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Baseline polysaccharide-specific antibodies may not consistently inhibit booster antibody responses in infants to a serogroup C meningococcal protein–polysaccharide conjugate vaccine

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ABSTRACT

Negative correlations between baseline antibody concentrations and increases in antibody concentrations (after booster doses of vaccines) have been reported previously. Such correlation coefficients are widely reported by statisticians to be subject to mathematical coupling. Negative correlations may be attributable partly or wholly to the combination of mathematical coupling and measurement error (or other short term fluctuations in measurements) and therefore not clinically interpretable. In this study we re-analysed the serum antibody responses from five clinical trials of serogroup C meningococcal conjugate vaccine (MenCV) given to infants for priming followed by boosting with MenCV or a meningococcal A/C polysaccharide vaccine (MenA/C) at 12 months of age. Using Pearson's correlation method to assess the effect of pre-booster MenC-IgG concentration on the relative increase in MenC-IgG concentration post-booster, a significant negative correlation was observed for all the groups, indicating that high pre-booster antibody was associated with a smaller rise in antibody post-booster. We tested two additional statistical methods that account for mathematical coupling. Using Blomqvist method of adjustment to assess the plausible extent of bias, correlation coefficients were still negative providing error variance was low. The other method, a multilevel modelling specification of Oldham's method appeared not to be appropriate. In contrast, using Pearson's correlation method a consistent negative correlation between carrier protein-specific baseline antibody concentration and the increase in MenC-specific antibody concentration was only observed following booster immunisation with the protein-polysaccharide conjugate vaccine but not following the MenA/C plain polysaccharide vaccine. These findings suggest that analysis of the inhibitory effect of baseline antibody on the response to booster immunisation is challenging and should account for the possibility of mathematical coupling and measurement error. That an inhibitory effect of baseline antibody cannot be assumed a priori is supported by observations in animal models, which show that baseline antibody can both suppress or enhance the antibody response to a specific antigen.

1. Introduction

Vaccines are frequently administered to individuals with pre-existing serum antibody specific for the vaccine antigen. This occurs because of either (i) presence of transplacentally acquired maternal antibody in infants, (ii) the existence of naturally acquired antibody following exposure to the organism from which the vaccine is derived (or related organisms) through carriage, (iii) the administration of prior doses of vaccine, or (iv) in the case of polysaccharide conjugate vaccines, the prior and/or concomitant administration of vaccines containing the protein carrier (i.e. routine diphtheria toxoid, tetanus toxoid and pertussis vaccines in, for example, Hib-TT, MenC-TT/-CRM_{197} and PnC-TT/-DT/-CRM_{197}

Abbreviations: CRM197, a mutant of diphtheria toxoid used as carrier protein in diverse protein polysaccharide conjugate vaccines; GMC, geometric mean concentration; Hib, *Haemophilus influenzae* type b; MenC, meningococcal serogroup C polysaccharide; MenA/C, meningococcal A and C polysaccharide vaccine; MenCV, serogroup C meningococcal conjugate vaccine; MMR, measles, mumps and rubella vaccine; 9vPnC-MenCV, combined 9-valent pneumococcal/meningococcal serogroup C conjugate vaccine; DT, diphtheria toxoid; SD, standard deviation; TT, tetanus toxoid.

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antigens) that is also present in the vaccine. Various studies in animals have reported that the presence of antibody at baseline can either suppress or enhance the antibody responses to the specific antigen to which they are targeted [1–3]. Possible mechanisms for enhancement of antibody response by baseline antibody include (1) the capture of small quantities of antigen by pre-existing IgG (especially subclasses IgG1, IgG2a and IgG2b) which then targets the antigen to antigen-presenting-cells, leading to more efficient activation of T cells, as IgG–antigen immune complexes are taken up more efficiently than antigen alone via FcγR+ expressed on antigen-presenting-cells; (2) IgM and IgG3 enhancement of the antibody responses through the activation of complement, which will then lower the threshold for B-cell activation [3]. Mechanisms proposed for suppression of antibody responses may be (1) formation of IgG/antigen immune complexes which may be eliminated by phagocytic cells before they can activate specific B cells; (2) epitope masking by IgG which may prevent B-cells from recognising and responding to the antigen [1–3]. The effect of baseline antibody on the activation of B cells is not well understood and yet is likely to have important implications for the design of schedules for re-immunisation.

