Meeting report: Towards better risk stratification, prevention and therapy of invasive GBS disease, ESPID research meeting May 2022

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ABSTRACT

The European Society of Pediatric Infectious Diseases (ESPID) hosted the third Group B Streptococcus (GBS) Research Session in Athens on 11th May 2022, providing researchers and clinicians from around the world an opportunity to share and discuss recent advances in GBS pathophysiology, molecular and genetic epidemiology and how these new insights can help in improving prevention and control of early- and late-onset GBS disease. The meeting provided a state-of-the-art overview of the existing GBS prevention strategies and their limitations, and an opportunity to share the latest research findings. The first presentation provided an overview of current GBS prevention and treatment strategies. In the second presentation, the genomic and antimicrobial resistance profiles of invasive and colonizing GBS strains were presented. The third presentation explained the association of intrapartum antibiotic prophylaxis (IAP) with the development of late-onset disease (LOD) and the interplay of host innate immunity and GBS. The fourth presentation evaluated the role of genomics in understanding horizontal GBS transmission. The fifth presentation focused on the zoonotic links for certain GBS lineages and the last presentation described the protective role of breastmilk. Talks were followed with interactive discussions and concluded with recommendations on what is needed to further GBS clinical research; these included: (i) the development of better risk

stratification methods by combining GBS virulence factors, serological biomarkers and clinical risk factors; (ii) further studies on the interplay of perinatal antimicrobials, disturbances in the development of host immunity and late-onset GBS disease; (iii) routine submission of GBS isolates to reference laboratories to help in detecting potential clusters by using genomic sequencing; (iv) collaboration in animal and human GBS studies to detect and prevent the emergence of new pathogenic sequence types; and (v) harnessing the plethora of immune factors in the breastmilk to develop adjunct therapies.

1. Introduction

Group B Streptococcus (GBS) is the leading cause of neonatal infections and is associated with high mortality and long-term neurodevelopmental impairments, which can result from early-onset disease (EOD, ≤7 days of birth) or late-onset disease (LOD, >7 days after birth) [1]. Several GBS vaccines are currently being tested in clinical trials, but are not yet licensed for use [2]. GBS vaccines administered to expecting mothers have great potential to protect infants by preventing GBS disease. While we wait for them to become available, and since immunization will not prevent GBS disease in very preterm infants, it is important we optimize current methods and procedures that allow us to identify newborns at risk of or with early- and late-onset GBS disease to provide timely and targeted prophylaxis or treatment, and to develop alternative prevention and control strategies.

Understanding the epidemiology of disease-causing GBS has been instrumental in developing prevention and control strategies. The use of whole genome sequencing has revolutionized GBS disease surveillance and provides a powerful tool to monitor changes in GBS epidemiology and understand GBS transmission patterns. This, combined with insights into GBS virulence and host factors associated with GBS colonization and/or invasive disease [3], is valuable for developing new GBS disease prevention strategies and therapeutic tools.

Researchers and healthcare professionals gathered at the third GBS Research Session, organized as part of European Society of Pediatric Infectious Diseases (ESPID) meeting in Athens, Greece, on 11th May 2022, to share and discuss recent advances in GBS pathophysiology, molecular and genetic epidemiology and how these new insights can help to improve current prevention and control strategies for newborns with increased risk of GBS disease.

2. Overview of the presentations

The third ESPID GBS Research Session brought together researchers and clinicians. The hybrid format allowed a broader geographical and disciplinary reach than previous meetings and enabled key researchers in the field to present their work. The session had a total of six presentations from experts in the field, which are summarised below.

