Title page

Title: Understanding the reactogenicity of 4CMenB vaccine: comparison of a novel and conventional method of assessing post-immunisation fever and correlation with pre-release *in vitro* pyrogen testing

Authors names and affiliations

Given name: Marta; Family name: Valente Pinto

Oxford Vaccine Group, Department of Paediatrics, University of Oxford; NIHR Oxford Biomedical Research Centre, United Kingdom

Given name: Kimberly; Family name: Davis

Oxford Vaccine Group, Department of Paediatrics, University of Oxford; NIHR Oxford Biomedical Research Centre, United Kingdom

Given name: Nick Family name: Andrews

Statistics, Modelling and Economics Department, Public Health England, London, United Kingdom

Given name: David; Family name: Goldblatt

Immunobiology Unit, UCL, Great Ormond Street Institute of Child Health, Biomedical Research Centre, London, United Kingdom

Given name: Ray; Family name: Borrow

Vaccine Evaluation Unit, Public Health England, Manchester, United Kingdom

Given name: Jo; Family name: Southern

ImmunoAssay Group, National Infection Service, Public Health England, Porton, Salisbury SP4 0JG, UK.

Given name: Ida Karin; Family name: Nordgren

Division of Biotherapeutics, The National Institute for Biological Standards and Control, United Kingdom

Given name: Caroline; Family name: Vipond

Division of Bacteriology, The National Institute for Biological Standards and Control, United Kingdom

Given name: Emma; Family name: Plested

Oxford Vaccine Group, Department of Paediatrics, University of Oxford; NIHR Oxford Biomedical Research Centre, United Kingdom

Given name: Elizabeth; Family name: Miller

Department of Infectious Disease Epidemiology, Faculty of Epidemiology & Population Health, London School of Hygiene & Tropical Medicine

Given name: Matthew D Family name: Snape

Oxford Vaccine Group, Department of Paediatrics, University of Oxford; NIHR Oxford Biomedical Research Centre, United Kingdom

Corresponding author

Marta Valente Pinto

Oxford Vaccine Group, Department of Paediatrics, University of Oxford

NIHR Oxford Biomedical Research Centre

Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Oxford, United Kingdom OX3 7LE

Telephone: +44 1865 611 400

Email: marta.valentepinto@paediatrics.ox.ac.uk

Abstract

Background: Better understanding of vaccine reactogenicity is crucial given its potential impact upon vaccine safety and acceptance. Here we report a comparison between conventional and novel (continuous) methods of monitoring temperature and evaluate any association between reactogenicity and the monocyte activation test (MAT) employed for testing four-component capsular group B meningococcal vaccine (4CMenB) batches prior to release for clinical use in Europe.

Methods: Healthy 7-12-week-old infants were randomised in two groups: group PCV13 2+1 (received pneumococcal conjugate vaccine 13 valent (PCV13) at 2, 4 and 12 months) and group PCV13 1+1 (received reduced schedule at 3 and 12 months). In both, infants received the remaining immunisations as per UK national schedule (including 4CMenB at 2, 4 and 12 months of age).

Fever was measured for the first 24 hours after immunisations using an axillary thermometer and with a wireless continuous temperature monitoring device (iButton®). To measure the relative pyrogenicity of individual 4CMenB batches, MAT was performed according to Ph. Eu. chapter 2.6.30 method C using PBMCs with IL-6 readout.

Results: Fever rates detected by the iButton® ranged from 28.7%-76.5% and from 46.6%-71.1% in group PCV13 2+1 and PCV13 1+1 respectively, across all study visits. The iButton® recorded a higher number of fever episodes when compared with axillary measurements in both groups (range of axillary temperature fevers; group PCV13 2+1: 6.7%-38%; group PCV13 1+1: 11.4%-37.1%). An agreement between the two methods was between 0.39 and 0.36 ($p<0.001$) at 8 hours' time-point post primary immunisations. No correlation was found between MAT scores and fever rates, or other reported adverse events.

Conclusions: It is likely that conventional, intermittent, fever measurements underestimates fever rates following immunisation. 4CMenB MAT scores didn't predict reactogenicity, providing reassurance that vaccine batches with the highest acceptable pyrogen level are not associated with an increase in adverse events.

Clinicaltrials.gov identifier: NCT02482636

Key words: 4CMenB; reactogenicity; Monocyte activation test; MAT score; iButton®

Introduction:

Vaccine reactogenicity is a key concern when implementing a new vaccine in an immunisation program. The number and severity of adverse events following immunisation (AEFI) can decrease public and health care provider acceptance of new vaccines due to safety concerns, which can consequently compromise immunisation coverage(1). With increasing vaccine hesitancy and refusal around Europe and the world, a better understanding and improvement of the methods to detect AEFI can increase vaccine trust (2, 3). 4CMenB (Bexsero®) has now been successfully used around the world and a few countries, including the UK, have already introduced the vaccine into the national immunisation programs with demonstrated effectiveness against group B meningococcal (MenB) disease (4-6). Concerns about high levels of reactogenicity associated with this vaccine have been debated among the scientific community, clinical practitioners and parents and need to be taken into consideration with the expected increased use (5). High post-immunisation fever rates have been reported, especially when co-administered with other routine immunisations (7, 8). The introduction of 4CMenB in the UK in 2015 (9) led to an increase in the number of hospital admissions and invasive procedures due to fever after immunisation in the infant population, despite recommendations for parents to administer prophylactic paracetamol to infants receiving 4CMenB (10, 11).

