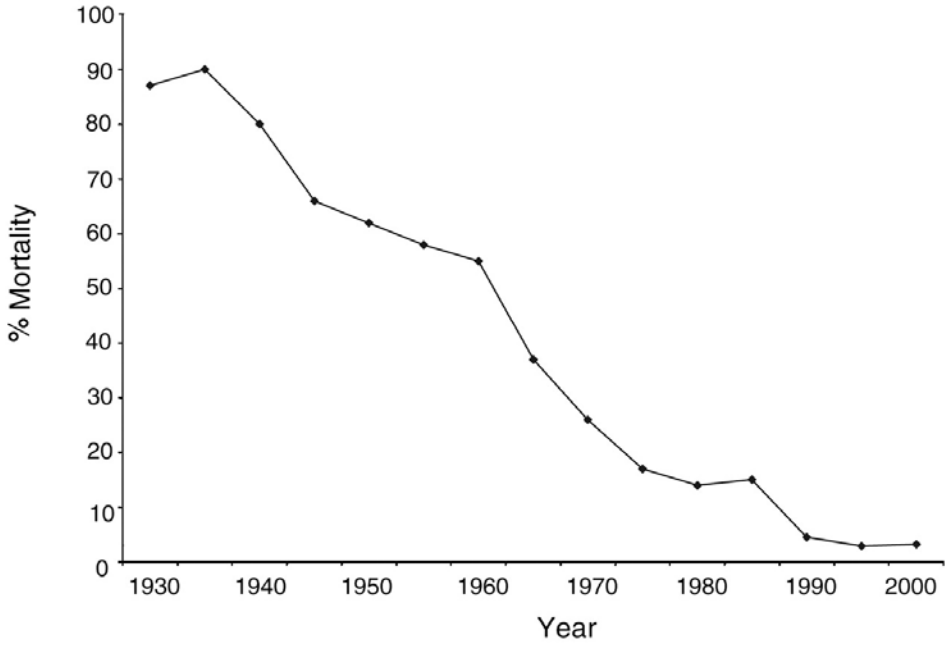

Figure 6 Case fatality rate of invasive GBS disease in infants reported from Yale University School of Medicine, New Haven, USA [46] (Figure reprinted with permission)

Morbidity and sequelae of invasive GBS disease in infants

There are only few studies on long term disabilities of invasive GBS disease. Mild disability has been defined as having a condition which is prevalent among children of the same age, but not typically associated with meningitis (middle-ear disease, squint, febrile convulsions, behavioural problems). Moderate disability impairs functioning, but is not severe. The child can attend mainstream school with or without additional support. Moderate disability includes mild neuromotor disability, intellectual impairment, and epilepsy controllable by treatment, and hydrocephalus without complications. Children with severe disability are unable to attend mainstream school and/or have severe motor impairment, significant intellectual impairment, severe seizure disorders, and severe visual or auditory impairment [95].

Reports from UK have shown 25% disability from GBS bacteraemia, with severe disability in 7.0%, moderate disability in 13.9% and mild disability in 4.5% of the infants [96]. In infants with neonatal meningitis due to GBS, disability in 50% has been reported. This included severe disability in 13.3%, moderate disability in 17.3% and mild disability in 18.4% of the infants [88;89;97]. The most commonly reported sequelae from meningitis have been cerebral palsy, blindness, deafness and hydrocephalus [89]. A retrospective study of 78

patients with EOD in the Netherlands found that the sequelae occurred mostly in patients with symptoms of GBS infection within six hours after birth. Early treatment of sick infants resulted in lower lethality, but not in less sequelae [98].

Pathogenesis of neonatal GBS infections and virulence factors of GBS

GBS can reach the foetus in uteri through ascending infection of the placental membranes and amniotic fluid. Alternatively, the newborn may become contaminated with the organism on passage through the birth canal. Invasive neonatal disease may be caused by both virulence factors in GBS and host factors.

The GBS virulence includes factors that obstruct immunological defence mechanisms and the ability to penetrate epithelial and endothelial cellular barriers to reach the bloodstream and deeper tissues. GBS produce toxins that directly injure or disrupt host tissue, and also produce factors that provoke inflammatory pathways which may aggravate the disease [99;100]. GBS colonisation of pregnant women and lack of maternal antibodies to GBS are also important factors contributing to invasive neonatal disease. (An outline of stages in molecular and cellular pathogenesis of neonatal GBS infection is shown in Figure 7).

Colonisation of the female genital tract

Maternal vaginal colonisation with GBS is essential for both early colonisation of the newborn infant and EOD. There is also a relationship between the degree of vaginal colonisation (the inoculum size) and risk of vertical transmission of GBS and risk of disease in the newborn [101]. The gastrointestinal tract is the primary reservoir of GBS, and vaginal colonisation probably represents dissemination from this source [65].

Adherence to human cells

To establish colonisation, GBS bind efficiently to human vaginal cells, with maximal adherence at the acidic pH characteristic of vaginal mucosa. The ability of adherence to other human cells like alveolar epithelium and endothelium and brain endothelium is probably also important for the pathogenesis of neonatal sepsis [102]. Molecules that appear to play an important role in adherence are the surface proteins, C5a peptidase (a bifunctional protein, which enzymatically cleaves C5a and mediates adherence to fibronectin) and laminin-binding

protein in the bacteria and in addition extra-cellular components as fibronectin in the host [99;100].

Penetration of host cellular barriers

GBS can traverse and penetrate intact placental membranes, weaken their tensile strength and promote rupture and premature delivery by several mechanisms [103]. The bacteria proliferate easily in the uterine cavity and a large inoculum can therefore be swallowed by the foetus and delivered to the foetal lung. GBS spreads from the initial pulmonary focus to the bloodstream and is circulated through other organs and tissues. An important factor in the cellular damage is β-haemolysin/cytolysin. The cytolytic, proinvasive and proinflammatory effects of GBS are partly neutralized by dipalmotyl phosphatidylcholine (DPPC), the major phospholipids constituent of human lung surfactant [104]. This may in part explain the elevated risk of premature surfactant-deficient neonates to suffer severe GBS lung injury and invasive disease. Cellular invasion is shown to correlate with the virulence potential of GBS strains. Clinical isolates of GBS from infants with invasive GBS disease invade epithelial cells better than strains from the vaginal mucosa of asymptomatic women [105].

Direct cytotoxicity to host phagocytes and inactivation of complement

The *cylE* –encoded β-hemolysin/cytolysin toxin, which is associated with the bacterial surface membrane [106], produces direct cytolytic injury to macrophages and induces macrophage apoptosis [107]. GBS also contribute to poor mobilisation of neutrophils by production of C5a peptidase, an enzyme that cleaves and inactivates human C5a, a complement component that is important in neutrophil chemotaxis [108].

Impairment of myocardial function

GBS directly impairs cardiomyocyte viability and function through β-hemolysin/cytolysin that possibly affects maintenance of normal calcium in intact cardiomyocytes and potentially leads to cell death. Experiments in rabbits have shown that infusion of GBS leads to lower cardiac output and decreased mean arterial pressure. This is caused by myocardial dysfunction rather than decreased vascular resistance [109].

Avoidance of the host's defence mechanisms

A number of virulence factors of GBS seek to prevent effective opsonophagocytic killing by the host. The most important virulence factor is the sialylated GBS polysaccharide capsule and surface proteins that can act in concert with capsular polysaccharide [110]. Serotype II strains displaying both components of the c protein antigen are more resistant to phagocytotic killing than are serotype II lacking c protein [111]. GBS have also been shown to penetrate and survive within several human cell types and especially within the phagolysosome of macrophages [112]. This capacity of cellular invasion may explain the ability of GBS to traverse placental membranes, the alveoli of the infant lung, and the neonatal blood brain barrier.

Host factors

Phagocytic cells including neutrophils and macrophages are central in the immunologic response to the penetration of GBS into lung tissue or bloodstream of the newborn infant. Effective uptake and killing by these cells require opsonisation of the bacterium by specific antibodies in the presence of complement [110]. Neonates are particularly susceptible to invasive disease due to quantitative and qualitative deficiency in phagocytic cell function, specific antibodies, or the classic and alternate complement pathways. A significant transplacentally transfer of maternal antibodies does not begin until the third trimester of pregnancy, 60 % of maternally derived IgG is transported to the foetus during the last 10 weeks of pregnancy [55]. Thus preterm infants are not protected by sufficient amounts of specific antibodies. Pregnant women without specific antibodies against GBS will also have babies who are more susceptible to GBS infections even if most infants born to women without specific antibodies remain healthy [55].

Activation of inflammatory responses

The clinical course of GBS sepsis and meningitis is dramatic with a high morbidity and mortality due to an excessive inflammatory response [55;113;114]. A powerful cytokine response and clinical signs of inflammation in neonates with GBS disease is in conflict with the picture of neonates as immunodeficient with impaired antimicrobial properties against GBS and tolerant to infective agents [114]. However, because the neonatal innate immune system is unable to eliminate GBS at the site of microbial invasion, a general inflammation with SIRS and sepsis is more likely to appear [113].