3. Current GBS prevention strategies: Where do we stand?

The session started with Linde Snoek, (Amsterdam University Medical Centre, Netherlands) providing an overview of the current worldwide strategies to manage early-onset Group B Streptococcus (EOGBS) infections. To reduce negative long-term consequences, health practitioners should aim for prevention or timely treatment of newborns with an early-onset infection, while minimizing antimicrobial exposure for healthy infants. However, it remains challenging to identify newborns with an invasive GBS infection due to absent or non-specific symptoms in the early stages. In most European countries and the United States, GBS prevention guidelines recommend universal screening of pregnant women in late gestation or during labour, followed by intrapartum antimicrobial prophylaxis (IAP) for those who are confirmed GBS carriers [4,5]. This results in

approximately one-third of pregnant women being exposed to antimicrobials [6]. In the Netherlands and the United Kingdom, a risk-based strategy is used, where IAP is recommended for pregnant women only if they have certain risk factors, such as intrapartum fever or prolonged rupture of membranes [7,14]. However, in almost half of all newborns with early-onset GBS infection, maternal risk factors are absent, and infections in these newborns can therefore not be prevented under current risk-based policies [8]. The downside of both screening-based and risk-based prevention is that they cause substantial overtreatment with antimicrobials, potentially selecting for antimicrobial resistance (AMR) in GBS and other bacterial pathogens and disrupting neonatal intestinal microbiome establishment, with the risk of adverse consequences later in life [9]. Also, while adherence to EOD prevention guidelines is of great importance to prevent neonatal GBS disease, a recent review showed inadequate adherence to maternal GBS screening guidelines and IAP administration in multiple European countries, ranging from 23 % adherence in Germany to 89–96 % adherence to screening guidelines in France [10]. Prospective studies in Greece and the Netherlands also found low compliance rates to screening and risk-based GBS prevention guidelines, respectively [11,12]. To try and improve on the performance of current GBS management methods, the neonatal early-onset sepsis calculator (EOSC) was developed. This is an online tool to estimate the risk of EOD based on maternal information, including GBS carriage status, and neonatal clinical findings, leading to management recommendations for the newborn [13]. The EOSC has been implemented in multiple hospitals in California [13]. The updated National Institute for Health and Care Excellence (NICE) guideline in the United Kingdom has incorporated the use of the EOSC as an alternative strategy, under certain conditions [14]. In Wales, the EOSC was also incorporated in the national EOD guideline [15]. The EOSC is being implemented in a limited number of hospitals in the Netherlands within a research context [16]. Another approach, used in Italy, is based on serial clinical observations (SCO) of the newborn followed by antimicrobial treatment when certain symptoms develop [17]. The EOSC and SCO strategies were designed to reduce unnecessary antimicrobial treatment [18–20]. However, a recent study in the Netherlands showed poor sensitivity of the EOSC in identifying EOD patients, especially shortly after birth [21]. Therefore, better EOD risk stratification methods are needed, which could also include, for example, results from a maternal bedside polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP) test that could identify not only the presence of GBS, but also the invasive potential of the isolate if the molecular assay would include detection of key virulence factors. Another strategy could be adding blood biomarkers like Creactive protein in cord blood to improve the predictive accuracy of currently used approaches [22]. Longitudinal studies are needed to determine the impact on the long-term outcome in newborns who receive delayed initiation of antimicrobial therapy, so that risks associated with delayed treatment can be weighed against those associated with preventative (over)treatment.

4. AMR profile and genetic basis of hypervirulent GBS lineage causing disease in the Netherlands for 28 years (1987–2015)

In the second presentation, Uzma Khan (Wellcome Sanger Institute, United Kingdom) presented her findings on the genetic basis and AMR profile of 1,345 invasive and 694 colonizing GBS strains from the Netherlands (between 1987 and 2020). Previously, Jamrozy et al. [23] showed that the increased incidence of neonatal invasive GBS disease in the Netherlands (1985–2015) was caused by the expansion of two GBS sub-lineages of clonal complex (CC) 17: sub-lineage CC17-A1 and sublineage CC17-A2 [23]. The expansion of both CC17-A1 and CC17-A2 was attributed to the acquisition of phage phiStag, which appeared in 1997 in Dutch invasive GBS isolates [23]. Dr Khan showed in her talk that phiStag was also prevalent in more than half of CC17-A2 carriage isolates. The close phylogenetic relationship between CC17 carriage and invasive isolates confirms that carriage and invasive isolates belong to the same genetic population. Another important finding was the high