No definitive mechanism accounting for the reactogenic nature of 4CMenB when administered with routine vaccines, has yet been described, but the presence of known pyrogens such as lipooligosaccharide (LOS) and other non-endotoxin pyrogens present in outer membrane vesicle (OMV) component of the vaccine are thought to be contributory(12).

In this study we aimed to better understand the reactogenic profile of 4CMenB when used concomitantly with other routine immunisations by analysing temperature data collected by a novel method that measures body temperature continuously, the iButton®, used during a previously reported clinical trial comparing two immunisation schedules incorporating 4CMenB (13). This small wireless system records body temperature once per minute for a maximum of 34 hours (14), in contrast to traditional, intermittent, methods of temperature measurement in clinical trials (15). This non-invasive system therefore theoretically has the advantage of allowing identification of all fever episodes during the immediate post-vaccine period, in which there is a higher probability of febrile reactions.

Furthermore, we used this enhanced collection of reactogenicity data to interrogate the relationship, if any, between clinical reactogenicity and the 4CMenB pyrogenicity testing performed prior to batch release (the monocyte activation test (MAT) (16, 17), which quantifies interleukin-6 (IL-6) response by ELISA).

Methods:

Study design:

Data were collected in the Sched 3 study, a multicentre, randomised, open-label clinical trial, reported by Goldblatt et al (13). Briefly, healthy 7 to 12-week-old infants were randomised to receive either the 13 valent pneumococcal vaccine (PCV13; Prevenar 13®, Pfizer, New York, NY, USA) at 2, 4 and 12 months of age (PCV13 2+1), or at 3 and 12 months (PCV13 1+1). Other routine vaccines were administered to all infants at the same schedule, i.e. 4CMenB (Bexsero, GlaxoSmithKlein, Rixensart, Belgium) at two, four and 12 months of age, DTaP-Hib-IPV (Infanrix–IPV-Hib, , GlaxoSmithKlein, Rixensart, Belgium) at two, three and four months of age, oral rotavirus vaccine (RV) (Rotarix, GlaxoSmithKlein,

Rixensart, Belgium) at two and three months of age, MenC/Hib (Menitorix, GlaxoSmithKlein, Rixensart, Belgium) at 12 months of age and MMR (MMRVAXPRO, MSD vaccines, Lyon, France) at 13 months of age. As per Public Health England guidelines, parents were advised to administer three doses of paracetamol in the first 24 hours following 4CMenB at two and four months of age, one dose immediately after the vaccine and a second and third dose four to six hours after (18). All vaccines (including 4CMenB) were obtained through routine National Health Service (NHS) supplies. The full description of the study design and reports of the primary objective outcomes are available in *Goldblatt* et al (13).

Objectives:

A secondary objective of this study was to assess reactogenicity of the study vaccines, including a full description of local and systemic reactions in the week following vaccination at two, three, four and 12 months of age as recorded by parents using a paper diary. The full analysis of local and systemic reactions identified in this study are described elsewhere (Davis, K et al, Lancet ID *in press*). This paper focus on a description of fever events and temperature variation after immunisation, plus any correlation of reactogenicity predicted by the Monocyte Activation Test (MAT) with measured local and systemic reactions.

Body temperature records:

Body temperature was recorded in the same period by parents using a digital thermometer placed in the axilla at around four, eight and 20 hours after immunisation, and every 24 hours thereafter, for a period of seven days. Fever was defined as a temperature ≥38ºC as per Brighton Collaboration Fever Working Group (15). Temperature was also measured by the Thermochron iButton® (Maxim Integrated Products Inc, San Jose, CA, USA). The device was applied to the infant's abdomen, as shown in figure 1, and used to record the infant's

body temperature continuously in the first 24 hours after immunisation. After 24 hours, the parents sent the device via post to our Department to be downloaded and analysed.

Temperatures recorded below 35ºC were considered to be non-physiological and excluded from the analysis.

Figure 1: iButton® application method in the infant abdomen

Monocyte activation test (MAT)

MAT assessment was performed on the 4CMenB batches used in this study by the National Institute for Biologic Standards and Control (NIBSC) as per routine practise prior to release. Peripheral blood mononuclear cells are stimulated with Bexsero® and IL-6 release is quantified by ELISA testing as compared to a reference batch of vaccine (16). The assay is based on data held at NIBSC on vaccine batches released for use in clinic, where the initial 371 batches passing the batch release test were assigned a score of one to five based on the lowest to the highest quintiles of relative pyrogenic units (RPU).