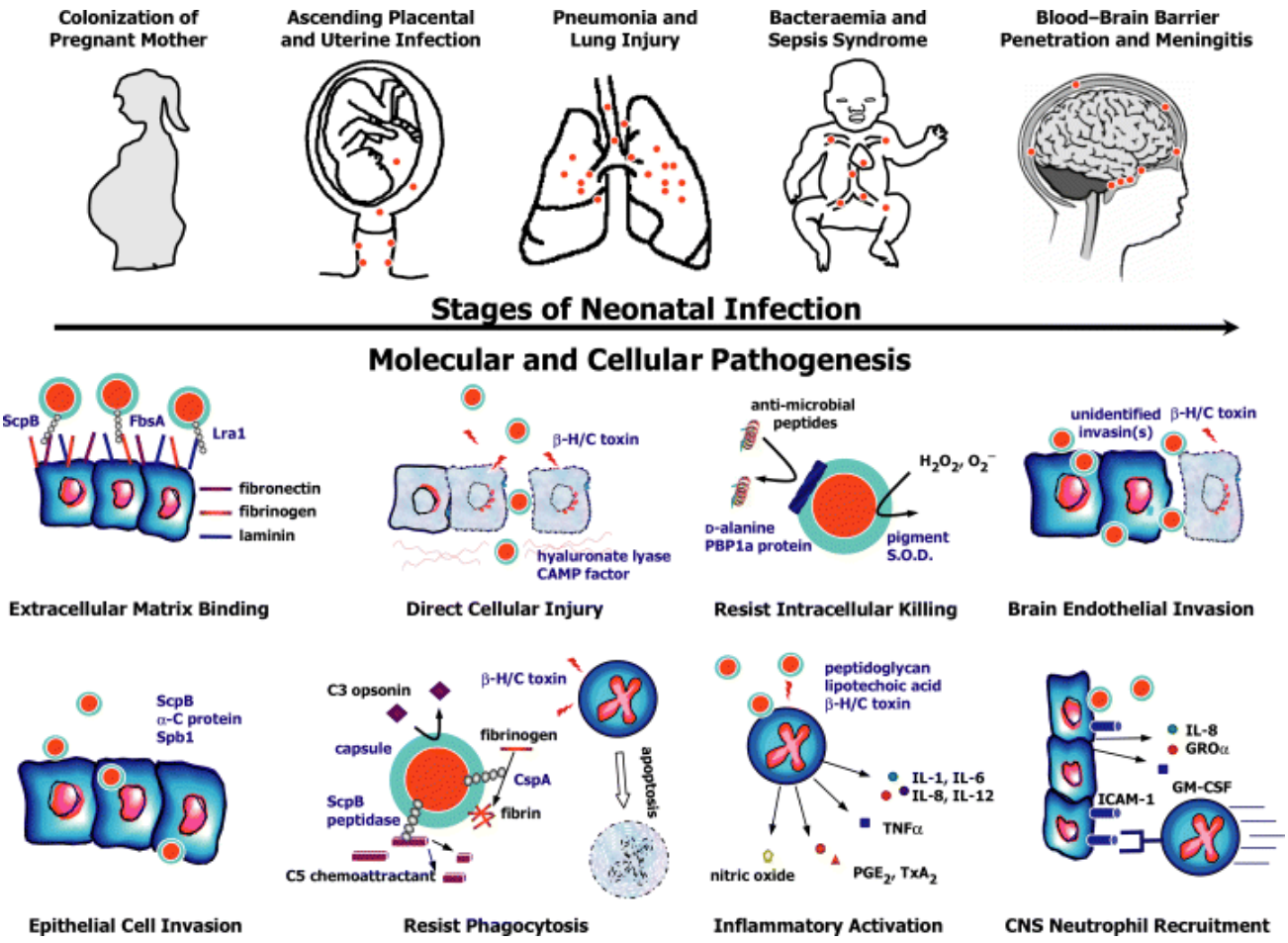

Figure 7 An outline of stages in the molecular and cellular pathogenesis of neonatal GBS infection [115] (Reprinted with permission).

(β-H/C: beta-haemolysin/cytolysin. S.O.D.: superoxide dismutase. IL: interleukin. TNFα, tumour necrosis factor-alpha. PGE_2: prostaglandin E_2. TxA2:thromboxane A_2. GROα: growth-related oncogene-alpha. ICAM-1:intercellular adhesion molecule 1. GM-CSF:granulocyte-macrophage colony-stimulating factor).

Risk factors for EOD

GBS colonisation

Maternal vaginal colonisation with GBS is essential for both early colonisation of the newborn infant and the risk of EOD. Women with heavy (dense) colonisation are at greater risk of having an infant with EOD [101;116-121]. Reported colonisation rates in pregnant women vary from 10-35% (Table 4).

Racial differences in colonisation rates have been observed. A study from The Netherlands where the participating women originated from 72 different countries showed that Asian women had a lower colonisation rate and African women a higher colonisation rate than European women [125]. This coincides with studies from USA which have shown a higher colonisation rate in African American women than in white and Hispanic women [130].

Table 4 Prevalence of maternal colonisation of GBS in different countries

Country	1996-2006	References
Sweden	25.4%	[67]
Norway	34.8%	[122]
Germany	16%	[123]
UK	21.3% 13.59%	[96;124]
The Netherlands	21%	[125]
Iceland	24.3%	[126]
Italy	11.3% 17.9%	[127;128]
USA	21%	[120]
New Zealand	20%	[129]
Zimbabwe	24%	R.Mawenyengwa (unpublished data)

Preterm and low birth weight (LBW) infants

Preterm and LBW infants have an increased risk of EOD with a progressive increase in risk for neonatal sepsis with decreasing gestational age (GA) and birth weight [101;121;131]. Hakansson et al showed that even infants born at 37 weeks GA had a three fold increased risk of EOD compared with infants born at 40 weeks [67].

Prolonged rupture of the amniotic membranes

Prolonged rupture of the amniotic membranes (PROM) for >18-24 hours before delivery increases the risk of neonatal GBS disease. Large published series indicate that PROM >18 hours occurs in 12.5% of deliveries and is associated with an OR of 7.28 (95% CI: 4.42-12.0) of invasive GBS disease [101;118;121;131;132].

Fever

Intrapartum temperatures >37.5ºC [132] and >38.0ºC [133] are associated with an increased risk of neonatal GBS infection [54;101;118;132]. It is not known whether the risk of EOD is higher with a temperature of 40.0ºC than with a temperature of 38.5ºC [101].

Chorioamnionitis

Intrapartum fever accompanied by two or more additional signs, including foetal tachycardia, uterine tenderness, foul-smelling vaginal discharge, or maternal leucocytosis, occurs in 1.0 % to 3.8 % of deliveries. These symptoms are mainly due to chorioamnionitis which is associated with neonatal GBS disease rates ranging from 6% to 20% [101;121;132;134].

GBS bacteruria

Infants born to women with GBS bacteruria during pregnancy are more frequently and more heavily colonised with GBS, and may be at increased risk for invasive GBS disease, however the different studies published are not conclusive [101;119;135].

Maternal antibodies

An infant's susceptibility to GBS is increased when the level of anticapsular antibodies to the infecting serotype is low. This is the case when the maternal antibody level is low and also when infants are born before 34 weeks gestation, since transplacental transport of immunoglobulin G is reduced early in gestation [136;137].

Previous infant with invasive GBS disease

Although having had a previous infant with invasive GBS disease is accepted as placing a mother at high risk in subsequent pregnancies, only a few instances have been reported in which neonatal GBS infection followed more than one pregnancy in the same mother [79;138]. However, women may remain colonised with the same strain of GBS for prolonged periods and may fail to develop protective levels of type-specific serum antibodies despite long-term colonisation [139]. It is therefore likely that the risk in subsequent pregnancies is higher for women who have had a child with EOD GBS disease, even if this risk cannot be quantified.

Race

Heavy colonisation with GBS has been identified more frequently in African American women than in white American women or Asian women. The incidence of EOD in African American infants is also higher. The higher colonisation rate may explain the higher risk of both early- and late-onset GBS disease among African Americans, but whether socioeconomic factors and differentiated health care also influence the risk of GBS disease is not known. In addition, findings of high colonisation rates in Scandinavian women may challenge the hypothesis of more GBS disease in populations with high colonisation rates [122;130;140].

Other risk factors

Gestational diabetes [67] and frequent vaginal exams [54], are reported to increase the risk of having a baby with EOD.

Prevention of neonatal GBS infections

Development of preventive strategies

The first attempt to prevent GBS disease in neonates was giving antepartum antibiotics to pregnant women colonised with GBS [141]. Oral and intramuscular regimens were tried, but were found to cause only a temporary drop in vaginal colonisation. It is believed that GBS remain in the colon and recolonise the birth canal once the antibiotics are stopped. In 1979 a report claimed that a single dose of ampicillin given to the mother intrapartum could interrupt the transmission of GBS from mother to baby [142]. Later, Boyer and Gotoff demonstrated a reduction in EOD if antibiotics were given intrapartum [143]. In early 1990s, guidelines for intrapartum antibiotics prophylaxis (IAP) were issued in North America. However, IAP use was not widely adopted and national standards for IAP administration were not implemented in USA until 1996, when CDC issued consensus guidelines recommending that health-care providers use either risk-based or culture-based screening to identify candidates for IAP. In 2002, a population-based study, demonstrated that routine screening of all pregnant women at 35-37 weeks' gestation and IAP for carriers prevented more cases of EOD in USA than the risk-based approach. This led to the universal prenatal screening recommendation in 2002 [65]. In Norway, guidelines for prevention of GBS were issued in 1998 and new guidelines in 2007/2008, recommending IAP to women with risk factors (www.legeforeningen.no).