proportion (18 %) of multi-drug resistant (MDR) carriage and invasive GBS strains, with resistance to aminoglycosides, macrolides and tetracyclines, with all these isolates belonging to the CC17-A2 sub-lineage. Almost all of these MDR isolates (46/47, 97.8 %) were also positive for the phiStag phage. Additionally, all CC17 MDR isolates harboured integrative conjugative element ICESag37, which is known to carry multiple AMR genes [24]. The ICESag37 element was also found in MDR CC17-A2 isolates from multiple countries other than The Netherlands. Therefore, this ICE element is probably responsible for disseminating these multiple AMR genes in CC17-A2 isolates globally. However, since GBS remains susceptible to antimicrobials commonly used in clinical practice, such as penicillin and amoxicillin, it is unclear whether the spread of these resistance genes directly led to the expansion of sublineage CC17-A2. Dr Khan and her team hypothesize that these resistance genes could have led to more efficient colonization or transmission through other mechanisms than purely selection based on antimicrobial exposure, or that the presence of these AMR genes is strongly associated with other genetic variants that improve fitness [25]. Whereas 85 % of CC17 isolates originated from normally sterile body sites (blood/CSF), 60 % of isolates from another common GBS lineage, CC1, were from non-sterile sites (rectovaginal), indicating that CC1 is more likely to be associated with colonisation than with invasive disease. Future studies should investigate differences in surface protein genes between the colonizing and invasive isolates, and their role in colonisation and invasive potential. These important results on characterizing the genetic determinants of invasive and colonizing GBS lineages show that it is possible to identify hypervirulent GBS lineages. As genome sequencing is becoming more accessible in clinical practice, this may be of value to characterize GBS isolates from newborns to improve GBS EOD prediction and inform disease management as needed, or to incorporate virulence markers in nucleic acid based point-of-care tests based on PCR or LAMP.

5. Pathogenesis and immunity of GBS meningitis

In the third presentation, Philipp Henneke (University Medical Center Freiburg, Germany) explored why IAP, which prevents earlyonset GBS sepsis, may be associated with an enhanced risk to acquire LOD [26] and discussed neonatal immune mechanisms that may facilitate sepsis developing from colonizing bacteria. Among pregnant women colonized with GBS in the anogenital tract, half will transfer the organism to their newborn infant. Yet, GBS sepsis occurs in less than 1 % of GBS positive infants [26], implying that GBS normally reaches its niche without health implications for its infant host. At barrier tissues such as the intestine and the skin, macrophages exert critical immunity to mucocutaneous pathogens like GBS, in part via recognition of bacterial nucleic acids by endosomal Toll-like receptors [27,28] in a cell differentiation state dependent fashion [29]. In the first postnatal weeks, embryonic lamina propria macrophages are replaced by bone marrow derived macrophages. This process depends on microbial signals as well as circulating monocytes [30–32]. Perinatal antimicrobial treatment interferes with intestinal macrophage development [32,33] in addition to impacting on bacterial clearance by decreasing circulating neutrophils [34]. In the central nervous system (CNS), macrophages differ with respect to origin: meningeal macrophages and microglia share a common prenatal progenitor, whereas perivascular macrophages originate from perinatal meningeal macrophages [35]. In the CNS, origin and differentiation has functional implications, as engrafted bone-marrow-derived macrophages functionally diverge from resident yolk sac-derived macrophages/microglia [36]. In multiples with simultaneous late-onset GBS sepsis, usage of perinatal antimicrobials and negative GBS-culture from the mother near birth were associated with GBS sepsis in the offspring after day 7 [26]. Thus safe incorporation of GBS into the individual microbiota requires a delicate interplay of host innate immune and microbiota development at defined body sites. It is tempting to speculate that early GBS exposure may foster hostpathogen and/or pathogen microbiota adaptation conferring individual resistance to the organism.