In this study to scrutinise if differences in the measured relative pyrogenicity of the vaccine batches could be distinguished in corresponding groups of patients, the same rank-based scoring system was used to assign vaccine batches within the larger dataset rank. A total of seven batches were used during this study (supplementary table 1).

Statistical analysis

A descriptive and comparative analysis of both axillary temperature and iButton® data was performed for each vaccine time point (two, three, four and 12 months) based on the highest

temperature recorded in the 24 hours post-vaccination for iButton and across the seven days post-vaccination for axillary recording, with fever defined as ≥38°C. To compare the iButton® fever rates and mean temperatures in different study groups, the Chi-squared test and the two-sided t-test were used. The time to first fever was determined by Kaplan Meier survival analysis, represented by failure curves and statistical significance differences between the study groups calculated using log-rank test.

To determine if the time to first fever was different between visits for the infants that had at least one episode of fever recorded when using the iButton®, a one-way repeated measures ANOVA was run after normalising data between visits using a log10 transformation. Concordance correlation between the two methods was determined by the use of the Lin coefficient, represented by Bland-Altman plots. For the concordance evaluation we used the exact time (hours and minutes) that the axillary temperature was recorded by the parents and compared it with the temperature recorded at the same time by the iButton® device.

Fever rates by MAT scores graded from one to four were compared by Fisher's test at each visit. The trend in fever, as well as other adverse reactions, by MAT score was also assessed across all MenB visits using random effects logistic regression adjusting for visit with individual as the random effect. Separate analyses comparing fever by MAT score were done using temperature records from axillary measurements and iButton®.

The proportion of adverse reactions by group were calculated with 95% exact confidence intervals and the groups compared by Fisher's exact test.

The data were analysed in STATA version 13, based on the safety data set which is "As Treated".

Results:

Study population

A total of 213 infants were enrolled in the Sched 3 study (PCV13 2+1=106 infants and PCV13 1+1= 107 infants), with a median age at enrolment of 60 days in group 1 and 59 days in group 2.

Fever rates identified with the iButton®:

In the 24 hours after administration of 4CMenB and concomitant vaccines the percentage of participants with at least one episode of temperature above 38 °C (as recorded by the iButton®) ranged from 28.7% to 76.5% in group PCV 2+1 and 46.6% to 71.1% in group PCV 1+1 (table 1). In both groups, these rates were higher at the 12 months immunisations (group PCV 2+1: 75/98; group PCV 1+1: 64/90), when prophylactic paracetamol was not recommended.

Fewer participants had temperatures above 38 °C following visit 2, when 4CMenB was not administered, and the number of participants with at least one episode of fever was significantly higher in group PCV 1+1 (receiving RV, DTaP-Hib-IPV and PCV13), than group PCV 2+1 that received RV and DTaP-Hib-IPV alone (group PCV 2+1=28.7% (n=101); group PCV 1+1=46.6% (n=103), p=0.008) (table 2).

Across all visits the mean time to first fever varied between nine and 10.5 hours after immunisation, with no statistical differences between groups at each time-point as represented by Kaplan-Meier curves (figure 2) or between visits (repeated measures ANOVA $f(3,269)=1.14$; $p=0.33$).

Figure 2: Kaplan-Meyer curve showing cumulative percentages of individuals between groups at different time points

Comparison of continuous temperature monitoring and intermittent temperature evaluation:

Two different methods of temperature detection in individual participants are displayed in figure 3, showing data from infants with at least one fever episode in the 24 hours after immunisation with simultaneous records from both methods, pooled across both study groups (n= 144 participants at the two month visit, 79 at three months, 113 participants at four months and 138 participants at the 12-month visit).

At each visit detected fever rates in the 24 hours following immunisation were between 2.9 (4 month visit) and 7.7 (3 month visit) fold higher with the iButton compared with intermittent axillary temperatures; across all visits the iButton had a 4.1 fold higher detection rate (456 compared with 111). In the same period for all febrile episodes and immunisation visits between 69.9% and 87.4% were detected only by the iButton®, 2.5% to 4.4% were detected by axillary thermometer alone and 10.1% to 26.8% by both methods (figure 3).

Figure 3: Venn diagram representation of individuals with at least one episode of fever in the first 24 hours after immunisation at different timepoints (all participants of the study from both groups with both measurements available)

Temperatures taken with axillary thermometers at 4, 8, 20 and 24 hours after vaccines were compared with temperatures recorded at the same time with the iButton®. The agreement between the methods using Lin's concordance correlation was weak at 4 hours (0.16-0.26) for all visits and 24 hours post-immunisation for all visits (0.20-0.12), except V3 (ρ_c ;95%CI: 0.32;0.07-0.55) . Eight hours post-immunisation the methods showed a better agreement for

visits 1 to 3 (V1: 0.390; V2: 0.337; V3: 0.355, V5: 0.158). Figure 4 demonstrates the agreement identified in this study at the first visit as a representative example.