Risk factor based strategy

In general, risk factor based strategies recommend IAP to women with prolonged rupture of membranes (>18-24 hours), gestation <37 weeks, intrapartum fever, previous GBS infected infant or GBS bacteriuria detected during the current pregnancy. However, this might be practiced differently in different countries. In Norway, IAP is recommended if GBS bacteriuria is detected during the current pregnancy, if the woman had a previous GBS infected infant, and if fever >38.0ºC. If GBS is detected by chance during the pregnancy, IAP is also indicated if labour starts before gestational week 37 and if prolonged rupture of membranes >18 hours. If there is premature rupture of membranes during pregnancy, culture and antibiotic treatment is recommended if GBS is detected (www.legeforeningen.no).

Screening strategy

In USA, rectovaginal specimens from pregnant women with gestational age 35-37 are cultured to detect GBS colonisation and IAP is recommended to colonised women. Figure 8 and 9 show more details of the screening strategy.

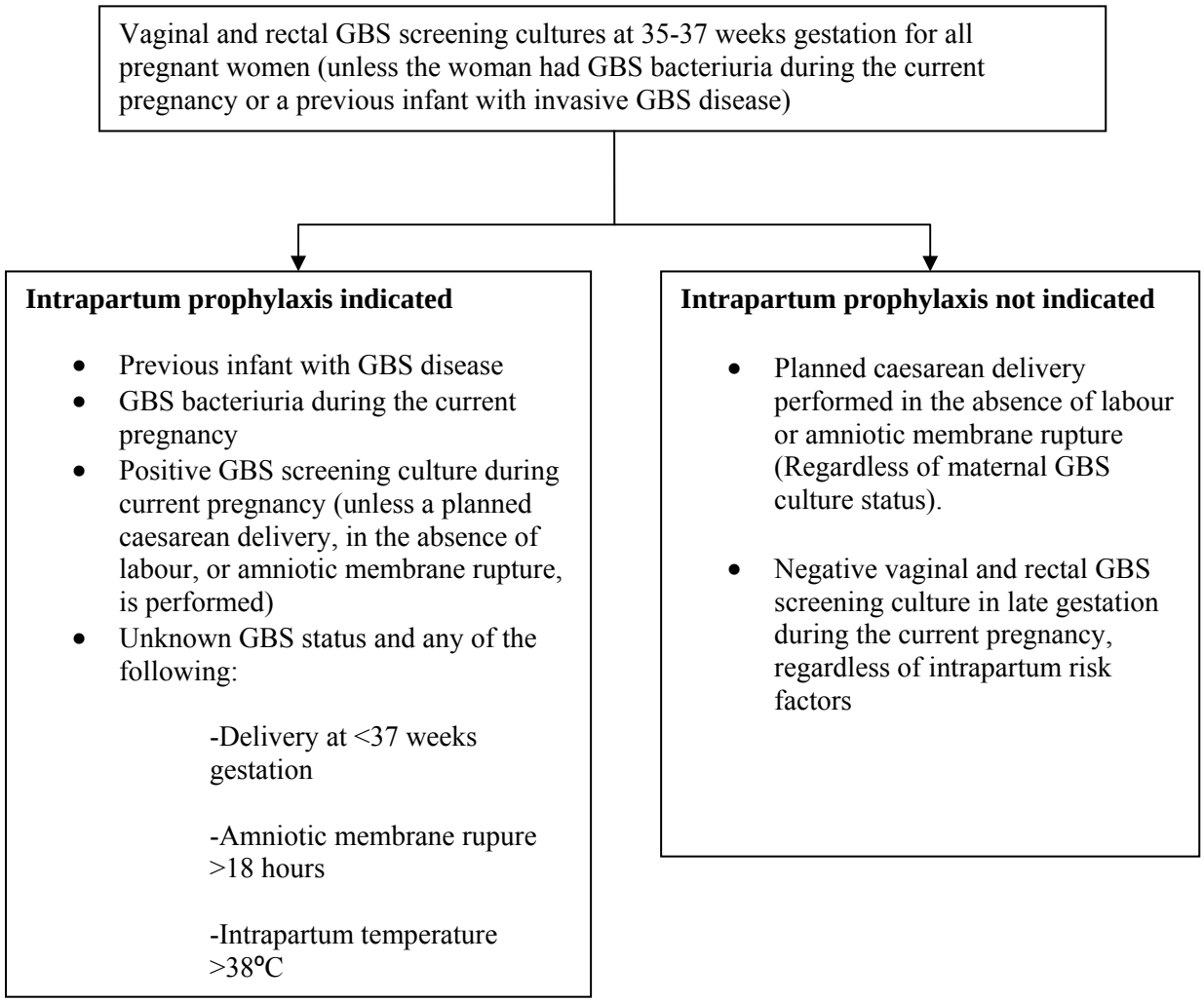

Figure 8 Screening strategy and indications for intrapartum antibiotic prophylaxis as recommended by CDC, 2002 [65].

Recommended:	Penicillin G IV every 4 hours until delivery
Alternative:	Ampicillin IV every 4 hours until delivery
If penicillin allergic:	
Patients not at high risk for anaphylaxis:	Cefazolin IV every 8 hours until delivery
Patients at high risk for anaphylaxis and GBS susceptible to clindamycin and erythromycin:	Clindamycin IV, every 6 hours until delivery or Erythromycin IV every 6 hours until delivery.
GBS resistant to clindamycin or clindamycin or susceptibility unknown:	Vancomycin every 12 hours until delivery

Figure 9 Regimens for intrapartum antibiotic prophylaxis as recommended by CDC [65].

Methods for detection of GBS colonisation in pregnant women

Culture

The "gold standard" of GBS screening is culture performed at 35-37 weeks gestation from swabs collected from both the vagina and the rectum. The use of selective media (agar plates and broth) for culture supplemented by antibiotics like colistin (10 μg/ml) or nalidixic acid (15 μg/ml) are recommended [65]. The selective agar plates may be examined after 24 hours while the inoculated selective, enrichment broth is incubated for 18-24 hours and then subcultured onto sheep blood agar. If GBS is not identified after the incubation of 18-24 hours, the blood agar plate should be reincubated and examined at 48 hours to identify suspected organisms. Suspected colonies may be tested using slide agglutination tests for specific identification [144]. Studies have shown that the use of standard direct blood agar plating rather than selective, enrichment medium leads to false negative culture results in as many as 50% of pregnant women colonised by GBS [144].

The culture taken at 35-37 weeks of gestation, may not accurately predict genital tract colonisation during labour because colonisation may be transient and colonisation may occur after the time of screening. Studies have shown sensitivities of a positive test (the ability to predict vaginal colonisation at time of labour) in week 35-37 from 54% to 91% [125] [101;145;146].

Antigen tests

GBS strains can also be identified by the production of group B Lancefield antigen [147]. Consequently, many latex agglutination tests and imunoassays that detect this antigen for GBS identification have been developed for rapid detection of GBS colonisations without previous culture. However, even if the specificity has been high (98-100%), the overall sensitivity of these commercially available immunological assays has been low and not sufficiently accurate for routine use in the intrapartum detection of women colonized with GBS [148].

DNA hybridisation

Probe hybridisation for GBS targets specifically the GBS ribosomal RNA. The method has been shown to be suitable to identify GBS from 18h to 24 h cultures in selective enrichment

broth with a sensitivity of 94.7-100% and specificity of 96.9-99.5% compared with culture [149]. The sensitivity is much lower when incubation is shorter. Thus, available probe hybridization methods are suitable for GBS identification from overnight cultures in selective enrichment broth, but are poorly sensitive for direct detection and identification of GBS from recto vaginal swabs obtained from pregnant women during labour [149].

Polymerase chain reaction (PCR)

Identification of GBS can be made by detecting a part of the DNA; the genetic target, which is unique for GBS. DNA extraction from GBS is dependent on lysis of the bacteria which sometimes may be challenging due to the robust polysaccharide capsule and cell wall of GBS.

The PCR starts with the denaturation step where double-stranded target DNA is denatured (melted) into single stranded DNA by increasing the temperature to approximately 95°C. The temperature is then lowered to approximately 55-58°C; this permits the annealing of the specific PCR primers to the single stranded target DNA. Finally, for efficient synthesis of DNA copies, the temperature is adjusted to be optimal for the DNA polymerase activity (extension), normally 72°C (Figure 10). To amplify target DNA the cycles through these temperatures are repeated several times (25 to 40 depending on the application). During a successful PCR process, several millions copies of the target DNA (amplicon) are made. In conventional PCR the amplicon accumulation is usually detected by gel electrophoresis. In real-time PCR, the amplicon accumulation is detected and measured during each PCR cycle using a fluorescent reporter.

