Neonatal invasive disease caused by *Streptococcus agalactiae* in Europe: the DEVANI multi-center study

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Research Article

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Abstract

Purpose

Group B streptococcus (GBS) remains a leading cause of invasive disease, mainly sepsis and meningitis, in infants < 3 months of age and of mortality among neonates. This study, a major component of the European DEVANI project (Design of a Vaccine Against Neonatal Infections) describes clinical and important microbiological characteristics of neonatal GBS diseases. It quantifies the rate of antenatal screening and intrapartum antibiotic prophylaxis among cases and identifies risk factors associated with an adverse outcome.

Methods

Clinical and microbiological data from 153 invasive neonatal cases (82 early-onset[EOD], 71 late-onset disease [LOD] cases) were collected in eight European countries from mid-2008 to end-2010.

Results

Respiratory distress was the most frequent clinical sign at onset of EOD, while meningitis is found in > 30% of LOD. The study revealed that 59% of mothers of EOD cases had not received antenatal screening, whilst GBS was detected in 48.5% of screened cases. Meningitis was associated with an adverse outcome in LOD cases, while prematurity and the presence of cardiocirculatory symptoms were associated with an adverse outcome in EOD cases. Capsular-polysaccharide type III was the most frequent in both EOD and LOD cases with regional differences in the clonal complex distribution.

Conclusions

Standardizing recommendations related to neonatal GBS disease and increasing compliance might improve clinical care and the prevention of GBS EOD. But even full adherence to antenatal screening would miss a relevant number of EOD cases, thus, the most promising prophylactic approach against GBS EOD and LOD would be a vaccine for maternal immunization.

Introduction

Streptococcus agalactiae, also termed Group B streptococcus (GBS), is a frequent causative agent of invasive bacterial infections in infants and contributes substantially to neonatal morbidity and mortality globally [1]. When occurring during the first six days of life, the infection is termed early-onset disease (EOD), and between days 7 and 89, late-onset disease (LOD). In EOD cases, infected neonates rapidly develop bacteremia with or without sepsis and/or pneumonia and less commonly meningitis. In LOD cases, neonates are usually bacteremic without focus and often develop meningitis [2, 3]. The main risk

factor for EOD is maternal rectovaginal colonization with GBS at the end of the pregnancy; additional established risk factors include prolonged rupture of membranes (ROM) before delivery (> 18 hours), intrapartum fever (≥ 38°C), GBS bacteriuria during the current pregnancy, a previous infant with GBS disease, and preterm delivery at less than 37 weeks of gestation [4]. Risk factors and the transmission route for LOD are less well established; the GBS may be acquired from the mother or from environmental sources [5, 6].

With the implementation of prevention strategies in many countries, the incidence of infant GBS disease globally has declined to an estimated 0.49/1,000 live births, with a probable underestimation in developing countries [7, 8]. The incidence in Europe is similar, with important regional differences [5, 7, 9–11]. The EOD incidence has been successfully reduced by universal antenatal screening and appropriate intrapartum antibiotic prophylaxis (IAP) programs for colonized pregnant women [7]. These include a standardized culture-based detection of GBS in a vaginal-rectal swab in late pregnancy (between 35 and 38 weeks, depending on national guidelines) combined with intravenous administration of antibiotics during labor to GBS-positive women. Alternatively, a risk factor-based approach for IAP is implemented in some countries [4]. National guidelines in Europe propose different strategies, though efforts are being made to standardize these recommendations [12]. Whatever the recommended strategy is, these measures have failed to substantially reduce the incidence of LOD [13].

GBS express a type-specific capsular polysaccharide (CPS), one of the most important GBS virulence factors [14]. To date, ten antigenically distinct types have been described (Ia, Ib, II to IX) [15]. GBS isolates can be further characterized by detection of surface proteins such as the pili, which are also known virulence factors [14, 16]. Furthermore, multi-locus sequence typing (MLST) characterizes the isolates into distinct sequence types (ST) clustered into clonal complexes (CC), which may provide information on the pathogenicity of the isolates. CC17, as an example, represents a hyper-virulent cluster of clones that is increasingly found in LOD cases and frequently causes meningitis, while also containing an emerging sub-lineage of multi-drug resistant isolates [17–19]. Usually, GBS isolates are considered uniformly susceptible to penicillin with the exception of very few invasive isolates initially described in Japan and later in the USA presenting reduced susceptibility to penicillin (PenRGBS) and other β-lactam antibiotics [20]. Today, in Japan, these isolates have increased up to 14% and higher. More recently, rare cases of PenRGBS have been reported from Canada, China, England, Germany, Italy, Korea [21]. More widespread is the increased resistance to erythromycin and clindamycin [17, 21, 22] and emerging resistance to fluoroquinolones [21].