Whilst the inhibitory effect of maternal antibody is well documented [4,5] the situation is less certain for individuals given booster doses of vaccines following immunisation at an earlier time-point, because of concerns with statistical methods and their interpretation in published studies. Many studies in humans have used correlation or linear regression to investigate how the absolute or relative increase in antibody concentration following a booster dose of vaccine relates to pre-booster antibody concentration. These studies have reported a negative correlation between baseline antibody concentration and the increase in antibody concentration after a booster dose of vaccine [6–9]. Other studies have investigated the ratio between pre- and post-booster antibody concentration (fold increase) in individuals with different baseline antibody concentrations (using an arbitrary cut-off) and commonly reported higher fold increases in the groups with lower pre-booster antibody titre [6,7,10].

However, the use of simple correlation or linear regression to assess the relationship between baseline values and changes in value has been criticised by statisticians for many years, since it is prone to bias [11,12]. The issue of concern is mathematical coupling between the two variables, i.e. the fact that the change score (which is correlated with the baseline value) is derived from a simple equation which includes the baseline value itself (change is calculated as post value minus baseline value). This is an important issue, for example, if measurement error over-estimates the observed value of pre-booster antibody concentration (i.e. the reading is higher than its true value), then the reported change in antibody concentration will be lower than its true value (since the higher value of pre-booster antibody is subtracted in calculating the increase in antibody concentration) [12]. If measurement error decreases the observed value of pre-booster antibody concentration, then the values of the pre-booster antibody concentration will be lower than it should be and the values of the change in antibody concentration will be higher. Thus, a negative correlation is a consistent result arising solely from the combination of mathematical coupling and measurement errors [13]. Measurement error here is deemed to include short term fluctuations in true values of the antibodies being measured, and any random errors inherent in handling and storage of serum samples, as well as true measurement error of the antibody levels. Systematic errors attributable to storage or handling may not affects results. For instance, a constant percentage deterioration in antibody counts is equivalent to a constant value being subtracted from all values on the logarithmic scale, so will not affect reported associations between baseline and changes on the log scale (which we are using in this paper). The mathematical issues apply similarly to comparing the strengths of associations in sub-sets with different degrees of protection at baseline.

In response to these issues, statisticians have developed alternative methods [11–13,21], to assess the relationship between change and baseline values, namely Oldham's method and Blomqvist's formula, which have different merits. Blomqvist's formula is based on knowledge of within person fluctuations in measurements ("error variance"). This may be obtained from a separate data set, containing repeated measurements of antibody concentrations taken over a short period of time. In the absence of such data, sensitivity analyses may be undertaken to determine the effect of a plausible range of estimates of within person error variances [11]. Oldham's method correlates the change between pre- and post-treatment values with the mean of the pre- and post-treatment values. This method is said to reduce the effect of mathematical coupling and be less prone to bias. Under the relatively plausible assumptions that the measurement error/short term fluctuations terms of pre- and post-treatment values are independent of each other, and that both have the same variance, then this method reduces the problems arising from mathematical coupling. These assumptions are plausible in comparison to the assumption of no measurement error/short term fluctuations required for validity of the standard correlation or linear regression methods. However, Oldham's method is likely to be somewhat biased towards a positive association between the change and mean (of medium term averages of baseline and of follow-up concentrations) values. Since both change and mean involve addition of follow-up values, any measurement error in these values induces a positive bias.

Oldham's technique can be extended to allow consideration of additional covariates or confounders (e.g., age and gender of study participants, variation in the dose of vaccine antigens, study in meta-analyses), or to allow individuals to be measured varying numbers of times. This requires use of multi-level modelling (MLM) techniques [12]. With correct model specification and without additional covariates, the results are equivalent to those obtained from Oldham's method.

In the present study, we used standard correlation methods, Blomqvist adjustment method and an MLM version of Oldham's method to assess the effect of pre-booster MenC-IgG concentration on the relative increase in MenC-IgG concentration following booster immunisation in five studies we previously conducted. Since estimates of within person fluctuations in measurement ("error variance") are not available, Blomqvist adjustment was based on a range of plausible estimates for these values.