Figure 4: Bland- Altman scatterplots representing the concordance between axillary temperature and the iButton temperature at the first immunisation visit (2 months); ***A****: V1 concordance 4 hours after immunisation;* ***B****: V1 concordance 8 hours after immunisation.;* ***C****: V1 concordance 20 hours after immunisation;);* ***D****: V1 concordance 24 hours after immunisation*

MAT scores:

The MAT scores of the most common 4CMenB batches used in this study were obtained following study completion and participants at each visit categorised according the MAT score of the 4CMenB vaccine they received (Table 3, data pooled across both study groups). The maximum MAT score obtained in the vaccine batches used in this study was four. By chance, for the two primary doses of 4CMenB at two and four months of age the administered vaccines most commonly had a MAT score of 1 (two-month vaccines: 106/184; four month vaccines: 130/175). For the booster dose of 4CMenB given at 12 months of age, the most common MAT score was 2 (91/154).

The relative percentage of individuals with fever (as measured per-axilla) for each MAT score were similar after the first 4CMenB dose (p=0.885) and although more variability was observed after the second primary dose, no statistically significance was detected (p=0.323) (table 3). 44% of the participants receiving a booster dose of 4CMenB of a batch with a MAT score of 2 had fever. No association was found between the MAT score and fever using data across all timepoints (table 4). Similarly, there was also no association between MAT scores and fever

rates when measured by iButton® (two month vaccines p=0.09; four month vaccines: p= 0.226 booster dose (12 month immunisations): p=0.163).

The broader range of solicited adverse events following immunisation are shown in Table 4. There was no evidence that the trend across MAT scores for these reactions were different for the first and second dose of 4CMenB compared with the third dose; therefore, combined scores are shown. In the evaluation of all adverse reactions by batch score (all visits together – two, four and 12 month visits), only diarrhoea was considered to be within the limit of statistical significance (p=0.05), with higher number of events in the individuals that received a batch classified as 3 (41.7%) or 4 (41.2%) (Table 4).

Discussion:

The identification of fever is an important measure of vaccine reactogenicity, given its potential to cause discomfort, parental anxiety and healthcare consultation (10, 19, 20). In this study we have demonstrated that continuous measurement of fever detects over four times as many febrile participants in the 24 hours after immunisation as conventional methods of intermittent measurement.

The iButton® has the advantage of being a wireless, non-invasive, tolerable device when applied to human skin with a very low percentage of adverse reactions, that can be easily used in the paediatric population (21, 22). The exploration of different methods to continuously monitor temperature is not a new concept in medical research, but previously these methods were difficult to apply, not designed to be used in ambulatory patients and sometimes invasive (when used in animal models) (23-25). The iButton® has been used in adults and children to assess circadian rhythms in sleep/wake research and in adults post cardiac surgery (21, 22, 26).

A recent paper by Balla et al (27) compared temperature measurement by iButton® and intermittent rectal measurement in inpatient adults, and concluded the former had a relatively low sensitivity of 40%, but high specificity of 100%. Despite this high degree of specificity, the application of the iButton® in immunisation trials, particularly in infants, is very scarce.

Our study shows that the rates of fever detected by the axillary thermometer are much lower than those detected by the iButton®, but also lower than the rates reported previously in 4CMenB vaccine clinical trials using similar intermittent methods (7, 8). We showed that rates of fever detected by the iButton® in the study population that received the current UK schedule varied from 28.7% to 76.5% with rates detected by the axillary thermometer varying from 6.7% to 38.0%.

In addition to the advantage of potentially detecting more episodes of fever, that might be missed by intermittent methods, the use of a continuous method could allow for a better determination of the expected temperature trends related to the type of vaccine administered. A better description of febrile reactions could also help with diagnosing potential coincidental medical conditions causing non-vaccine related fevers. Although this possibility might only be applicable in the setting of clinical trials, when used in combination with other methods it could provide important information. The use of temperature trends as a predictor of severity in septic patients has shown that differences in amplitude and frequency of temperature curves can help to differentiate septic versus non-septic patients (28). A better understanding of these events could help clinicians identify children whose fever is unrelated to any recent immunisations received.

Another challenge with the current intermittent temperature monitoring systems is the identification of the best method to measure temperature for each age group. In the UK it is standard practice to measure axillary temperature in paediatric clinical trials. Other methods such as rectal temperature is the method of choice in other European countries (29-31). The Brighton collaboration doesn't define a gold standard method to measure temperature (15), helping to explain the variation between countries.

Our study shows that the rates of fever detected by the axillary thermometer are lower than the rates reported previously in 4CMenB vaccine clinical trials using similar intermittent method (7, 8). In a study by *Gossger* et al the rates of fever measured using axillary temperature varied between 51 to 61% when 4CMenB was administered in combination with other routine vaccines (7). One of the possible explanations for the lower rates of fever detected by axillary thermometer in our study was the use of three doses of paracetamol in the first 24hours after immunisation, as a prophylactic measure. This recommendation was implemented in the UK after the introduction of the 4CMenB vaccine in the National Immunisation Program and was not applied consistently in clinical trials such as *Gossger* et al.