Even if efficient to prevent numerous EOD cases, current preventative strategies do not appear to be adequate for the expected control of GBS disease, therefore, additional efforts are urgently required. Vaccines for pregnant women are promising approaches to protect infants through placentally transferred antibodies, they have been successfully tested in early phase clinical studies [23–25]. Vaccine development programs require detailed microbiological characterization of circulating strains [26]. Current efforts are being made to develop conjugated CPS-based or protein-based (e.g. pili) vaccines, and

to define correlates of seroprotection to facilitate the assessment of vaccine efficacy [27], which has been shown to be mediated mainly by opsonophagocytic antibodies [28].

The DEVANI (Design of a Vaccine Against Neonatal Infections) consortium was established in 2008 as a pan-European initiative to assess neonatal GBS disease burden in Europe, to provide clinical and microbiological information for vaccine design, and to improve laboratory performance in diagnosing GBS colonization and infection [29]. The DEVANI project also included an investigation of serological correlates of neonatal protection through the analysis of CPS- and pilus-specific immune responses in sera from 984 GBS colonized and 473 non-colonized pregnant women who delivered healthy neonate and in mothers of 153 infants with GBS disease [28]. From this cohort, Fabrini et al. demonstrated that IgG levels against CPS and pilus proteins were significantly higher in GBS colonized women delivering healthy neonates than in mothers of babies with GBS disease or non-colonized women. Here, we report on the clinical and microbiological characteristics of invasive neonatal GBS infections along with frequencies of maternal risk factors of 153 neonatal cases collected over a 2.5-year acquisition period in eight European countries.

Methods

Study design and participating countries

The DEVANI study design has been widely described by Rodriguez-Granger et al. [29]. In summary, for the description of invasive neonatal disease, clinical characteristics and GBS isolates from cases have been collected between mid-2008 and end-2010 in eight European countries (Belgium, Bulgaria, Czech Republic, Denmark, Germany, Italy, Spain and the UK). The study has been approved by local ethics committees of each participating institution, and informed consent for study participation has been obtained from the legal caregivers. According to the estimated number of births per participating institution, the expected number of eligible cases of GBS invasive disease ranged from 100 to 200. The number of reported cases from each participating country is shown in the online resource figure S1a and S1b. Overall, 82 EOD cases and 71 LOD cases were included in the study.

Data collection and case definitions

Eligible cases were identified through daily surveillance according to the following inclusion criteria: GBS isolation from blood or cerebrospinal fluid (CSF) in infants aged 1–6 days (EOD) or ≥ 7 days up to 89 days (LOD) or ≥ 90 days (very late-onset disease [VLOD], included in the LOD groups). For each case, obstetrical information, clinical signs at onset of disease, paraclinical assessment of infection, microbiological characteristics of GBS strains and information on management and outcome were collected and saved in a secured online database according to the defined criteria of the DEVANI project. Infants were regarded as preterm if they were born earlier than 37 weeks of gestation.

Collection of GBS isolates and strain characterization

Cultures of clinical specimens collected from normally sterile sites, such as blood and CSF, were processed according to local microbiological procedures. All GBS isolates were sent to national central laboratories. GBS identification was confirmed by an agglutination method for the detection of the Lancefield group B antigen [30], stored and further characterized. Capsular serotyping was performed by standardized latex agglutination tests using the Strep-B latex kit (Statens Serum Institut, Denmark) [30, 31]. Multiplex polymerase chain reaction (PCR) assay was used to determine capsular genotype as described previously [32–34]. Repeated external quality assessments had been organized and ensured the accuracy of the results for the different typing methods [29]. Isolates were further characterized by MLST to identify the STs and CCs [18, 19].

Clinical and laboratory assessment

C-reactive protein (CRP) levels and chest X-rays were analyzed according to local standards. Clinical examinations were performed by local medical staff and reported using a standardized online tool, that was the DEVANI web database, by members of the local study groups. CRP values were considered significantly elevated above a threshold of 10 mg/l.

Statistical analysis of risk factors

The relative risk values were calculated by Graphpad prism 9 using the Koopman asymptotic score and Fisher's exact test to determine the p-value. Two-tailed Fisher's exact test was calculated with the online tool "Graphpad Quickcalcs" (https://www.graphpad.com/quickcalcs/). CRP values were compared using unpaired t-test. P-values below 0.05 were considered statistically significant.