2. Materials and methods

Data were obtained from five studies previously conducted in Oxford, UK [10,14–20]. In these studies children were primed at 2, 3, 4 months of age with either serogroup C meningococcal conjugate vaccine (MenCV), or a combined *Haemophilus influenzae* type b and serogroup C *Neisseria meningitidis* tetanus toxoid conjugate vaccine (Hib-MenC-TT), or a combined 9-valent pneumococcal-group C meningococcal conjugate vaccine (9vPnc-MenCV). They were boosted at 12 months of age with either MenCV or meningococcal A/C polysaccharide vaccine (MenA/C), or Hib-MenC-TT. A total of 6 different vaccine schedules were used across the 5 studies (see Table 1) and the children were separated into 6 groups according to their vaccine schedule. Each dose of MenCV contained 10 μg of *N. meningitidis* group C oligosaccharide (MenC), however, they contained between 12.5 and 25 mcg of CRM_{197} [10,14–20]. They were manufactured either by Novartis Vaccines and Diagnostics (studies 1 and 3) [10 MacLennan, 2000 #36, 16], or by Wyeth Vaccines (studies 2, 4 and 5) [14,17,19].

Table 1
Summary describing the studies from which the data were taken for the present analysis.

Study no	Study name	Group of vaccine schedule	Priming at 2, 3, 4 months	Boosting at 12 months	References
1	Novartis M14P5E1	1	MenCV	MenCV	[10]
2	Wyeth Combo study	1	MenCV	MenCV	[14,15]
		2	MenCV	MenA/C	
		5	Pnc9-MenC	MenCV	
		6	Pnc9-MenC	MenA/C	
3	Novartis MenC study	1	MenCV	MenCV	[16]
		2	MenCV	MenA/C	
		7[a]	HepB	MenCV	
		8[a]	HepB	MenA/C	
4	Wyeth MenC study	1	MenCV	MenCV	[17,18]
		2	MenCV	MenA/C	
		7[a]	HepB	MenCV	
		8[a]	HepB	MenA/C	
5	GSK Hib-MenC study	4	Hib-MenC	Hib-MenC	[19,20]
		3	MenCV	Hib-MenC	

[a] Vaccine schedule not used in the present study.

Each dose of MenA/C contained 50 μg of MenC, however, only 1/5th of the MenA/C was administered in studies 2 and 4 [14,17], and the full dose of MenA/C was used in study 3 [16].

The combined Hib-MenC-TT vaccine contained 5 μg of MenC, the combined Pnc9-MenC contained 10 μg of MenC. All the protein–polysaccharide conjugate vaccines used in each of the 6 studies had CRM_{197} (a mutant of diphtheria toxoid) as the carrier protein, except the combined Hib-MenC-TT, which used the tetanus toxoid as carrier protein. The PnC9-MenC vaccine contained 38.5 mcg of CRM_{197} [14]. The Hib-MenC-TT contained 12.5 mcg of tetanus toxoid [19].

The concentration of IgG specific to MenC was available before the booster at 12 months of age and after the booster at 13 months of age for the children participating in all the studies. The concentrations of IgG specific to diphtheria toxoid (DT) before the booster were available for all the studies except study 4, and the concentrations of tetanus toxoid (TT) before the booster were available for all the studies except studies 2 and 4. For any particular study, the concentration of either MenC, diphtheria or tetanus-specific IgG was measured in one of four laboratories (Oxford Vaccine Group, UK; Novartis Vaccines (ex-Chiron Vaccines), Emeryville, California; Wyeth, Rochester, NY; GlaxoSmithklineBiologicals, Belgium). The results were pooled and analysed to assess the effect of pre-booster MenC-specific IgG concentration on the relative increase in MenC-IgG concentration after the booster. The analysis was performed first using the Pearsons's correlation method, then using Blomqvist's adjustment method to assess the plausible extend of bias to the standard correlation method, and finally using the MLM specification of Oldham's method [21] for each group across 5 studies receiving 1 of 6 different vaccine schedules (see Tables 1 and 2).

2.1. Blomqvist method

For each group, and indeed for each study within groups 1 and 2, Blomqvist adjustment was used. This adjusts the regression coefficient of changes on baseline score (the unadjusted values are obtained directly from our data sets). The adjusted regression coefficients are converted to correlation coefficients using the standard formula; correlation of baseline and changes = (regression coefficient of changes on baseline) SD (baseline)/SD (changes), where SD = standard deviation. The baseline error variances used were as follows: the error variance was assumed to be 10%, and 50% of the total baseline variance in the specific group and study of interest. The error variance at follow-up was assumed to be firstly the same as that at baseline, then half that at baseline, and then just 10% of that at baseline. Error variance in the change scores is calculated as the sum of error variances at baseline and at follow-up (by independence of these error terms). It is plausible that the error variance at follow-up will be smaller than at baseline, since the mean values are substantially higher, so it may be less likely that they will be out by a factor of two (or any other chosen factor), and it is these factor differences that are relevant when working, as here, on the logarithmic scale. For groups with more than one study, a pooled correlation coefficient was derived by means of meta-analysis (inverse variance method on Fisher transformation of correlation coefficients).