6. Horizontal GBS transmission: Insights from NICU clusters and adult disease

In the fourth presentation, Elita Jauneikaite (Imperial College London, United Kingdom) discussed her work on potential horizontal transmission in LOD cases in young infants in hospital settings. Enhanced surveillance was undertaken for all late-onset GBS in a high dependency neonatal intensive care unit in London for two years. It revealed four distinct clusters caused by GBS sequence type (ST)1 serotype V (4 cases), ST23 serotype Ia (3 cases), ST139 serotype Ib (2 cases) and ST17 serotype III (2 cases), respectively [37]. Genome sequencing showed that 11/12 outbreak isolates were genetically linked to at least one other LOD isolate, suggesting horizontal transmission [37]. To further our understanding of potential GBS transmission in hospital settings, a systematic review was carried out to identify reported outbreaks of invasive GBS (iGBS) disease in hospital settings; the review identified 30 hospital clusters in 11 countries over an approximately 50-year period, of which 26 involved neonates and four involved adults [38]. Although hospital outbreaks of iGBS disease are considered to occur infrequently; they are likely to be under-reported. An additional key finding from the review was that long intervals (~50 days) between cases of late-onset GBS might impede the detection of GBS outbreaks [38]. To build on this work, Dr Jauneikaite and colleagues investigated 410 GBS genomes from iGBS disease in infants in the UK and the Republic of Ireland between 2014 and 2015 [39]. Cases with ≤10 single nucleotide polymorphisms (SNPs) differences were linked with their epidemiological data and investigated as potential clusters. In total, seven potential clusters of infant GBS disease were identified, four of which had not previously been described in publications, emphasizing the importance of routine submission of GBS isolates to reference laboratories to detect potential clusters using genomic sequencing [39]. Future studies should also include an investigation into multiple GBS serotype/genotype co-colonisation [40] and its association with risk of LOD, transmission routes of recurrent or relapsing iGBS disease, and the potential for GBS carriage and environmental contamination in nosocomial settings.

7. GBS in babies, adults and animals: Is there a link?

In the fifth presentation, Ruth Zadoks (Sydney School of Veterinary Science, Australia) discussed GBS in animals, babies and adults and whether there is a potential zoonotic link for certain GBS lineages. GBS, also known as Streptococcus agalactiae, is not only a commensal of the lower gastrointestinal and urogenital tract in humans and the leading cause of invasive disease in neonates. It is also a major cause of mastitis in dairy cattle, sepsis in tilapia (the third most commonly farmed fish species in the world) and skin and soft tissue infections in camels and sporadically infects many other animal species, including amphibians, reptiles, cartilaginous fish, boney fish, aquatic mammals, and terrestrial mammals [41–43]. In addition, insects can be infected experimentally [44]. Only birds are not known to be susceptible to GBS. Based on epidemiological and evolutionary data, transmission between humans and animals happens in both directions [41]. For example, an outbreak of foodborne GBS disease in Singapore in 2015 was attributed to the consumption of fish with GBS genotype ST283, which is not known to be hypervirulent in babies but caused severe invasive disease in previously healthy adults, resulting in sepsis, meningitis and osteoarthritis [45]. The emergence of ST283 is traced to the 1980s, in parallel with the emergence of intensive fish farming, and possibly due to the acquisition of fish-associated virulence genes by human-derived GBS [46]. Likewise, the acquisition of a lactose operon for fermentation of milk sugar may have contributed to the emergence of GBS as a mastitis pathogen in cattle [47]. Reverse zoonotic transmission, i.e. transmission of GBS from humans to animals, is more common than animal-to-human transmission, and may hinder the success of animal disease control campaigns [41]. In the 1950s and 1960s, mastitis control programmes led to the successful elimination of bovine GBS [48–50] in several European countries. However, in the early 21st centry, GBS re-emerged in cattle in northern Europe