In another 4CMenB study by *Vesikari et al* (8), rates of fever were higher still than in *Gossger et al*, although in infants the method of choice was rectal temperature measurement. In this population, 65.3% of the individuals that received 4CMenB with routine immunisations had a temperature ≥38.5ºC in the first six hours after their immunisations (8).

Both rectal and axillary methods of temperature measurement are suitable for infants until the age of two years (32). In newborns, although both methods can be used and are considered accurate, rectal temperatures are consistently higher when compared with axillary

temperatures, and the mean variation between the two methods in the same individual is wide (33). One area for future research would be to compare the iButton technology deployed here with intermittent rectal measurements to determine if the correlation is better than with axillary measurements.

To our knowledge this is the first study to correlate the results of pre-release pyrogen content testing of 4CMenB by MAT with clinical outcomes. The application of MAT has been shown to be a suitable cell-based method to measure the release of IL-6, a pro-inflammatory cytokine which mediates the fever response (16, 17). Across the 371 NIBSC reference batches, the RPU values ranged from 0.5-1.7 RPU. The batches in the study ranged from 0.85 to 1.24, falling into the range scored 1-4. Using the MAT test to measure the pyrogenic potential of new vaccines such as Bexsero is novel and whilst extensive validation was carried out and the limits of pass/fail based on batches shown to be safe in a clinic trial, actual clinical information of any differences identified in the RPU of the vaccine and a reaction in patients was unknown. The results of this study provide reassurance to the regulatory laboratory that the differences in the RPU within batches which pass the release tests do not manifest as differences in the reactogenicity in the clinic. Also, no evidence was found in this study that higher MAT scores were associated with a higher number of adverse reactions. This emphasises the role of MAT scores being used for batch release, with all batches that meet the release criteria being suitable for clinical use.

This study has some limitations that could have influenced some of the results and also could make comparisons with other studies more challenging. In previous 4CMenB studies, the use

of paracetamol was only as required, unlike the recommendations to parents made in this study. This approach could bias fever rates and cause prolongation of time to first fever, which would limit comparisons with other studies. Also, any correlation between MAT scores and 4CMenB reactogenicity, could potentially be 'masked' by use of prophylactic paracetamol for the infant vaccines, or (unrecorded) variability in the reactogenicity of the concomitantly administered vaccines, We also assumed was that paracetamol was given to the infants as per recommendation, however this was only specifically recorded for the first paracetamol dose (when the study team was present for administration) but subsequent dose administration was assumed. However, the data collected represents the current day to day practice in the UK, and help demonstrate that batches approved for release based on the MAT scores are associated with clinically acceptable reactogenicity.

Another limitation is regarding the limited numbers used to understand potentially correlations between MAT scores and 4CMenB batches. The lack of correlation observed in this study cannot be overinterpreted and a larger sample would be needed to confirm these findings.

Final conclusion

The use of a continuous measurement method in this clinical trial allowed us to more accurately define the presence of fever episodes after UK routine immunisation in infants when compared with the standard intermittent method, and increased use of this methods in vaccine clinical trials should be encouraged. Reactogenicity of the infant vaccine schedule is partially attributed to the 4CMenB vaccine which is tempered by the prophylactic use of paracetamol at the time of immunisation. The findings of this study are that reactogenicity seen in infants immunised with 4CMenB did not correlate with the MAT scores, providing reassurance that the defined threshold for a vaccine to pass the MAT safety test for human use is appropriate, although these findings need to be confirmed with larger sample sizes.

Conflicts of interest

MVP is a member of the Portuguese National Immunisation Technical Advisory Group - (Comissão Técnica de Vacinação da Direcção Geral de Saúde). MDS acts on behalf of the University of Oxford and Oxford Vaccine Group (OVG) as Chief or Principal Investigator on clinical trials sponsored and/or funded by vaccine manufacturers including Pfizer and GSK and JP, MVP, and EP are employed by the OVG. DG has served on ad-hoc advisory boards for GSK, Merck and Sano and is a National Institute of Health Research (NIHR) Senior Investigator. The UCL GOSICH Lab (DG) has received contract research funding from GSK, Merck and Sano. RB performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur. KD, JS, NJA, and EM declare no competing interests.

Funding and approvals

This study was ethically approved by the Oxfordshire Research Ethics Committee (reference number 15/SC/0387) and is registered on the EudraCT clinical trials database (2015-000817-32) and ClinicalTrials.gov (NCT02482636).

This study was funded by NIHR and the Bill and Melinda Gates Foundation.

Contributors statement

EM, JS, NJA, RB, and DG designed the trial, JS, JP, MVP, EP, and MDS oversaw the clinical trial, clinical data collection and clinical data management. NJA and MVP conducted the statistical analysis. MVP wrote the first draft of the paper and all authors contributed to subsequent drafts. All read and approved the final version of the report.