Results

Clinical characteristics of neonatal invasive disease

Overall, 153 neonatal cases with invasive GBS disease were collected. 82 EOD cases were included in the study, all of which had a positive blood culture, while six patients (7.3%) additionally had GBS culture-positive meningitis (as shown in the online resource fig. S1d). Sixty-six of the EOD cases (80.5%) were term neonates and the overall median gestational age was 39 weeks (fig. S1d). Seventy neonates (86.4% of EOD cases) presented symptoms at birth or within the first day of life (fig. S1e). Of the 71 LOD cases (including seven VLOD) enrolled in the study, 67 (94.4%) showed bloodstream infection, while 17 (23.9%) had GBS culture-positive meningitis, i.e., four patients (5.6%) had a positive GBS culture only from the CSF and 13 (18.3%) had both a positive blood and a positive CSF culture (fig. S1d). Among the LOD cases, 69% were term infants while 22 (31.0%) were preterm infants, and the overall median gestational age is 38 weeks (fig. S1c). The median age at disease-onset was 34 days, the maximum was 114 days (fig. S1f). The cumulative age-dependent incidence, including both EOD and LOD cases, is presented in fig. S2. The sex ratio male/female was 1.16 for the EOD cases and 0.97 for the LOD cases (Fig. S1g).

The clinical presentation differed between EOD and LOD cases (Fig. 1a). At onset, in EOD cases, respiratory symptoms were predominant (67.1%), while LOD cases more frequently presented with fever

(66.2%) and neurological signs (52.1%). Cardiocirculatory and gastrointestinal (GI) symptoms were equally frequent in EOD and LOD cases (Fig. 1a). Chest X-rays were obtained from 46 (56.1%) of EOD cases and from 30 (42.2%) of LOD cases. As expected, EOD cases were more likely to have an abnormal X-ray finding than LOD cases (58.7% vs 23.3%, p = 0.0043), with “interstitial diffuse thickening” being the most common sign (Fig. 1b). Preterm neonates with EOD exhibited a different clinical presentation dominated by apnea (p = 0.0026) and cardiocirculatory symptoms (p = 0.0434) and a tendency to more GI symptoms and less fever than term neonates (Fig. 1c). CRP levels were determined in 73 EOD cases (89.0%) and 61 LOD cases (85.9%). Maximum CRP levels during the disease course ranged from < 3 to 701 mg/l (median = 41 mg/l, interquartile range [IQR] 10–102 mg/l) in EOD and from < 3 to 479 mg/l (median = 55 mg/l, IQR 17–133 mg/l) in LOD cases. CRP levels remained below the threshold value of 10 mg/l in 13 EOD and 7 LOD cases (Fig. 1d).

Microbiological characteristics of GBS isolates from neonatal invasive disease

A CPS type assessed by latex agglutination and genotyping was assigned to all isolates. For EOD, the distribution showed a predominance of type III, V and Ia (50.0%, 20.7% and 17.1%), followed by types II, IX, IV and Ib (as shown in online resource fig. S3a). For LOD cases, type III was predominant with 77.5%, followed by types Ia (15.5%), V, II and IV (fig. S3b). The distribution and *in vitro* expression of the three pilus variants assessed in 146 out of 153 neonatal strains had been previously reported [28]. MLST data is available for 79/82 (96.3%) of EOD and for 56/71 (78.9%) of LOD isolates. It revealed a heterogenous CC distribution in EOD cases, with 32.9% of the isolates clustering in CC17 while, overall, ten different CCs were detectable (fig. S3a). CC17 was found only in CPS type III isolates and accounted for 27/39 (69.2%) of the CPS type III EOD cases for which MLST data was available. In LOD, CC17 is responsible for 40/43 (93.0%) CPS type III cases, i.e., 71.4% of all LOD cases for which MLST data was available (fig. S3b). Thus, CC17 (as does CPS III) represented a significantly higher proportion among LOD (71.4%) than EOD (34.2%) cases (rate ratio [RR] 2.09; confidence interval [CI] 1.49–2.99). The prevalence of CC17 in EOD cases differed substantially between different European countries, ranging (within countries with more than four reported cases) from 18% in the UK to 67% in Italy. The same was observed among LOD cases, the prevalence of CC17 ranged from 40% in Spain to 86% in Italy. Only five different CCs were found among the LOD isolates (fig. S3b).

Risk factors for invasive neonatal disease

Across all cases (EOD and LOD), antenatal maternal GBS colonization status had been performed for 73 mothers (47.7%). In countries with recommendations for universal screening, among cases’mothers, that is a selected population where preventive strategy failed, rates of screening cultures were 72.7% (8/11) in Belgium, 39.5% (15/38) in Germany, 66.7% (32/48) in Italy, 54.2% (13/24) in Spain and 33.3% (1/3) in Czech Republic. Reasons for the low reporting rate were not assessed but likely include at least premature delivery before timing of screening, lack of adherence to guidelines by health care providers, refused screening offers by pregnant women and incomplete data traceability. In countries without universal screening, GBS screening rates were 30% (3/10) in Denmark, 6.7% (1/15) in the UK and 0% (0/4) in

Bulgaria. In 33 of the EOD cases (40.2%), antenatal maternal GBS colonization status had been assessed. Of those, 48.5% (n = 16) were positive (Fig. 2a). Of the 16 EOD cases with a positive antenatal screening result, only seven mothers (43.8%) had received IAP, and the first dose had been given less than four hours before birth in three cases, leaving a total of four mothers within our cohort of 82 EOD cases (4.9%) having received appropriate IAP (> 4 hours). 19/39 LOD cases (47.5%) with antenatal screening results were born to mothers with confirmed positive GBS carrier status (Fig. 2a), four (21.1%) of whom received adequate antenatal IAP.