2.2. MLM version of Oldham's technique

For each group a MLM model was fitted using multilevel software (MLwiN version 2.0.01; http://www.cmm.bristol.ac.uk), where the outcome of the model was the log of the MenC-IgG concentration. Pre- and post-treatment measures were fitted as repeated measures within subject. For groups with more than one study, a 3-level model was fitted with study at level 3, subjects at level 2, and time (i.e. pre- and post-booster coded as -1 and +1 respectively) at level 1. A 2-level model was used for groups with one study, with subjects at level 2 and time at level 1. The correlation coefficient was derived from the covariance between the random slope and random intercept of the model [21]. This gives the correlation between mean (of pre- and post-booster) values and changes.

Furthermore, in order to assess the role of carrier-specific antibodies on the MenC-antibodies response to booster immunisation, we assessed the correlations between the change in MenC-IgG concentration and the pre-booster diphtheria toxoid/tetanus toxoid-IgG concentration (depending of the carrier protein contained in the vaccine used for booster) using Pearson's correlation method, for the studies where pre-booster diphtheria toxoid/tetanus toxoid IgG concentrations were available. For groups with more than one study, a pooled correlation coefficient was derived by means of meta-analysis (inverse variance method on Fisher transformation of correlation coefficients). Note that there is no mathematical coupling when looking at correlation between baseline protein-carrier specific-IgG concentrations with changes in MenC-IgG concentrations, as the baseline variable is not the same as the variable used for the "change".

Table 2
Analysis of the effect of pre-booster MenC specific IgG concentration, and the relative increase in MenC specific IgG concentration after the booster, using unadjusted Pearson's correlation coefficients, the Blomqvist adjustment on these correlations, and then using the MLM technique.

Group	Priming 2,3,4 months Boosting 12 months	Pre-boost MenC-IgG GMC (μg/ml) (95% CI)	Post-boost MenC-IgG GMC (μg/ml) (95% CI)	Men-C IgG GMR (95% CI)	Unadjusted Pearson's correlation (pre and change) (95% CI)	Estimated true correlation (pre and change), using Blomqvist method					MLM correlation between mean (of pre and post) and change (95% CI)
						10% error var, same at f/u	50% error var, same at f/u	10% error var, half at f/u	50% error var, half at f/u	10% error var, 10% that at f/u	50% error var, 10% that at f/u	
1 (n = 202)	MenCVMenCV	1.81 (1.59, 2.06)	36 (32, 40)	20 (17, 23)	−0.58* (−0.48, −0.67)	−0.57	NA	−0.55	NA	−0.53	−0.08	−0.15* (−0.29, −0.02)
2 (n = 130)	MenCVMenA/C	1.87 (1.61, 2.17)	11 (8.5, 13)	5.7 (4.5, 7.3)	−0.5* (−0.36, −0.62)	−0.51	NA	−0.48	NA	−0.46	NA	0.21* (0.04,0.39)
3 (n = 41)	MenCVHib-MenC-TT	1.11 (0.8, 1.55)	3.7 (2.9, 4.7)	3.3 (2.6, 4.3)	−0.68* (−0.47, −0.82)	−0.71	NA	−0.67	NA	−0.64	−0.2	−0.35* (−0.67, −0.03)
4 (n = 134)	Hib-MenC-TT Hib-MenC-TT	0.73 (0.62, 0.85)	10 (8.5, 12)	14 (11, 16)	−0.58* (−0.46, −0.68)	−0.56	−0.42	−0.55	−0.3	−0.54	−0.25	−0.02 (−0.19,0.15)
5 (n = 44)	9vPnc-MenCV MenCV	0.51 (0.41, 0.63)	6.5 (4.6, 9.2)	13 (8.9, 19)	−0.44* (−0.17, −0.65)	−0.42	−0.28	−0.42	−0.26	−0.41	−0.25	0.45* (0.13,0.76)
6 (n = 44)	9VPnc-MenCV MenA/C	0.4 (0.32, 0.49)	2.2 (1.7, 2.9)	5.6 (4.4, 7.3)	−0.48* (−0.21, −0.68)	−0.45	−0.16	−0.44	−0.13	−0.43	−0.11	0.09 (−0.19, 0.38)

Error variances for Blomqvist adjustment are taken to be 10% and 50% of the total baseline variance, and at follow-up are taken to equal the error variance at baseline, or to be half of it, or to be 10% of baseline error variance. NA, not available, missing values for Blomqvist adjusted correlations are a result of the follow-up variance being too small to support such large error variances as assumed.
* Correlation that are statistically significant.