Different PCR-based assays for identification of GBS have been developed. The assays are based on a variety of genetic targets. Examples are the *cfb* gene, which encodes the CAMP factor, the *sip* gene, encoding the surface immunogenic protein Sip, and *pts*I gene (phosphotransferase) [122;150;151]. The PCR assays also differ by different lysis methods and different probe detection formats (TaqMan probes, fluorescence resonance energy transfer (FRET) probes or molecular beacons) [152]. Studies of different GBS specific PCR assays have shown that they are both sensitive and specific compared to culture [122;150;153;154].

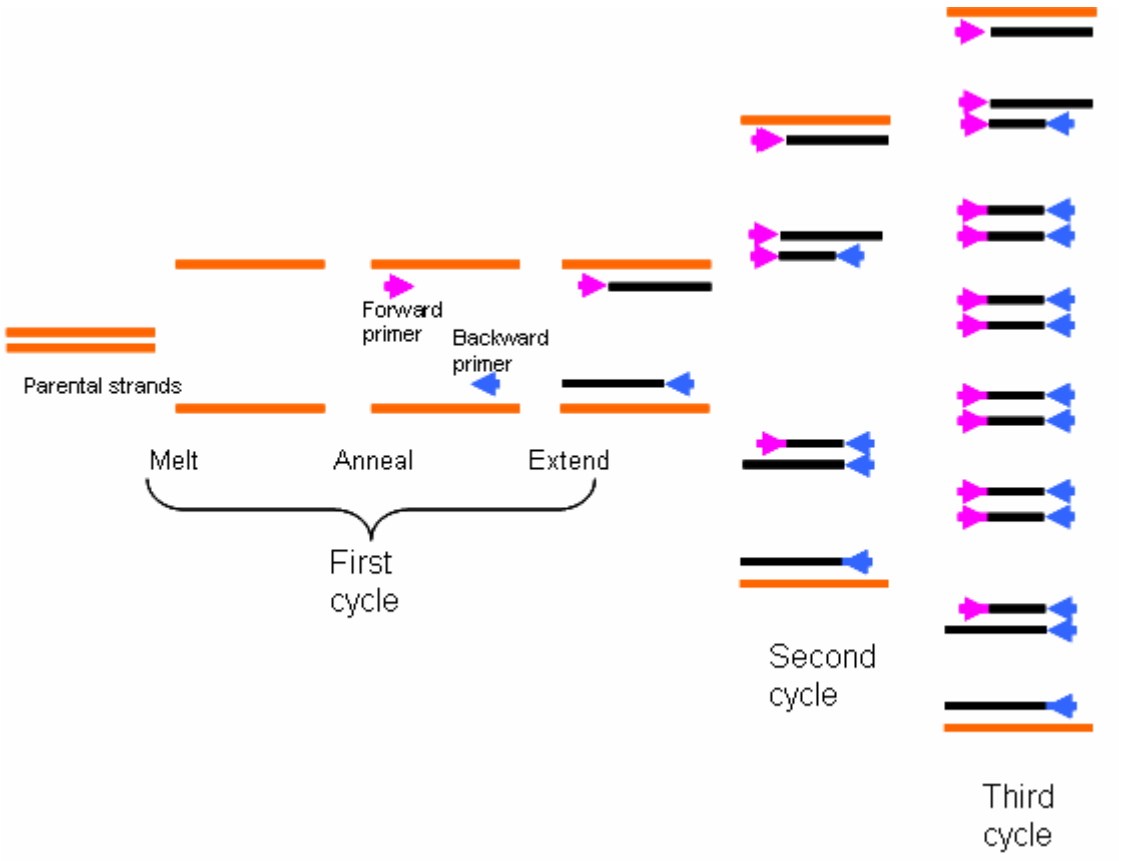

Figure 10 General outline of the first three cycles in PCR. During the first three cycles of the PCR process, 22 copies of a part of the parental strands, defined by the specific primers, have been made.

Vaccines

Maternal antibody deficiency to GBS is associated with increased neonatal susceptibility to invasive GBS disease [155]. Immunization of women during or before pregnancy could prevent peripartum maternal disease and protect infants from perinatally acquired infection by transplacental transfer of protective IgG antibodies [75;156].

The group B antigen, which is common to all strains, does not seem to be important for specific immunity to GBS infection. Maternal antibodies against the group B specific antigen do not protect against neonatal infection [157]. However, serotype-specific antibodies to GBS capsular polysaccharide (CPS), have been shown to cross the placenta, promote opsonophagocytosis and killing of GBS [137;158].

Early studies showed low immunogenicity in response to the polysaccharide capsule of GBS alone [159], but by combining the GBS polysaccharide with tetanus toxoid, an excellent immune response could be produced [156;160]. Also several of the surface protein antigens induce protective immunity in animal models [17]. Vaccine trials have shown that if surface proteins are conjugated to CPS, they enhance the immunogenicity of the CPS [161-163]. Alternative approaches to vaccines are based on surface proteins of GBS [161;164], on the recognition of immunogenic pili that extend from the surface of the bacterium [164], and on fusion proteins [165]. A summary of the status of GBS vaccine research is presented in Table 4.

A successful GBS vaccine could reduce mucosal bacterial colonisation and produce both humoral and mucosal immunity, and is expected to prevent more cases of neonatal disease than the current strategies with IAP [96;166]. However, trials of vaccine efficacy and safety are required for licensing of the vaccines. Such efficacy trials are likely to use substitute outcomes based on serological markers of a protective immune response, since trials to assess neonatal infection would need to be extremely large. Extensive post-marketing surveillance for effectiveness and safety would be an essential part of a licensing strategy.

The prime obstacle to the development and testing of a GBS vaccine is probably the spectre of the liability associated with vaccine delivery in pregnant women [167;168]. Concerns for the safety of the mothers and foetuses require exhaustive and costly evaluation of candidate vaccines and the issue of liability is both serious and complex. Potential challenges other than medico-legal issues include lack of protection passed to infants born prematurely, the unknown effects on neonates' immune responses and regulatory issues [169]. In order to successfully proceed in this field of maternal immunisation, it is necessary to define the actual risk, so that studies can be appropriately designed to demonstrate safety.

Studies of concerns that would be associated with GBS vaccination during pregnancy from the perspectives of pregnant women and health care providers have been performed [170]. Given all the factors involved in deciding whether to accept a vaccine or not, it appeared that being well informed about GBS was the most important factor. For any vaccine to be implemented, effective strategies for building public and individual trust are critical. These strategies need to be weighed against the pros and cons of the current IAP strategy as well as vaccination [170].

Table 4 Summary of GBS vaccine research and development

Vaccine target	Advantages/approach	Limitations
Capsular carbohydrate		
Unmodified polysaccharide vaccine (type III serotype)	Phase I trials (1988) indicated that the vaccine was safe and well tolerated [171]	Only 60% of the recipients showed an immune response; Requirement to improve immunogenicity of the CPS
Conjugate polysaccharide vaccine	Type III serotype: increase in immunogenicity when coupled to an immunogenic protein (tetanus toxoid (TT)); Conjugate vaccine with all nine currently identified GBS serotypes (Ia, Ib, II, III, IV, V, VI, VII and VIII) prepared and tested preclinically [172-175] (1988-2002)	Capsular conjugate vaccines of this type need to be multivalent in order to provide sufficient coverage against prevalent serotypes
Conjugate bivalent polysaccharide vaccine	Bivalent vaccine (GBS type II-TT and type III-TT) combined and administered; Well tolerated [176] (2002)	Further testing is warranted to investigate immune interference when more than two GBS CPS conjugate vaccines are simultaneously administered
Conjugate multivalent polysaccharide vaccine	Proposed that effective GBS vaccine in the United States includes five major serotypes (Ia, Ib, II, III and V). It is anticipated that multivalent vaccines will include each conjugate vaccine prepared separately [176] (2002)	Formulation of a GBS conjugate vaccine for use in one geographic area might not be effective in other regions
Proteins		
C5a peptidase	Present in all strains and serotypes of GBS; Little or no antigenic variability; Capable of inducing antibodies that are opsonically active [177]. Immunization induces serotype-independent protection (2001)	Progress as a potential vaccine is unknown
β-Component of the c protein	Elicits protective immunity in animal models [163] (1992)	This protein is only present in a minority of strains that cause infection (~20%)
LmbP	Expressed by most GBS strains (2005)	Progress as a potential vaccine is unknown [178].
Sip	Present on all GBS strains; Induces protective antibodies; Recombinant SIP protein protected mice infected with numerous GBS strains [179]	Biological function is not well understood; No recent reports of progress towards the development of a vaccine [178;180;181]
LrrG	Highly conserved protein antigen that induces protection [182].	Progress as a potential vaccine is unknown
Fusion protein from N-terminal of Rib and α	More immunogenic than one derived from the repeats and was immunogenic even without adjuvant (2007).	Antibodies to the N-terminal fusion protein protected against infection in mice and inhibited bacterial invasion of epithelial cells [165].
PI-2b pilus antigens	Present in all tested GBS strains, protective in mice (2008) [164].	Progress as potential vaccine is unknown

CPS, capsular polysaccharide; LmbP, laminin binding protein; Sip, surface immunogenic protein.