Acknowledgments

We thank the participants and their parents, the Buckinghamshire NHS Child Health Information Service for their assistance, the Oxford Vaccine Group nurses in Oxford, PHE study nurses in Gloucestershire and Hertfordshire for their help in recruitment, vaccination and follow up of participants. The report is based on independent research commissioned and funded by the NIHR Policy Research Programme (Evaluation of suitability of vaccines for the national immunisation programme. National Vaccine Evaluation Consortium, grant number 039/0031— grant holder EM) and the Gates Foundation (OPP1126431—grant holder DG). The views expressed in the publication are those of the author(s) and not necessarily those of the of the NIHR or the Department of Health and Social Care, or the Bill & Melinda Gates Foundation.

References

1. O'Leary ST, Maldonado YA. Adverse Events Following Immunization: Will It Happen Again? Pediatrics. 2017;140(3): e2017160

2. European Centre for Disease Preventation and Control. Let's talk about hesitancy. Stockholm: ECDC; 2016.

3. Larson H, de Figueiredo A, Karafillakis E, Rawal M. State of vaccine confidence in the EU 2018. European Comission; 2018.

4. Parikh SR, Andrews NJ, Beebeejaun K, Campbell H, Ribeiro S, Ward C, et al. Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. The Lancet. 2016;388(10061):2775-82.

5. European Centre for Disease Preventation and Control. Expert opinion on the introduction of the meningococcal B (4CMenB) vaccine in the EU/EEA. Stockholm: ECDC; 2017.

6. Ladhani SN, Andrews N, Parikh SR, Campbell H, White J, Edelstein M, et al. Vaccination of Infants with Meningococcal Group B Vaccine (4CMenB) in England. N Engl J Med. 2020;382(4):309-17.

7. Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. JAMA. 2012;307(6):573-82.

8. Vesikari T, Esposito S, Prymula R, Ypma E, Kohl I, Toneatto D, et al. Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine (4CMenB) administered concomitantly with routine infant and child vaccinations: results of two randomised trials. The Lancet. 2013;381(9869):825-35.

9. Ladhani SN, Campbell H, Parikh SR, Saliba V, Borrow R, Ramsay M. The introduction of the meningococcal B (MenB) vaccine (Bexsero®) into the national infant immunisation programme:New challenges for public health. J Infect. 2015;71(6):611-4.

10. Nainani V, Galal U, Buttery J, Snape MD. An increase in accident and emergency presentations for adverse events following immunisation after introduction of the group B meningococcal vaccine: an observational study. Arch Dis Child. 2017. 312941

11. Murdoch H, Wallace L, Bishop J, Robertson C, Claire Cameron J. Risk of hospitalisation with fever following MenB vaccination: self-controlled case series analysis. Arch Dis Child. 2017;102(10):894-8.

12. van der Pol L, Stork M, van der Ley P. Outer membrane vesicles as platform vaccine technology. Biotechnol J. 2015;10(11):1689-706.

13. Goldblatt D, Southern J, Andrews NJ, Burbidge P, Partington J, Roalfe L, et al. Pneumococcal conjugate vaccine 13 delivered as one primary and one booster dose (1 + 1) compared with two primary doses and a booster (2 + 1) in UK infants: a multicentre, parallel group randomised controlled trial. Lancet Infect Dis. 2017. 18(2):171-179

14. van Marken Lichtenbelt WD, Daanen HA, Wouters L, Fronczek R, Raymann RJ, Severens NM, et al. Evaluation of wireless determination of skin temperature using iButtons. Physiol Behav. 2006;88(4-5):489-97.

15. Michael Marcy S, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004;22(5-6):551-6.

16. Vipond C, Sutherland J, Nordgren K, Kemp G, Heath A, Care R, et al. Development and validation of a monocyte activation test for the control/safety testing of an OMV-based meningococcal B vaccine. Vaccine. 2019;37(29):3747-53.

17. Valentini S, Santoro G, Baffetta F, Franceschi S, Paludi M, Brandini E, et al. Monocyte-activation test to reliably measure the pyrogenic content of a vaccine: An in vitro pyrogen test to overcome in vivo limitations. Vaccine. 2019;37(29):3754-60.

18. Public Health England Meningococcal: the green book, chapter 22 2013, last update 20 September 2016

19. Zhou W, Pool V, Iskander JK, English-Bullard R, Ball R, Wise RP, et al. Surveillance for safety after immunization: Vaccine Adverse Event Reporting System (VAERS)-United States, 1991-2001. MMWR Surveill Summ 2003;52(1):1-24.

20. Murdoch H, Wallace L, Bishop J, Robertson C, Claire Cameron J. Risk of hospitalisation with fever following MenB vaccination: self-controlled case series analysis. Archives of Disease in Childhood. 2017;102(10):894-8.