Analysis was carried out using Stata (version 10, StataCorp, USA) and MLwiN (version 2.0.01, University of Bristol, UK). Data of all analyses were log transformed prior to analyses to meet the normal distribution assumption.

3. Results

MenC-IgG antibody data were available from 646 children, who had been immunised according to 1 of 6 different schedules (Table 1). The children were primed with either MenCV, combined Hib-MenC-TT, or combined 9vPnc-MenCV. Boosting was with either MenCV, MenA/C, or Hib-MenC-TT. All groups had a pre-booster MenC-geometric mean concentration (GMC) below the postulated correlate of protection of 2 μg/ml [22,23]. However, the GMC of MenC-antibody of groups 1, 2, and 3 primed with a monovalent MenCV was ≥1 μg/ml, in contrast to the other groups primed with a combined vaccine containing the MenCV. Following booster immunisation, all groups had a significant increase in MenC-IgG concentration, and all the groups had a MenC-GMC above 2 μg/ml (Table 2).

Using Pearson's correlation method to assess the effect of pre-booster MenC-IgG concentration on the relative increase in MenC-IgG concentration post-booster, a significant negative correlation was observed for all the groups (Table 2). As already discussed, measurement error and short term fluctuations in pre-booster levels could cause, or contribute to such associations. The Blomqvist adjustment shows how much of an influence this bias may plausibly have, by showing estimated correlation coefficients between underlying true values at baseline and changes (i.e. taken from baseline and follow-up values averaged over short term fluctuations without measurements error, Table 2). Blomqvist adjusted correlation coefficients remain negative in most cases except when the error variance is high. For example for group 6, primed with 9vPnc-MenCV and boosted with MenA/C, where the adjusted correlation is −0.16, when 50% of the baseline variance is attributable to error, even assuming the same error variance at follow-up.

Using the MLM technique to assess the relationship between mean (of pre-and post-booster) MenC-IgG concentration and the relative increase in MenC-IgG concentration post-booster, contrasting results were observed with either a positive, negative or no correlation (Table 2).

To assess the relationship between carrier-protein specific antibody concentrations and the increase in MenC-specific antibody concentrations Pearsons's correlation coefficient was used. Note that in this case the use of Pearsons's correlation is valid since the variables concerned are not mathematically coupled. A negative correlation was observed when a protein–polysaccharide conjugate vaccine (MenCV or Hib-MenC-TT) was used for booster. This was significant for 3 out of the 4 such groups (Table 3). In contrast, there was no correlation when the plain-polysaccharide MenA/C vaccine was used as booster (Table 2).

4. Discussion

The effect of pre-existing antigen-specific serum antibody on response to subsequent doses of vaccine is not well understood. Studying this phenomenon in humans, outside the settings of transplacental antibody transfer and clinical situations where antibodies are administered passively (e.g. hepatitis B immunoglobulin in individuals exposed to an infectious source), is limited to observational studies using correlation or regression analysis. In the current study using standard correlation methods the increase in MenC-antibody concentration was consistently negatively correlated with pre-booster MenC-antibody concentration as previously described [6–9]. Negative correlations, in other disease areas,

Table 3
Correlation between the change in MenC-IgG concentration and the pre-booster carrier protein-IgG concentration using Pearson's correlation method.