Aims of the study

- To survey the incidence of invasive GBS disease in infants and adults and detect possible trends in the distribution of capsular types, surface proteins and susceptibility to erythromycin and clindamycin in invasive GBS strains in Norway during the period 1996 to 2006

- To characterise isolates from infants with invasive GBS disease in Norway in 2006 and 2007 by phenotypic and molecular methods and investigate whether phylogenetic lineages are associated with specific characteristics like antibiotic susceptibility, geographic origin, early or late onset GBS disease and lethality

- To evaluate a real-time PCR targeting the *sip*-gene in detecting GBS colonisation in pregnant women at delivery and compare the performance with optimised GBS culture

- To assess to what extent time affects the logistics of intrapartum screening and administration of intrapartum antibiotic prophylaxis and thereby the feasibility of intrapartum screening

Material and methods

Study population

In Paper I-II, data on incidence of invasive GBS disease in the Norwegian population were obtained from The Norwegian Surveillance system for Communicable Diseases (MSIS), in Paper I from patients in all age groups and in Paper II from infants <3 months. The GBS strains from patients with invasive disease in Norway were sent from the laboratories at the respective hospitals to the reference laboratory at St. Olavs Hospital for further characterisation. For paper III, we collected specimens from 250 pregnant women at the maternity ward at St. Olavs Hospital, Trondheim, Norway. For paper IV, data on arrival at the maternity ward of 836 pregnant women, and time of delivery at St. Olavs Hospital, Trondheim, were analysed.

Detection and identification of GBS

Methods of detecting and identifying GBS from pregnant women and from invasive strains are described in paper I, II and III.

Phenotypic characterisation of invasive GBS strains

Capsular typing and typing of surface proteins were performed by indirect fluorescent antibody tests (FAT). From January 2006, these methods were replaced by PCR methods detecting capsular polysaccharide synthesis gene clusters and genes encoding surface localised proteins as described in paper I. The susceptibility testing of strains is described in Paper I and Paper II.

Genotypic characterisation of invasive GBS strains

Capsular typing and typing of surface proteins were performed by PCR methods detecting capsular polysaccharide synthesis gene clusters and genes encoding surface localised proteins as described in Paper I and Paper II.

MLST For each strain the seven housekeeping genes *alcohol dehydrogenase (adhP)*, phenylalanyl tRNA synthetase (*pheS*), amino acid transporter (*atr*), glutamine synthetase (*glnA*), serine dehydratase (*sdhA*), glucose kinase (*glcK*), and transketolase (*tkt*) were amplified and sequenced using primers and conditions as described by Jones et al [183]**.** Sequencing was performed using the BigDye terminator Cycle Sequencing Kit v 3.1 (Applied Biosystems) followed by capillary electrophoresis on ABI 3130x Genetic Analyzer. The sequence traces for each of the seven gene loci were assigned an allele number, and each strain was assigned a sequence type (ST) according to allelic profile, by submission of the sequences to the *S. agalactiae* MLST database. Genetic comparison of the strains was performed by constructing a dendrogram showing the relationship among STs using MEGA3 (version 3.1) [184]. Strains were grouped into clonal complexes (CC) using the eBURST software program [41]. The term singleton ST refers to STs that did not cluster into a clonal complex (CC).

PFGE Bacterial chromosomal DNA was digested with *Sma*I [185], before separation by electrophoresis using the CHEF XA Mapper (Bio-Rad. Richmond, CA, USA). The PFGE profiles were compared both by manual inspection and by cluster analysis using Bionumerics (version 5.10, Applied Maths, Sint-Martens-Latem, Belgium).

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Decision and cost effectiveness analysis

In the decision analysis (Paper IV), a complex "decision tree" was modelled by means of the software program, TreeAge Pro version 1.2 (Tree Age Software, Inc., Williamstown, MA). The "decision node" was the choices between three different strategies: No intervention, antepartum screening and intrapartum screening. There were five principal outcomes of the strategies; no GBS disease, full recovery after invasive disease, moderate disability after invasive disease, severe disability after invasive disease and death due to invasive disease (Figure 11). The different strategies included many possibilities of outcome and in the model there are 63 chance nodes and 126 different outcomes.

The information obtained in the cost effectiveness analysis was the cost of gaining one quality adjusted life year (QALY) when a health intervention is applied and compare the cost per QALY for different strategies. The number of QALYs gained by an intervention represents the number of years of perfect health that would be obtained by providing that intervention. To differentiate between a year of life gained and a healthy year of life gained,

one must consider the effects of disease on both quantity and quality (utility) of life separately. In short: individuals who consider themselves to be in perfect health would rate their utility as one, while someone who would just as soon be dead might rate his or her life as a zero. Someone with a chronic debilitating disease might rate (utility score) her life as 0.7, indicating that she values her life as only worth seven tenths of a year lived in perfect health. The total number of QALYs in a health state is equal to the product of utility score and the number of years lived in that health state. An infant with a serious neurological handicap due to invasive GBS disease as newborn might have both a life expectancy that is shorter than a healthy infant and also a lower quality of life. In the cost effectiveness analysis, the cost of an intervention ("No intervention", "culture strategy" or "PCR strategy"), the quality of life, and the number of years of life gained were combined into a single cost effectiveness ratio. Cost effectiveness threshold or willingness to pay (WTP) represents the price which the society is willing to pay to gain one QALY.

Equation1: Cost effectiveness ratio:

$$\frac{\text{cost of intervention (screening, antibiotics)} - \text{costs prevented by intervention}}{\text{QALYs gained by intervention}}$$

In the study, comparisons between interventions were made using the incremental cost effectiveness ratio which is defined as

Equation 2: Incremental cost effectiveness ratio:

$$\frac{\text{total cost of intervention 1} - \text{total cost of intervention 2}}{\text{QALYs, intervention 1} - \text{QALYs, intervention 2}}$$

In paper IV, reference values for life expectancy and quality of life (utility scores) were obtained from published studies that reported utility values during childhood associated with disability due to meningitis or bacteraemia as summarised and presented in one comprehensive study [96].

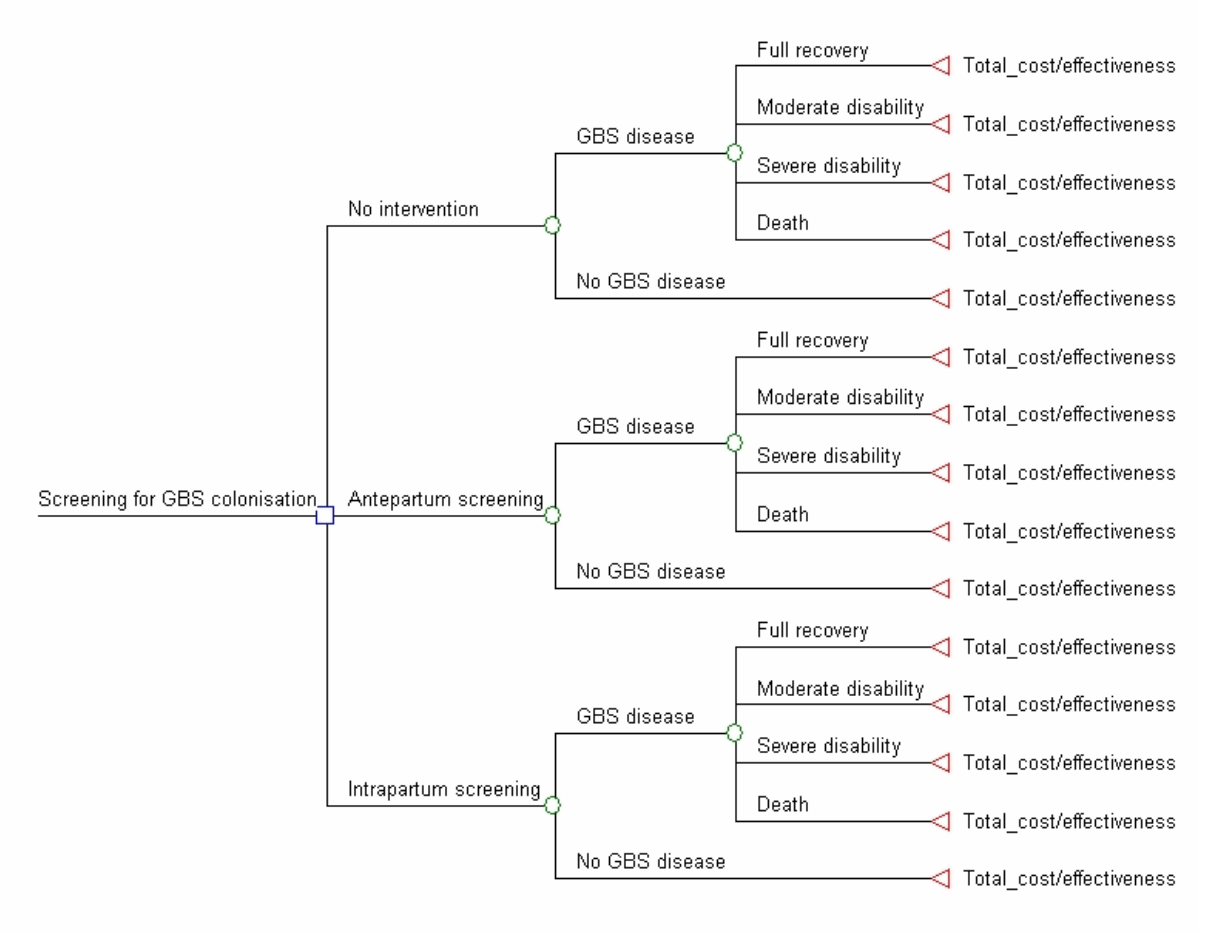

Figure 11 General outline of the decision model. The three strategies have all five end points. The costs included in the model are costs of screening and potential intrapartum antibiotic treatment of GBS colonised mother and hospital costs of a healthy or sick infant. Effectiveness is quality adjusted life year (QALY) of either a healthy or sick infant. Variables like risk of GBS colonisation of women, risk of invasive disease either sepsis or meningitis if colonised, time for intrapartum prophylaxis if intrapartum or antepartum screening, sensitivity of different screening methods and effect of antibiotic prophylaxis given 1-2 hours or more than 2 hours before delivery are not shown in this figure.