The delivery mode was vacuum/forceps-assisted in 10 EOD cases (13.0%); and 21 EOD cases (27.3%) required an emergency C-section (Fig. 2b). Established maternal risk factors for EOD included ROM (> 18h) in 14 EOD cases (19.7%), and intrapartum fever (≥ 38°C) in nine EOD cases (13.0%). GBS bacteriuria, a surrogate marker for heavy maternal colonization, was reported in 17.6% of EOD cases (n = 9). One mother (1.4%) had a previous infant with invasive GBS disease (Fig. 2c). Breastfeeding before disease onset was reported in 85.2% (52/61) of LOD cases (Fig. 2d).

Outcome of neonatal invasive disease

Antibiotic treatment was initiated in most EOD cases after the onset of symptoms (53/78; 67.9%), otherwise as prophylactic treatment. 10.0% (7/70) of EOD cases and 7.8% (5/64) of LOD cases had an unfavorable outcome, i.e., early neurological sequelae or death (Fig. 3a). Early neurological sequelae included all neurological abnormalities detected during the initial in-hospital treatment period, no later evaluation is included in the current study, as it was not part of the original study design. Potential predictive factors for an adverse outcome in EOD were the occurrence of cardiocirculatory symptoms (RR 3.00; CI 1.56–4.76) and prematurity (RR 4.09; CI 1.77–7.86) (Fig. 3b), while the diagnosis of meningitis or the detection of a CC17 strain were not significantly associated with an adverse outcome. Conversely, in LOD cases, meningitis was the only predictive factor for an adverse outcome (RR 2.95; CI 1.28–4.98), though 80% of meningitis cases recovered without early sequelae (Fig. 3c). Of note, this assessment was based on the clinical diagnosis of meningitis (n = 20), as opposed to a positive CSF culture (n = 17).

Discussion

The present study reports on the clinical and microbiological characteristics, as well as risk factors and outcome in 153 infant patients with invasive GBS disease from eight different European countries, a cohort collected within the framework of the EU-funded DEVANI project. Among the countries included in the DEVANI consortium, epidemiological and clinical data related to GBS neonatal disease were not equal, sometimes very poor or lacking.

Concordant with previous publications, GBS EOD presented shortly after birth with predominantly respiratory symptoms [35]. The time point of infection was indicative of intrapartum or in-utero GBS transmission from the colonized mother. At the time of the study, a screening-based strategy was recommended in Belgium, Czech Republic, Germany, Italy and Spain; nevertheless, mothers of numerous EOD cases had not been screened accordingly in these five countries, partly due to preterm birth before

reaching the recommended time point for screening. But among other reasons for the low reporting were likely lack of adherence to guidelines by health care providers, refused screening offers by pregnant women and incomplete data traceability. Indeed, poor adherence to existing recommendations has been identified as a factor limiting the effectiveness of antenatal screening [36]. On the other hand, a risk-based strategy was applied in the UK, Bulgaria and Denmark, however, a lower number of EOD cases' mothers had had antenatal screening. In summary, in our study, less than half of EOD cases' mothers (33/77) had an antenatal swab taken and of those, 48.5% were identified GBS-positive, which is concordant with previous studies [37, 38]. Concerning the presence of risk factors, the rate was even lower with 33% of parturients (to EOD cases) presenting with at least one risk factor, a frequently described limitation to this strategy of prevention [39, 40]. Thus, both strategies were likely to miss opportunities for preventing neonatal invasive cases. In addition, even with the knowledge of an existing positive screening result, IAP was not administered timely in a significant number of cases, a finding concordant with other publications [5, 6, 36, 38]. The reasons for this non-compliance had not been systematically analyzed but were potentially due to the limited time period between arrival in the hospital and birth. These considerations reflected some important limitations of both prevention strategies. The low sensitivity of antenatal screening methods needed to be improved. And, as most cases occurred in situations where testing or treating for GBS in pregnant women was not done according to the standard of care, emphasizing the necessity to more stringently adhere to the existing recommendations in order to improve the overall quality of care. They also highlighted the need to harmonize recommendations for prevention throughout Europe and for an alternative approach, i.e., maternal GBS immunization.