[47,51]. Many isolates belonging to newly emerged bovine lineages carry a tetracycline resistance gene, previously identified as a marker of human host adaptation, suggesting that these bovine isolates may have a human origin [25,47]. At the moment, there is no evidence of GBS strains shared between babies and animals, or for animal exposure or food of animal origin as a risk factor for iGBS in neonates. The main neonatal GBS clade, CC17, is a host-specialist, largely limited to humans. Other clades, e.g., ST23, are host generalists. The different host species provide host generalist GBS clades with access to different accessory gene pools, including virulence genes and AMR genes. Access of GBS to this broad pool of genes and selective pressures may contribute to the emergence of new GBS strains and transmission routes, potentially leading to new animal and public health threats [41,42]. Collaboration between human and animal GBS experts is needed to monitor and recognize such risks.

8. GBS - protection or transmission through breastmilk

In the sixth presentation, Kirsty Le Doare discussed the controversies around breastfeeding and GBS. GBS can be found in breastmilk in 3.5 % of women [26,52,53]. Whereas GBS is part of the microbiome in the breastmilk of healthy donors, it is also found in women with mastitis [54]. The entero-mammary circulation has been hypothesized as one possible pathway for GBS migration to milk, with acquisition through the infant's oral and skin microbiota as an alternative mechanism [55]. Although there are 30 case reports of iGBS disease with identical GBS strains concurrently identified in breastmilk, a case-control study of 92 cases with LOD and 268 healthy controls in Australia showed that breastfeeding was not associated with an increased risk of LOD (odds ratio 1.2 [95 % confidence interval 0.7–2.3]) [56]. In addition to its nutritional value, breastmilk contains bioactive factors that protect against respiratory and gastrointestinal diseases and commensal bacteria that contribute to the development of an infant's microbiome [57]. Higher secretory immunoglobulin A (SIgA) concentrations in breastmilk of Gambian women reduced infant colonization with GBS in the first three months of life [58]. Women with high anti-GBS IgG in serum had reciprocally high anti-GBS IgG and anti-GBS SIgA in breastmilk that persisted to two months post-partum [59]. Human milk oligosaccharides (HMOs) also play a role in preventing infection [60]. HMOs bind GBS in vitro[61], exert a bacteriostatic effect[62] against GBS and may reduce infant colonization [63]. In addition, interaction between breastmilk and GBS within the infant's intestinal tract HMOs alter biofilm growth [64] and affect bacterial cell wall permeability in a strain-dependent manner [65] resulting in higher susceptibility of GBS strains to clindamycin and gentamicin [66]. Secretor status (active or inactive copy of the FUT2 gene) determines a mother's ability to make certain protective HMOs like 2'-fucosyllactose (2'FL Maternal secretor status testing could be utilised in the future for clinical purposes as a breastmilk screening tool for GBS risk [67]. Further studies investigating how breastmilk may protect against GBS adhesion and invasion of the infant's intestine will help to inform more targeted vaccination strategies. Additionally, neonatal intestinal organoid models based on features of term and preterm infants' intestines will add to our understanding of the interaction between breastmilk and GBS within the infant's intestinal tract.

9. Summary of the meeting

The third ESPID GBS Research Session brought together researchers and clinicians to explore ways of improving prevention and control of early- and late-onset GBS infections The existing screening- and risk-based strategies for pregnant women aim to prevent EOGBS disease, but they have several limitations, including suboptimal sensitivity and specificity in predicting iGBS disease. Both strategies may lead to unnecessary antimicrobial treatment for healthy mothers and newborns who would be unlikely to get GBS disease, and risk-based strategies, in particular, miss many cases. Some challenges with GBS screening include intermittent GBS carriage during pregnancy, the potential of false-