Ethics

The study on GBS colonisation in pregnant women was reported to the Norwegian Social Science Data Services, and was approved by the Regional Committee for Medical Research Ethics. A research bio-bank was approved by The Norwegian Directorate for Health and is registered at The Norwegian Institute of Public Health.

Statistical analyses

Categorical data were compared by Chi-square or Fisher's exact tests.
Data on detection of GBS colonisation and data on interval between admission and delivery and time for intrapartum screening were collected and analysed in SPSS (SPSS Inc, Illinois, USA). Data on incidences of invasive GBS disease and data on GBS typing were collected and analysed in Windows Excel. Minitab and Pearson's Chi-square test were used for comparison of incidence in the two periods 1996-2000 and 2001-2006 and for comparison of proportions of serotypes. In cost effectiveness analyses, the analyses were performed with TreeAge Pro version 1.2 (TreeAge Software Inc., Williamstown, MA).

Results

Paper I: The overall mean incidence of GBS disease in adults (>19 years) increased from 1.34 cases per 100 000 in 1996-1998 to 3.1 cases per 100 000 in 1999-2006 ($p < 0.001$). The mean incidence in elderly (>70 years) increased from 3.9 per 100 000 in 1996-1998 to 9.15 in 1999-2006 ($p < 0.001$). The incidence of neonatal early-onset disease was stable during the period with 0.46 cases per 1000 live births. The case fatality of GBS disease in infants was in average 6.5% in 1996-2005. However, in 2006 the case fatality increased to 20%. Serotype III and V were predominant in 839 invasive GBS strains characterised; type III in infants and type V in the elderly. The distribution of surface proteins was stable from 1996 to 2005, but the detection rate of surface proteins in type III and V was low. Genes encoding surface proteins were detected in nearly all strains from 2006. The resistance to erythromycin and clindamycin increased from an average of 4% of invasive strains in the period 2003 to 2005 to 25.4% resistant strains in 2006.

Paper II: 96 GBS strains from nearly all infants registered with invasive disease in Norway 2006 and 2007 were characterised by phenotypical and molecular methods. MLST identified 27 sequence types and five clonal complexes (CCs): CC17, CC1, CC19, CC358 and CC23 which is similar to what has been observed in previous studies from other countries. Resistant strains were found in CC1 and CC19. PFGE performed on all serotype V strains identified 10 PFGE patterns belonging to four different clusters. The erythromycin and clindamycin susceptibility of each strain appeared to be associated with its PFGE cluster profile. There also appeared to be an association between PFGE profile and early and late onset disease. MLST data did not correlate with phenotypical characteristics of invasive GBS strains from infants in Norway.

Paper III: The performance of a real-time PCR targeting the *sip* gene for detection of Group B streptococcus (GBS) colonisation in pregnant women at delivery was compared with optimised culture. Of samples from 251 women, 87 (34.7%) were GBS positive by culture and 86 (34.3%) were PCR positive. Using GBS culture as gold standard, the sensitivity of real-time PCR was 0.97 (95% CI: 0.90-0.99), and specificity was 0.99 (95% CI: 0.97-0.99).

The rate of GBS colonisation was lower in vaginal specimens than in rectal specimens both by culture and PCR. Real-time PCR targeting the *sip* gene was found to be fast and sensitive, well adapted for detection of GBS colonisation in pregnant women at term.

Paper IV Logistics of intrapartum screening and intrapartum antibiotic prophylaxis to pregnant women colonised with GBS were studied. Decision analysis was applied to compare cost effectiveness of antepartum culture at gestational week 35-37 and intrapartum PCR in screening for GBS. Of 836 registered pregnant women, 78.5% arrived before the four hours set as a minimum for providing intrapartum screening logistics and effective antibiotic prophylaxis. Depending on frequency of analyzing runs in the laboratory, 46% to 78.5% of the women could receive adequate antibiotic prophylaxis if intrapartum screening was performed. If "willingness to pay" (WTP) was at least $10972/QALY, both antepartum and intrapartum screening strategies were cost effective compared to no intervention. Culture was the most cost effective screening strategy, but PCR would give the best clinical outcome. However, the incremental cost/effect of PCR compared to culture was very high ($101153/QALY), and much higher than the established cut-offs for WTP ($50000/QALY - $70400/QALY).

Discussion

Epidemiology of GBS

While the incidence of EOD in Norway seemed to increase between 1985 and 1994, it stabilised and was unchanged from 1996 to 2006. The reported incidences of invasive GBS disease may be biased by different factors. In Norway, several neonatal units were established at the hospitals after 1980 and improved the neonatal care throughout the country. This may have enhanced the quality of diagnostics and increased the number of septic infants with a correct diagnosis. Thus, some of the observed increase in incidence of GBS disease in Norway from the late 1980s to the mid 1990s may be explained by structural changes of the health care system and not by a change of epidemiology. Another bias in statistics of neonatal disease, both in Norway and in other countries, is the neonatal sepsis of unknown aetiology. Bacterial growth is detected in less than 10% of blood cultures from sick neonates [186], and most infants treated for a neonatal infection are discharged from the hospitals with the diagnosis "unspecified sepsis in the newborn" (Figure 12).

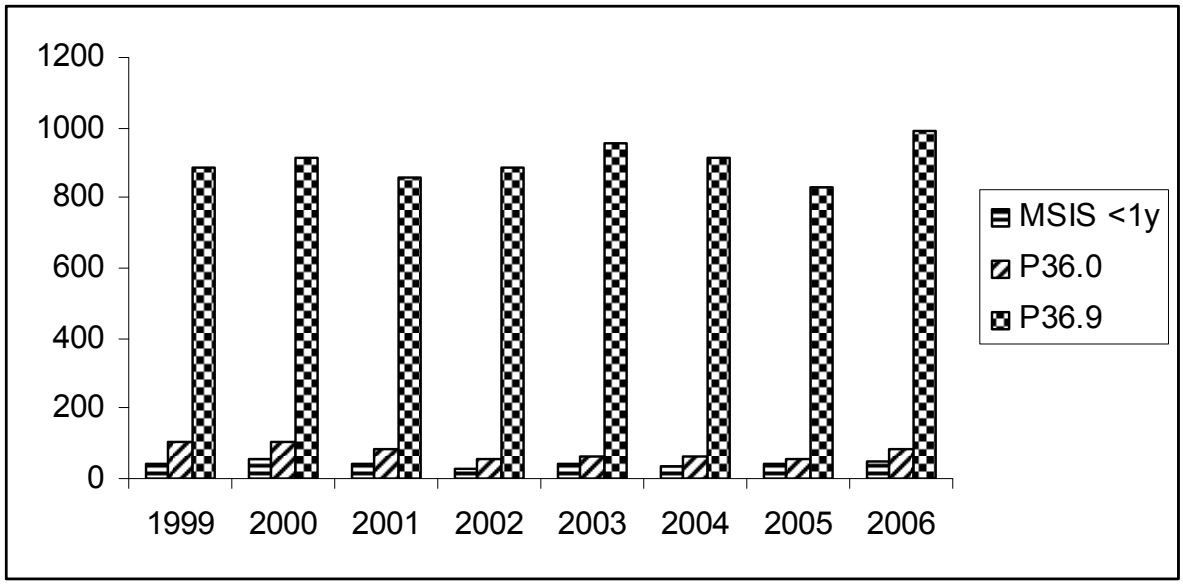

Figure 12 Cases with diagnosis P36.0 (GBS disease in newborn) and P36.9 (Sepsis in newborn, unknown cause) and cases of invasive GBS disease in infants <1year reported to

MSIS (Sources: Norwegian Patient Register and Norwegian Surveillance system for Communicable Diseases (MSIS))

Studies have suggested that the incidence of invasive GBS disease, if based on additional criteria other than culture, is at least three times higher than the incidence based on culture-proven cases [58;67;187]. Estimates of "true incidence" of invasive GBS disease are important in the context of both the burden of disease and for health policy issues, but might be less suitable for epidemiological studies. Local diagnostic routines and traditions, changing systems of health service financing, more focus at GBS disease in media and among paediatricians, gynaecologists and microbiologists might influence what diagnoses are being used. The ambiguity of incidence reports makes explanations of trends in GBS disease and differences between countries uncertain and incomplete. This also makes it more difficult to estimate the effect of different strategies to prevent EOD.