21. Smith ADH, Crabtree DR, Bilzon JLJ, Walsh NP. The validity of wireless iButtons® and thermistors for human skin temperature measurement. Physiological Measurement. 2010;31(1):95-114.

22. Hasselberg MJ, McMahon J, Parker K. The validity, reliability, and utility of the iButton® for measurement of body temperature circadian rhythms in sleep/wake research. Sleep Med. 2013;14(1):5-11.

23. Hamilos DL, Nutter D, Gershtenson J, Redmond DP, Di Clementi JD, Schmaling KB, et al. Core body temperature is normal in Chronic Fatigue Syndrome. Biol Psychiatry. 1998;43:293-302.

24. Kaaijk P, van der Ark AA, van Amerongen G, van den Dobbelsteen GP. Nonclinical vaccine safety evaluation: advantages of continuous temperature monitoring using abdominally implanted data loggers. J Appl Toxicol. 2013;33(6):521-6.

25. Mathur N, Glesk I, Buis A. Skin Temperature Prediction in Lower Limb Prostheses. IEEE J Biomed Health Inform. 2016;20(1):158-65.

26. Zornoza-Moreno M, Fuentes-Hernandez S, Sanchez-Solis M, Rol MA, Larque E, Madrid JA. Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 2011;28(4):330-7.

27. Zsuzsanna Balla H, Theodorsson E, Strom JO. Evaluation of commercial, wireless dermal thermometers for surrogate measurements of core temperature. Scand J Clin Lab Invest. 2019;79(1-2):1-6.

28. Drewry AM, Fuller BM, Bailey TC, Hotchkiss RS. Body temperature patterns as a predictor of hospital-acquired sepsis in afebrile adult intensive care unit patients: a case-control study. Crit Care. 2013;17(5):R200.

29. Silfverdal SA, Icardi G, Vesikari T, Flores SA, Pagnoni MF, Xu J, et al. A Phase III randomized, double-blind, clinical trial of an investigational hexavalent vaccine given at 2, 4, and 11-12 months. Vaccine. 2016;34(33):3810-6.

30. Martinon-Torres F, Safadi MAP, Martinez AC, Marquez PI, Torres JCT, Weckx LY, et al. Reduced schedules of 4CMenB vaccine in infants and catch-up series in children: Immunogenicity and safety results from a randomised open-label phase 3b trial. Vaccine. 2017;35(28):3548-57.

31. Berner R, Boisnard F, Thomas S, Mwawasi G, Reynolds D. Safety and immunogenicity of fully liquid DTaP(5)-IPV-Hib pediatric combination vaccine (Pediacel®) compared to DTaP(3)-HBV-IPV/Hib (Infanrix® Hexa) when coadministered with heptavalent pneumococcal conjugate vaccine (PCV7) as a booster at 11-18 months of age: a phase III, modified double-blind, randomized, controlled, multicenter study. Vaccine. 2012;30(35):5270-7.

32. Canadian Paediatric Society Statement. Temperature measurement in paediatrics - CP 2000-01. Paediatr Child Health. 2000;5(5):273- 6.

33. Uslu S, Ozdemir H, Bulbul A, Comert S, Bolat F, Can E, et al. A comparison of different methods of temperature measurements in sick newborns. J Trop Pediatr. 2011;57(6):418-23.

Tables

Table 1: Percentage of participants in each group with at least one fever episode (≥38°C) detected by the iButton® in the first 24 hours after immunisation or axillary measurement in the first seven day after immunisation, after each immunisation visit

	PCV2+1			PCV1+1		
Age	Axillary temp (1st 24h)	iButton® (24 hours period after immunisation)	p value (Pearson Chi-squared test)*	Axillary temp (1st 24h)	iButton® (24 hours period after immunisation)	p value (Pearson Chi-squared test)*
2 months	18/106 16.9%	69/99 69.7%	0.175	14/104 13.4%	72/101 71.3%	0.9
3 months	0/105	29/101 28.7%	-	10/105 9.5%	48/103 46.6%	0.024
4 months	20/101 29.4%	63/102 61.8%	0.001	16/102 15.6%	47/98 48.5%	0.09
12 months	25/99 25.2%	75/98 76.5%	0.04	18/88 20.5%	64/90 71.1%	0.11

*Evaluation of proportion of cases in each group

Table 2: iButton® fever rates and fever episodes characteristics in group 1 and group 2

	PCV2+1 (Total N=107)				PCV1+1 (Total N=106)				
	V1 (2 months) (N=99)	V2 (3 months) (N=101)	V3 (4 months) (N=102)	V5 (12 months)	V1 (2 months) (N=101)	V2 (3 months) N=103	V3 (4 months) (N=97)	V5 (12 months)	P values for differences