negative cultures, the need to use a specific culture method to ensure that GBS carriage is detected and the delay in receiving results from culture-based methods leading to inadequate IAP coverage [68,69]. Additionally, IAP does not prevent GBS disease in preterm infants, since screening is recommended at 35–37 weeks of gestation and many infants are born before screening occurs. Novel approaches such as the EOSC that combine maternal risk factors and clinical assessment of infants to produce a more nuanced risk stratification have reduced overtreatment of healthy infants, but have poor sensitivity [19,21]. Given that current EOGBS risk stratification methods have suboptimal sensitivity or specificity, we could draw from recent advances in molecular diagnostics and genomic epidemiology to address the issue. PCR and LAMP are commercially available and their potential for routine clinical use is already published or exploited. As genome sequencing is becoming quicker, more affordable and readily available, more robust risk prediction tools could be developed by combining GBS virulence factors and other information from genomes with serological biomarkers and patient clinical risk factors. In addition, it has been shown that whole genome sequencing plays an important role in disease surveillance, highlighting the potential for close to real time detection of hospital clusters of iGBS cases by routinely submitting GBS isolates to reference laboratories.

Another pressing issue is the increasing incidence of late-onset GBS, which calls for a better understanding of the pathophysiology of late onset GBS and novel approaches to its prevention. Perinatal antibotics lead to alterations in the newborn's intestinal microbiota and disturbances in the development of host immunity [26]. Associations between perinatal antimicrobials and the development of LOD have been found [26]. Given the long-term neurodevelopmental sequelae associated with GBS meningitis, which is common in LOD, further exploration of the role of CNS macrophages in neuronal damage is crucial. Furthermore, a growing body of evidence supports the role of molecules within breastmilk, especially HMOs, as antimicrobial and anti-biofilm agents against GBS, opening novel opportunities for potential adjuvants to current prevention and treatment strategies. Understanding how GBS adheres to and invades tissue in the infant intestine may provide insights into LOD, especially in preterm infants, who remain a significant risk group.

Finally, we should not forget that GBS is a multi-host pathogen shared between humans and animals. Recognizing that different hosts provide access to a large gene pool that can be acquired by GBS, with the potential for subsequent transmission between humans and animals in both directions is key to improving human and animal health.

10. Recommendations

• Since current guidelines and the EOSC for the prevention and treatment of early-onset infections have poor sensitivity, specificity, or both, better diagnostic tools and risk stratification methods are needed.

• The phiStag phage that potentially contributed to the expansion of two invasive GBS CC17 sub-lineages (CC17-A1 and A2) in the Netherlands was also present in most carrier isolates from the same sub-lineages. A proportion (18 %, 47/262) of CC17-A2 sub-lineage carriage and disease isolates were multi-drug (aminoglycosides, macrolides and tetracyclines) resistant, possibly encoded through mobile genetic element, ICESag37. In addition to this, almost all of these ICESag37 positive isolates (46/47, 97.8 %) carried phiStag phage. Genome sequencing could improve risk stratification based on such detailed GBS genomic characterisation.

• Perinatal antimicrobials interfere with intestinal macrophage development and decrease the number of circulating neutrophils. Together with the absence of maternal GBS carriage, perinatal

antimicrobials may be associated with the development of late-onset GBS disease in multiple births. Early exposure to GBS in newborns might provide individual resistance, leading to a decreased risk of late-onset GBS disease. This is an important hypothesis that should be studied further.

• Hospital outbreaks of invasive GBS among neonates and adults might be underreported. Routine submission of GBS isolates to reference laboratories could help in detecting potential clusters by using whole genome sequencing.

• GBS is a multi-host pathogen and transmission between animals and humans takes place. Therefore, a One Health approach, including collaboration in animal and human GBS studies, is required to understand the GBS pangenome, to detect and prevent the emergence of new strains and to optimise disease control in humans and animals alike.

• There is limited evidence that breastmilk is implicated in LOD or recurrence of GBS infections in babies. On the other hand, breastmilk contains a plethora of immune factors developed from the maternal immune system (sIgA, IgG, HMOs, microbiota) that play a role in prevention of GBS infection. Understanding these molecules and their role in preventing GBS infection may allow the development of adjunct therapies.

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