GBS LOD cases differed substantially with regards to clinical presentation and microbiological characteristics. A high proportion of cases caused by GBS CC17 (71.4% of all sequence typable strains), known to be hypervirulent, was detected in our LOD cohort, however, at a rate lower than recently published findings from the USA [20], but similar to findings from France and China [41, 42]. More than half of the LOD cases showed neurological symptoms, confirming GBS LOD to be a neurotropic disease. The transmission route in GBS LOD remains controversial, though transmission via breast milk has been reported [6, 43]. Other routes include neonatal colonization during birth [44], nosocomial infection through health-care workers [45] and others [6, 43, 46]. Nine LOD cases (14.8%) of our cohort had not received breast milk before the onset of symptoms. The colonization rate in mothers of LOD cases was roughly 50%, a rate similar to previously published findings [44], much higher than the ~ 20% in the general population [47]. Other than that, no information on the transmission route was available from our data. Known risk factors for LOD included prematurity and a positive antenatal GBS culture [48].

Adverse outcomes are still repeatedly reported with invasive GBS disease despite adequate treatment, and the preterm cohort is especially at risk [49]. In our EOD cohort, this is mostly associated with cardiorespiratory failure. Death from LOD had occurred in only one case in our cohort, 3/20 cases with clinical meningitis (15%) had survived with neurological sequelae though such sequelae were more frequently reported after GBS meningitis, [50]. However, no long-term follow-up data was available for our cohort. Overall, the mortality (5.7% in EOD and 1.6% in LOD) was lower than or similar to most published cohorts [41].

The study revealed relevant differences in clinical practice between the different participating countries such as the percentage of pregnancies with antenatal screening and general clinical practice such as performing an X-ray for neonates with invasive infections (data not shown). Antenatal GBS screening was recommended in many European countries between 35 + 0 and 37 + 0 weeks of gestation, but not always covered by health insurance resulting in difference of compliance and not generally recommended as in the UK, Bulgaria and in Denmark [51]. Among these countries as well as in other European countries, during the last decade, guidelines have not evolved significantly even if attempts towards a European consensus has been done but not yet concluded [12]. So, it seems imperative to harmonize the recommendations in order to improve the quality of care and to systematically assess effects of measures taken across Europe.

Microbiological characteristics showed a wide distribution between European countries, especially the frequency of isolates attributable to CC17 in LOD, which range from 40% in Spain to 86% in Italy. This is likely to impact on the disease course, e.g., the prevalence of meningitis in different countries.

Limitations of our study include the lack of information on the actual incidence, as no steps were implemented to guarantee that every case was reported. Furthermore, the observational period extends only during inpatient care, thus probably missing the detection of neurological sequelae that frequently become apparent only later in life [50]. This study did not include a control group of healthy neonates, thus clinical characteristics cannot specifically be attributed to GBS disease and the specificity of maternal risk factors cannot be determined. The delay in reporting is attributable to difficulties in data management that were only recently resolved. However, the clinical findings in our cohort are still relevant for to the actual situation of invasive GBS infections in neonates and young infants and reflect the spectrum of GBS disease nowadays. Even if the epidemiology of GBS disease has changed over the last decade, knowledge about the low acceptance rate of GBS screening and the low rate of appropriate intrapartal antibiotic prophylaxis is an eye-opener to GBS policy makers in current days. Indeed, our study includes data from eight European countries for which no or very few more recent epidemiological data has been published and where recommendations for prevention of GBS neonatal disease has not much changed.

Conclusions

This study describes the clinical and microbiological properties of 153 invasive infant GBS cases collected within the framework of the European DEVANI consortium. The originality of the study lies in the detailed description of clinical characteristics including specific features of premature infants. It identifies potential predictors of an adverse outcome, including cardiocirculatory symptoms and prematurity for EOD and meningitis for LOD. Microbiological characteristics were assessed via a harmonized protocol, ensuring comparability, and revealed important differences in strain prevalence throughout Europe.

The study emphasizes the importance of having standardized practical protocols and a better adherence for the prevention and care of invasive GBS disease. It also demonstrates that IAP indicated by a

prevention strategy, either screening-based or risk factor-based, fails in a substantial number of cases across Europe. Therefore, additional efforts, such as a GBS vaccine for pregnant women along with standardized European guidelines are imperative if we are to decrease the disease burden.

Declarations

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Competing interests

Financial interests: Margarit I, Maione D and Rinaudo D are employees of the GSK group of companies and hold shares in the GSK group of companies. Margarit I, Maione D and Rinaudo D are listed as inventor on patents owned by the GSK group of companies. Uffe B. Skov Sørensen and Mogens Kilian received personal consultancy fees from Suzhou VACMICRO Biotech Co., Ltd

Non-financial interests: Markus Hufnagel has been invited by Novartis to an advisory board meeting on juvenile idiopathic arthritis. Pierrette Melin has provided one scientific consultation for GSK vaccines.