A recent change in virulence of GBS strains in Norway? After the dramatic rise of case fatality of GBS in Norway in the first half of 2006, a survey of GBS cases in search for possible explanations was carried out by The National Institute of Public Health [188]. The survey did not reveal any single explanation of the increased lethality, but suggested that a more virulent serotype V could explain some of the fatal cases.

Type V emerged as an important serotype in invasive strains from neonates and adults in the 1990s and is now in some areas the most common serotype in adults [75;189]. In our material from 1996 to 2006, type V was detected in 10% of strains from infants and in 24% of strains from adults (Paper I). In 2007, 19% of invasive strains from infants had capsular type V (Paper II). In addition to be the cause of five neonatal deaths in 2006 and 2007, type V has also shown more resistance to erythromycin and clindamycin from 2005 on. In GBS strains from infants, 61% of type V strains were resistant in Norway, 2006 and 2007.

It has also previously been shown significant antibiotic resistance in type V strains [61;190;191]. A study by Diekema showed that resistant type V strains from the 1990s in USA did not represent a new subtype of GBS V [192]; the resistant strains had the same or a very similar PFGE pattern as the most common type V registered by CDC already in 1975 [193]. This subtype of type V has also been reported as common among type V strains in France and Germany [191;194] and in accordance with this our analysis suggested that also the major PFGE profile among Norwegian strains was similar to those previously reported.

In Norway, 4% of invasive strains from infants were resistant to erythromycin and clindamycin from 2003 to 2005, but in 2006 and 2007 almost 20% of the strains were

resistant (Paper I and Paper II). A tendency toward lower antibiotic susceptibility of GBS has also been shown in Sweden over the last years [60]. The increasing resistance to erythromycin and clindamycin in Norway and Sweden coincides with observations in other countries [61;190;191;195].

The first erythromycin-resistant strains of streptococci were reported from United Kingdom in 1959 [196]. Yet, resistance to erythromycin and clindamycin has not been an acknowledged problem until the 1990s when up to 41% of GBS isolates in USA and Canada were reported to be resistant [61;65].

The ORACLE I and II trials [197;198] concluded that erythromycin given to pregnant women with premature rupture of the membranes (pPROM) and spontaneous preterm labour was associated with a range of health benefits for the neonate, and a probable reduction in childhood disability. This statement probably led to an increased use of erythromycin in maternity wards. It is not known if frequent use of erythromycin in pregnant women can explain the increased erythromycin resistance of GBS. However, the increasing resistance among GBS strains, also in Norway, indicates that erythromycin to women with penicillin allergy should no longer be the drug of choice in pregnant women.

Molecular epidemiology

The study on molecular characteristics of invasive strains from infants did not indicate that sequence types and clonal complexes were associated with specific characteristics like susceptibility to erythromycin and clindamycin, geographic origin, early or late onset GBS disease and lethality (Paper II).

Some studies have found relatively more type III strains with sequence type (ST) 17 in invasive strains compared to colonising strains [199;200]. This has indicated that ST17 might represent a virulent clone of GBS. However, a study by Davies et al could not confirm the claimed association between sequence type and invasiveness [201]. In our material of invasive strains only, none of 23 cases with ST17 strains were fatal, none were resistant to erythromycin or clindamycin and they were equally distributed between early and late onset disease. With the possible exception of ST17, published reports suggest that GBS STs and clonal complexes (CCs) seem to be equally distributed in invasive and non-invasive strains [201-204]. Our study suggested a lack of association between ST and characteristics interesting to the clinician. These observations also coincide with observations of other species [37;205]; the clonal groups are observed at the same frequencies regardless of whether

the data are based on isolates from cases of invasive disease or asymptomatic carriage. In general, the MLST data alone do not provide information regarding the virulence potential of an isolate [37]. Possible explanations for this might be:

- There are no genetic differences between isolates regarding the tendency to cause disease.
- Other factors such as host susceptibility, within-host evolution and 'opportunistic' components of the bacteria are important.
- Strains differ in virulence potential, but the genetic basis for these differences is not linked (i.e. is not reflected by) to the MLST data [37]. "While MLST focuses on the stable core genome encoding essential housekeeping functions, 'specialist' phenotypes, which include clinically relevant properties, are more often encoded on accessory genes or elements, which can be rapidly disseminated throughout a population and equally rapidly lost."[37].

These observations emphasise the question of whether MLST is a relevant method for surveillance of disease (virulence), intervention (antibiotic resistance) or emergence (host or geographical source). Correlations between MLST genotype and clinical phenotypes are probably not an aim for MLST, and the clinician or epidemiologist will not be provided with reliable information regarding an isolate. Thus, neither our study nor any other studies indicate that MLST is the method of choice in surveillance of GBS disease. Instead, MLST provides a 'population framework', which means that isolates with similar or identical MLST genotypes are closely related, having descended from a recent common ancestor. "The ambition of MLST is to identify what an isolate is, not what it does" [37].

Invasive type V strains from infants in Norway, 2006 and 2007, were also analysed with PFGE (Paper II). Using PFGE we were to some extent able to discriminate between type V strains with different clinical properties as resistance to erythromycin and clindamycin, inducible clindamycin resistance and lethality. By visually comparing published gel images, similarities between a major PFGE profile of type V strains in our study and the most common PFGE pattern of type V strains published in the US, France and Germany could be inferred [191;192;194]. However, to make this comparison more accurate, it would have been necessary to run a new PFGE with all strains in the same gel.

Methods used for investigating local and global epidemiology should ideally be able to distinguish accurately between different strains within a bacterial species and assign isolates which have descended from a recent common ancestor to the same molecular type. Isolates

that only share a more distant common ancestor should be assigned to different molecular types. Neither MLST nor PFGE fulfil all these criteria and the methods are also resource-demanding. Consequently, there is a need for supplementary methods or may be even methods that can replace MLST and PFGE.

Methods for detection of GBS in pregnant women

The real-time PCR targeting the *sip* gene proved to be very sensitive and specific. The method can replace the culture method in detecting GBS colonisation in pregnant women at term.

PCR assays for GBS can detect as little as one colony forming unit (CFU) per PCR reaction [206]. The PCR method should therefore in theory be at least as sensitive as culture. Still, in our study, as in many other studies [146;207;208], PCR seemed to be slightly less sensitive than culture (0.97) (Paper III). This might have many possible explanations. To make a PCR test suitable for screening for GBS colonisation in pregnant women, all steps in the process have to be optimised; from the collection of specimens, via DNA extraction, to the actual PCR reaction with optimal primers and temperatures.

In our study, culture was favoured by receiving more test material than PCR. We dissolved the specimens in 550 µl Nacl and used 50 µl of this solution for culture and 300 µl for DNA extraction. The extracted DNA was eluated in 100 µl and only 2 µl of this eluate was analysed by PCR. This means that the methology favoured culture by a factor of 8.3 compared with PCR. The sensitivity of 0.97 compared with culture must therefore be considered very high.

The result of the culture is dependent on many steps in the process, the collection site, interval from the collection to the analysis [140], the storage, selective and enrichment media and proper interpretation of the bacterial colonies [144]. Thus, colonisation rates reported from different geographic areas and performance of alternative tests as PCR are influenced by the quality of the "gold standard" and might be an important bias in many studies.

Screening strategy

What is a QALY worth? In our study we used two different cost effectiveness thresholds or willingness to pay (WTP) (Paper IV). Assuming that the QALY (quality adjusted life year) is an adequate measure of health care outcomes, the question of what value that should be

placed on a QALY is not easily solved. WHO has suggested an estimate of 3 times a nation's per capita gross domestic product for each disability-adjusted life year (DALY) averted. In cost effectiveness analyses from USA, US $50 000 per QALY is often used. A study by Devlin and Parkin retrospectively examined 33 health technology decisions made by The National Institute for Health and Clinical Excellence (NICE) [209]. The study revealed that NICE did not always stuck to their own range of acceptable cost effectiveness. Factors like uncertainty of estimates and burden of disease could explain the probability of acceptance of extra costs per QALY. The use of willingness to pay (WTP) to value a QALY might also be problematic because the public preference for an increased unit of health may differ according to the characteristics of the population. It has been reported that people will pay more to avoid a decrease in health than to increase health [210]. The public usually gives a higher value to QALYs than policy-makers do and more wealthy countries put a higher value on a QALY than less wealthy countries.

Influence of time The study on intrapartum vs. antepartum screening in Paper IV showed that time influenced on the feasibility of intrapartum screening. A weakness of the study might be that the study was performed at one single hospital with a study population of 836 pregnant women. The population studied, and the routines for admission of pregnant women at the hospital might not apply to other areas. Still, the interval between start of labour and delivery is very variable and also depends on whether the woman is nullipara or not, but is probably not dependent of race and culture [211]. There is therefore reason to believe that routines for admission to a maternity ward do not differ much from hospital to hospital or from country to country. Nevertheless, a study from USA showed a median interval between admission and delivery of 7.9 hours [212], compared to the 12.9 hours in our study. This suggests that the feasibility of intrapartum screening might be even lower in a US population.