				(N=98)				(N=90)	between schedules
At least one fever episode (N=99)	69 (69.7%)	29 (28.71%)	63 (61.76%)	75 (76.53%)	72 (71.3%)	48 (46.6%)	47 (48.45%)	64 (71.11%)	V1(p=0.805)* **V2 (p=0.008)*** V3(p=0.069)* V4(p=0.398)*
Mean temperature (SD) (N=99)	36.75ºC (0.42)	36.29 ºC (0.41)	36.53 ºC (0.48)	36.75ºC (0.53)	36.78 ºC (0.42)	36.57 ºC (0.44)	36.47 ºC (0.45)	36.81ºC (0.58)	V1(p=0.607)◊ V2(p=0.999) ◊ V3(p=0.160) ◊ V4(p=0.766) ◊
Mean maximum temperature (SD)/range	38.18 ºC (0.47)/ 37ºC-39.4 ºC	37.68 ºC (0.45)/ 36.37ºC-39.1ºC	38.12ºC (0.60)/ 36.75ºC-39.5ºC	38.38ºC (0.76)/ 36.75ºC-40.62ºC	38.18 ºC (0.44)/ 37ºC-39.5ºC	37.92ºC (0.49)/ 36.37-39.5ºC	37.93ºC (0.59)/ 36.25ºC-39.5ºC	38.37ºC (0.82)/ 36.5ºC-40.37 ºC	

Legend: *Chi 2 test; ◊ 2-sided T-test;

Table 3: Monocyte activation test (MAT) scores for the 4CMenB vaccine batches used in the Sched 3 study and percentage of individuals with fever identified by axillary temperature measurements and by iButton® by MAT score

		Axillary Temperature			Ibutton ®		
Dose	MAT Score	n/N (temp>=38)	% (95%CI)	P-value	n/N (temp>=38)	% (95%CI)	P-value
1	1	18/106	17% (10.4-25.5)	0.885	65/103	63.1% (53.3-71.9)	0.095
	2						
	3	4/29	13,8% (3.9-31.7)		23/27	85.2% (65.9-94.5)	
	4	7/49	14,3% (5.9-27.2)		38/51	74.5% (60.6-84.7)	
2	1	39/130	30% (22.3-38.7)	0.323	71/124	57.3% (48.3-65.7)	0.226
	2						
	3	3/19	15.8% (3.4-39.6)		8/19	42.1% (22.1-65.1)	
	4	5/26	19.2% (6.6-39.4)		16/23	69.6% (47.8-85.1)	
boost	1	0/2	0% (0-84.2)	0.239	2/2	100%	0.163
	2	40/91	44% (33.6-54.8)		72/90	80% (70.3-87.1)	
	3						
	4	20/61	32.8% (21.3-46)		40/56	71.4% (58.1-81.9)	

Table 4: Solicited adverse events following 4CMenB classified according to MAT score of the 4CMenB vaccine received at that visit. (Data pooled across study groups and across visits at 2, 4 and 12 months of age).

	MAT score				
	1 (two batches, mainly used for 2 and 4 months imms)	2 (one batch -only used for 12 months imms)	3 (one batch used for 2 and 4 months imms)	4 (three batches, one used for 2 and 4 months imms, one for 4 and 12 months imms, one 12 months imms only)	P-value* (trend by MAT score)
Fever (≥38.0)	57/238 (24.0%)	40/91 (44.0%)	7/48 (14.6%)	32/136 (23.5%)	0.07
Feeding	131/237 (55.3%)	43/90 (47.8%)	22/48 (45.8%)	73/136 (53.7%)	0.81
Less active	128/237 (54.0%)	64/91 (70.3%)	31/48 (64.6%)	80/136 (58.8%)	0.95
Irritable	172/237 (72.6%)	77/91 (84.6%)	35/48 (72.9%)	109/136 (80.2%)	0.30
Crying	129/237 (54.4%)	58/91 (63.7%)	24/48 (50.0%)	171/136 (52.2%)	0.28
Vomiting	80/237 (33.8%)	15/91 (16.5%)	19/48 (39.6%)	41/136 (30.2%)	0.85
Diarrhoea	89/237 (37.6%)	25/91 (27.5%)	20/48 (41.7%)	56/136 (41.2%)	0.05
Redness	83/234 (35.5%)	49/91 (53.8%)	23/48 (47.9%)	62/136 (45.6%)	0.32
Swelling	47/234 (20.1%)	25/91 (27.5%)	15/48 (31.3%)	39/136 (28.7%)	0.26
Tenderness	87/235 (37.0%)	50/90 (55.6%)	20/48 (41.7%)	74/135 (54.8%)	0.10

*from random effects logistic regression adjusting for dose with individual as the random effect

Legend: V1: 2 months immunisation visit (first primary dose of 4CMenB); V3: 4 months immunisation visit (second primary dose of 4CMenB); V5: 12 months immunisation visit (booster dose of 4CMenB)

Supplementary table 1: 4CMenB vaccine batches used in Sched 3 study

Batch number (Sched 3)	**MAT Score**
146301	**1**
140501	**4**
152401A	**1**
152101	**3**
140901A	**4**
154301	**2**
15B701	**4**