All other authors have no financial or non-financial interests to disclose.

Authors' contribution

Lohrmann F wrote the first draft of the manuscript.

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All authors read and approved the final manuscript.

Ethics approval

The study has been approved by local ethics committees of each participating institution, and informed consent for study participation has been obtained from the legal caregivers. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent for publication: Not applicable.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1: Symptoms, signs and examination results

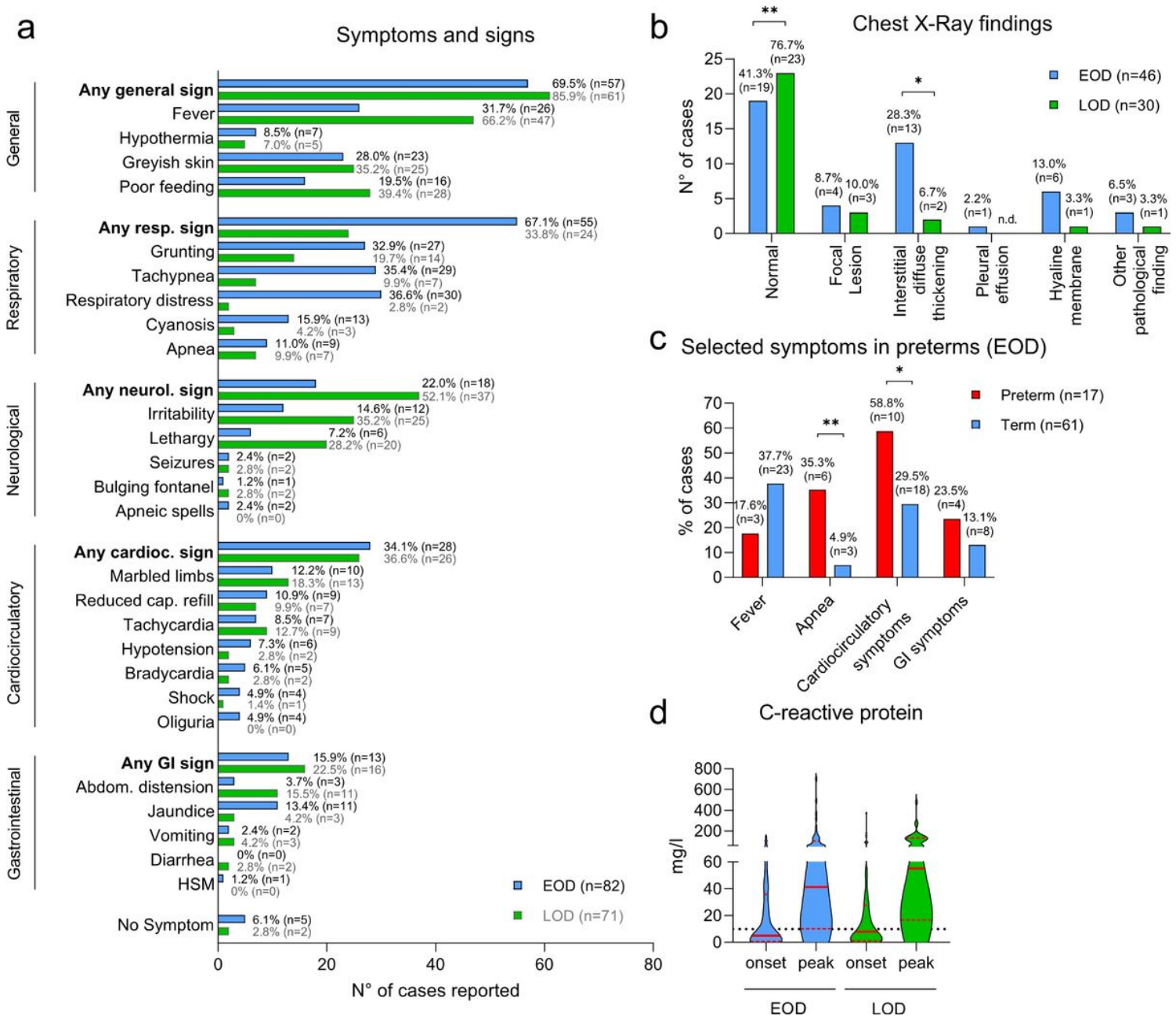

Figure 1

Symptoms, signs and examination results a. Symptoms and signs of EOD and LOD cases **b.** Chest X-ray findings of EOD and LOD cases. P-values from “Fisher’s exact test” are as follows: “normal findings”: p=0.0043; “interstitial diffuse thickening”: p=0.0364; all other differences are not statistically significant **c.** Selected symptoms of preterm versus term EOD cases. P-values from “Fisher’s exact test” are as follows: “apnea”: p=0.0026; “cardiocirculatory symptoms”: p=0.0434. The other comparisons are not statistically significant **d.** Serum C-reactive protein levels at disease onset and maximal levels of EOD and LOD cases. The red lines indicate median values, the dashed red lines the IQRs. The black dotted line indicates a level

of 10 mg/l, the threshold for this study. Differences between EOD and LOD are not significant (unpaired student's t-test)