Screening of low-risk women only The rationale of comparing screening strategies in low-risk women was based on a study of Colbourn et al. They claimed that infants of women with high risk for EOD would have an increased risk of non-GBS sepsis as well. Thus, IAP to all high-risk women would therefore be cost effective independent of GBS carrier status [97]. This is in contrast to previously published studies claiming that the main advantage of intrapartum PCR is the benefit of risk stratification on presentation for delivery and especially when women deliver prematurely. These conclusions have partly been based on a study by Schuchat et al suggesting that risk factors for EOD were unrelated to sepsis attributable to

other organisms [54]. They explained this by the possible different route of transmission between GBS and other organisms. For example, GBS is acquired from the mother in nearly all cases of EOD and only 7% of GBS cases presented after the first day of life. By contrast, 39% of non-GBS cases presented after the first day of life and consequently, may represent infections acquired during hospitalisation or through contact with others [54]. An exception was prematurity which is an important risk factor for both GBS and non-GBS infections presenting at day one. The applicability of the risk-based approach to prevention of other infections will depend on the prevalence of risk conditions among these cases and on the effectiveness of IAP against other causes of early onset disease.

Compliance with recommended strategy In 1998, recommendations for prevention of GBS disease in infants were introduced in Norway. But in contrast to the decrease in incidence observed in Australia and USA the last 10-20 years, the incidence of invasive GBS disease in infants in Norway has been unchanged (Paper I and Figure 5). Thus, even if the incidence is similar to what is observed in USA, this might indicate that there is a potential for improvements of preventive strategies in Norway.

Studies from USA have suggested that the benefits of the screening strategy compared with the risk-factor strategy stemmed from identifying GBS colonised women who did not present with obstetric risk factors, and that women who were GBS positive were more likely to receive intrapartum antibiotics than were women with obstetric risk factors in the risk cohort [54;65;212]. Two studies from Norway, although based on small numbers, suggest that hospital staff do not comply with recommended directives for prevention of GBS disease in infants [188] (G. Waal and I. Engedal. Intrapartum antibiotikaprofylakse ved terminfødsler, fødeavdelingen St. Olavs Hospital, 2004. Student thesis, NTNU 2008). The previously mentioned MSIS-report on invasive GBS disease in Norway from January 2005 to July 2006 showed that only one out of eight women with rupture of membranes more than 24 hours, and only two out of ten women giving birth at gestational age <33 weeks received IAP [188]. In addition, a retrospective study from St. Olavs Hospital showed that only 4/47 women with risk factors received IAP (Waal and Engedal. Student thesis, NTNU 2008). The student thesis also revealed that the directives in some instances were vague and made it difficult to deduce whether the staff had complied with the strategy or not. Also in Sweden there seem to be a low compliance with directives for IAP; only 14% of women with any risk factor had received IAP for at least two hours according to a study by Hakansson et al [67]. These studies indicate that even if a risk factor based strategy for prevention of invasive GBS

disease is recommended, only a few women with risk factors actually receive IAP. Possible explanations of this might be:

- It is difficult to implement extra routines in maternity wards [213]
- The directives for IAP are too vague
- Medical personnel in maternity wards (at least in Norway and Sweden) are reluctant to accept and implement directives that include more use of antibiotics. (There is an ongoing discussion whether peripartum antibiotics may result in more resistance in non-GBS bacteria [214], and also whether antibiotics might lead to a chronic abnormal gut flora that may have health consequences for the infant later in life [215]).

A better implementation of existing directions for IAP and more precise guidelines could possibly reduce the incidence of GBS disease in infants, also in Norway.

Main conclusions

- The incidence of invasive GBS disease in infants has been stable in Norway during the period from 1996 to 2006, and the incidence of early onset disease is similar to what has been observed in other developed countries.
- The incidence of invasive GBS disease in adults and elderly increased significantly from 1996 to 2006.
- Both phenotypical and genotypical characteristics of invasive GBS strains from infants in Norway are comparable to characteristics of strains reported from other countries.
- The Real-time PCR targeting the *sip* gene is suitable for detection of GBS colonisation in pregnant women at term.
- Intrapartum screening of low-risk pregnant women is not cost effective compared with antepartum screening in week 35-37 due to shortage of time and higher costs.

Future aspects

- **Improved methods for epidemiological typing**

  The available methods for epidemiological typing of GBS are expensive, take much laboratory recourses and do not fulfil all criteria for local and global epidemiology. A low cost typing method that is rapid, reproducible, easy to perform, with the portability like MLST and the ability to recognise the epidemic isolates would be a useful tool for outbreak management and surveillance of virulence, antibiotic resistance and detecting sources of virulent strains. Methods like multiple-locus variable number tandem repeat analysis (MLVA) based on differences in the variable number of tandem repeats (VNTR) on multiple loci on the chromosome of bacteria have shown to be useful in analysis of other bacteria than GBS. MLVA might be useful for epidemiological typing of GBS.

- **More sensitive tests for neonatal GBS sepsis**

  Today many cases of invasive GBS disease are probably undetected due to blood culture negative sepsis in neonates. This makes surveillance of GBS disease in infants and evaluation of preventive strategies difficult. To improve the surveillance and evaluation of different preventive strategies, more sensitive diagnostic tests for neonatal GBS sepsis are required.

- **Development of a vaccine against GBS**

  Even with improved methods to detect women at risk, intrapartum antibiotic prophylaxis will not eradicate early onset GBS disease and will have no effect on late onset GBS disease. In addition, possible negative effects of antibiotics make preventive strategies based on antibiotics inadvisable. A successful GBS vaccine could reduce mucosal bacterial colonisation, produce both humoral and mucosal immunity, and thereby prevent neonatal disease more effectively than the current strategies with intrapartum antibiotic prophylaxis.

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161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.

164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.
166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.

194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS
197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
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2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.

219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS

247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT
272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS

275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN
297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY

300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS
304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2s IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS
324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION

327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING
335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT
350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY

354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS
358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL.LIKE RECEPTOR – EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.
363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE AND STRENGTH TRAINING IN CORONARY ARTERY DISEASE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS
369. Anja Bye: GENE EXPRESSION PROFILING OF *INHERITED* AND *ACQUIRED* MAXIMAL OXYGEN UPTAKE – RELATIONS TO THE METABOLIC SYNDROME.
370. Oluf Dimitri Røe: MALIGNANT MESOTHELIOMA: VIRUS, BIOMARKERS AND GENES. A TRANSLATIONAL APPROACH
371. Ane Cecilie Dale: DIABETES MELLITUS AND FATAL ISCHEMIC HEART DISEASE. ANALYSES FROM THE HUNT1 AND 2 STUDIES
372. Jacob Christian Hølen: PAIN ASSESSMENT IN PALLIATIVE CARE: VALIDATION OF METHODS FOR SELF-REPORT AND BEHAVIOURAL ASSESSMENT
373. Erming Tian: THE GENETIC IMPACTS IN THE ONCOGENESIS OF MULTIPLE MYELOMA
374. Ole Bosnes: KLINISK UTPRØVING AV NORSKE VERSJONER AV NOEN SENTRALE TESTER PÅ KOGNITIV FUNKSJON
375. Ola M. Rygh: 3D ULTRASOUND BASED NEURONAVIGATION IN NEUROSURGERY. A CLINICAL EVALUATION
376. Astrid Kamilla Stunes: ADIPOKINES, PEROXISOME PROFILERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS AND SEROTONIN. COMMON REGULATORS OF BONE AND FAT METABOLISM
377. Silje Engdal: HERBAL REMEDIES USED BY NORWEGIAN CANCER PATIENTS AND THEIR ROLE IN HERB-DRUG INTERACTIONS
378. Kristin Offerdal: IMPROVED ULTRASOUND IMAGING OF THE FETUS AND ITS CONSEQUENCES FOR SEVERE AND LESS SEVERE ANOMALIES
379. Øivind Rognmo: HIGH-INTENSITY AEROBIC EXERCISE AND CARDIOVASCULAR HEALTH
380. Jo-Åsmund Lund: RADIOTHERAPY IN ANAL CARCINOMA AND PROSTATE CANCER

2009

381. Tore Grüner Bjåstad: HIGH FRAME RATE ULTRASOUND IMAGING USING PARALLEL BEAMFORMING
382. Erik Søndenaa: INTELLECTUAL DISABILITIES IN THE CRIMINAL JUSTICE SYSTEM
383. Berit Rostad: SOCIAL INEQUALITIES IN WOMEN'S HEALTH, HUNT 1984-86 AND 1995-97, THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
384. Jonas Crosby: ULTRASOUND-BASED QUANTIFICATION OF MYOCARDIAL DEFORMATION AND ROTATION
385. Erling Tronvik: MIGRAINE, BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM
386. Tom Christensen: BRINGING THE GP TO THE FOREFRONT OF EPR DEVELOPMENT
387. Håkon Bergseng: ASPECTS OF GROUP B STREPTOCOCCUS (GBS) DISEASE IN THE NEWBORN. EPIDEMIOLOGY, CHARACTERISATION OF INVASIVE STRAINS AND EVALUATION OF INTRAPARTUM SCREENING