EOD= Early-onset disease. LOD= Late-onset disease. GI= Gastrointestinal. HSM= Hepatosplenomegaly

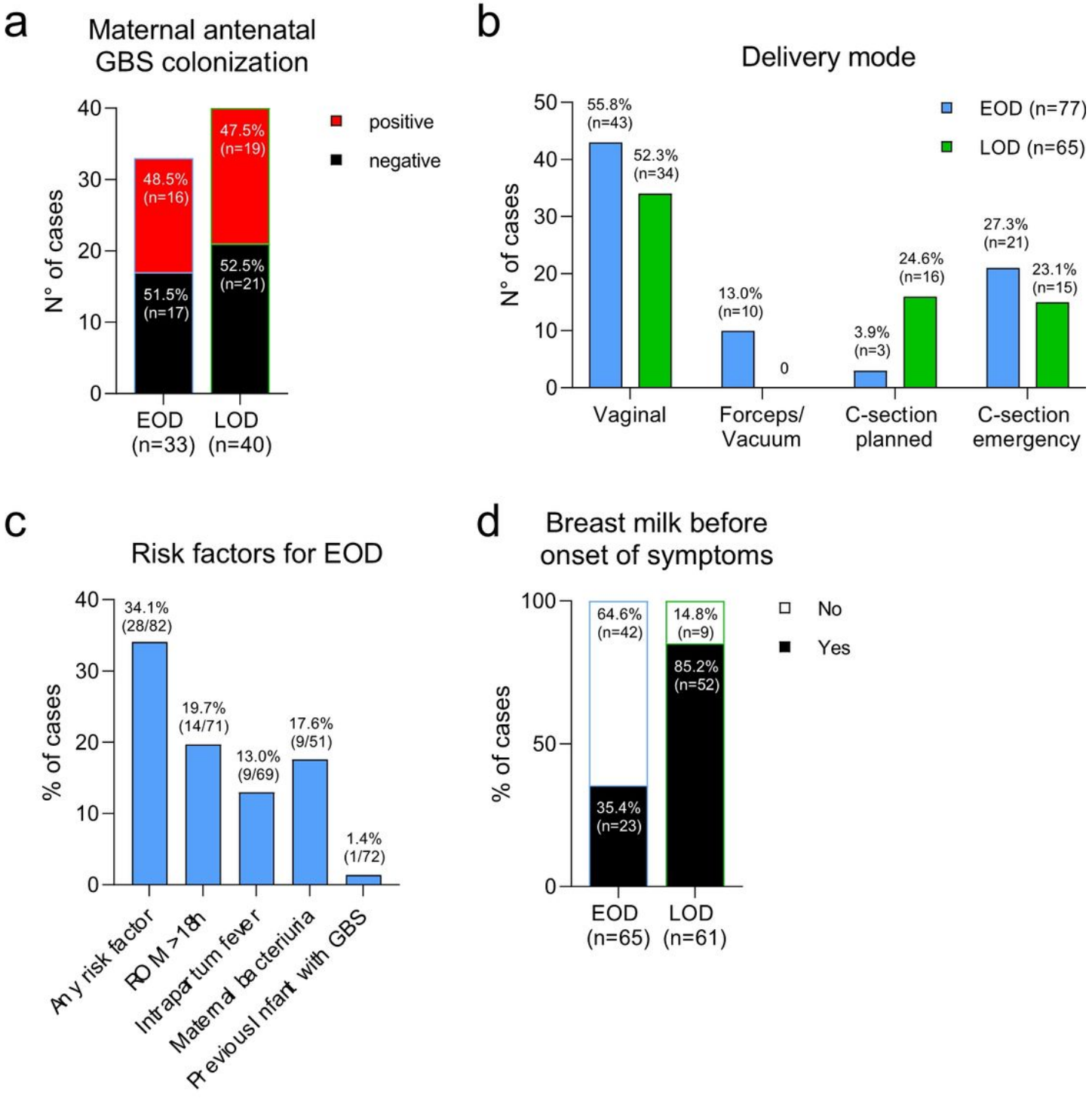

Figure 2

Maternal risk factors and GBS exposure a. Maternal GBS colonization status at the time of screening **b.** Delivery mode of EOD and LOD cases **c.** Frequency of established maternal risk factors for EOD cases **d.** Percentages of breast feeding before onset of symptoms of EOD and LOD cases

EOD= Early -onset disease. LOD= Late-onset disease. C-section= Caesarean section. ROM= Rupture of membrane

Figure 3: Outcome

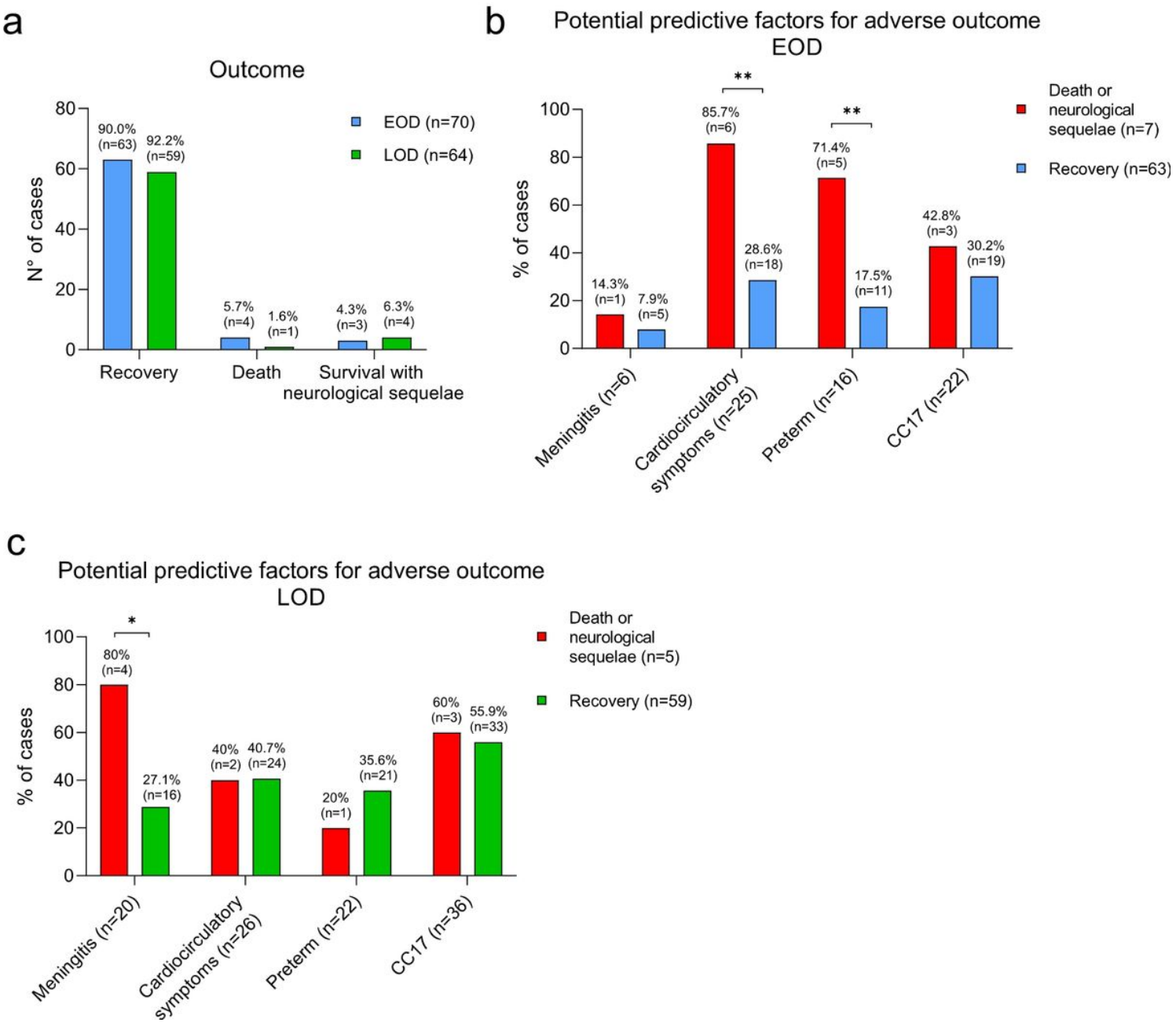

Figure 3

Outcome a. Outcome of EOD and LOD case **b.** Potential predictive factors for adverse outcomes in EOD cases. Statistical significance by “Fisher’s exact test” is reached for “cardiocirculatory symptoms”

(p=0.0055) and “preterm” (p=0.0056) **c.** Potential predictive factors for adverse outcomes in LOD cases. Statistical significance by “Fisher’s exact test” is reached for “Meningitis” (p=0.0300). Meningitis is based on the clinical diagnosis, not the positive CSF culture

EOD= Early-onset disease. LOD= Late-onset disease. CC17= Clonal complex 17

Supplementary Files

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