Group	Priming at 2, 3, 4 months	Boosting at 12 months	Study no.	No. of parti-cipants	Correlation between pre-boost carrier protein[a] IgG and change in MenCIgG on boosting (95% CI)	*p*-Value
1	MenCV	MenCV	1, 2, 3	110	−0.43 (−0.58, −0.26)	<0.0001
2	MenCV	MenA/C	2, 3	97	0.06 (−0.14, 0.26)	0.57
3	MenCV	Hib-MenC-TT	5	41	−0.21 (−0.49, 0.1)	0.19
4	Hib-MenC-TT	Hib-MenC-TT	5	132	−0.18 (−0.34,0)	0.04
5	9vPnC-MenCV	MenCV	2	35	−0.47 (−0.7, −0.17)	0.004
6	9vPnC-MenCV	MenA/C	2	38	−0.04 (−0.36, 0.28)	0.8

[a] Diphtheria toxoid-IgG was used for all the groups except groups 3 and 4 where tetanus toxoid was used because the Hib-MenC-TT contains tetanus toxoid as carrier protein.

between baseline and changes have been shown to result from the combined effect of mathematical coupling and measurement error [24,25], and so cannot be interpretable in a clinically meaningful way. Use of Blomqvist adjustment, on a range of plausible error variances, suggests that the true underlying correlation between baseline and changes remains negative in the cases of small error variance, as it can be expected assuming that there is a small coefficient of variation in the ELISA assay used [26,27] and assuming that there is little day to day fluctuation in MenC-IgG concentrations [10]. In contrast, using the MLM technique, to account for the mathematical coupling of the pre- and post-booster antibody concentration, a consistent negative correlation was not observed between the mean MenC-specific antibody concentration and the MenC-specific antibody increase post-booster. However, the use of the MLM method appears not to be appropriate as it may introduce a positive bias, since the follow-up values are added in calculating both baseline and changes, so any measurement error in follow-up term induces a positive bias. Furthermore, it does not directly measure the correlation between baseline and change (rather it uses mean and change). These data suggest that the inhibitory effect of baseline antibody on the immune response to booster may be accurate assuming that there is a low error variance (due to small measurement error due to the ELISA assay and little day to day fluctuations in measurements) [10,26,27].

The use of Oldham's method associated with MLM to account for mathematical coupling between baseline values and changes is supported by Tu et al. from a series of publications mostly on dental literature. However, this method has also been criticised as it is based on several assumptions (such as same error variance at baseline and at follow-up) which cannot be verified. Furthermore, this method does not directly measure the correlation between baseline and changes, whereas that is the correlation which is clinically relevant (rather than the Oldham's correlation between mean and changes). Others support the use of Blomqvist [11], although additional data (repeated measurements taken short intervals apart) are needed to apply this technique directly, which is not really feasible when doing vaccine trials in young children with limited blood draws. However, we have used the method suggested by Hayes et al. (1988) among others, which is effectively undertaking a sensitivity analysis, to estimate error variance, according to a range of plausible estimates. As discussed earlier, only a low error variance is likely to occur in the studies reported in the present manuscript.

Our study is limited by the heterogeneity of the different studies used. Indeed, since a variety of other factors varied amongst the infants studied [e.g. the dose of MenC used in priming and boosting, the role of combination versus monovalent vaccine in priming or boosting, the general level of pre-boost antibody (higher vs lower)] it was not possible to infer which ones were independently important due to the number of groups available for study in relation to the number of possible variables. Also, the antigen-specific IgG concentrations were measured in different laboratories, and although laboratories derived their anti-polysaccharide IgG protocols from two publications [26,28], the assays pooled, analysed and compared in this study have not been compared to each other directly, which should be acknowledged as a limitation of this study. However, the method used accounts for this heterogeneity (caused by assay variations or infant selection) between the different studies used.

There are a number of factors that might explain why pre-immunisation antibody concentrations may have a weaker inhibitory effect on vaccine immunogenicity than previously reported. First, the statistical methods used in previous studies did not account for the problems of coupling which are inherent in studies where a variable is measured in the same individual pre- and post-intervention (as discussed above). Second, antibody mediated inhibition may not be a significant immunological phenomenon in the context of booster immunisation of infants or at least it may only be significant at higher concentrations of pre-immunisation antibody than the ones observed in the infants of the present study. In several reviews of animal data, it has been reported that the antibody-feedback regulation on B cell responses to immunisation could be either positive or negative [1–3]. Finally, an alternative possibility is that even if antibody mediated inhibition occurs, the pre-immunisation antibody correlates with some other immunological parameter (e.g. immunological memory) that is more significant in enhancing the immune response than the antibody is at directly inhibiting it. It is thought that serum antibody is maintained by the differentiation of memory B cells into plasma cells. If the antibody levels at baseline reflect the size of the memory B cell pool available, then one might expect greater secondary antibody responses to be associated with higher levels of pre-existing antibody.

A striking finding of the current study was a consistent negative correlation between the carrier protein-specific baseline antibody concentration and the increase in MenC-specific antibody concentration following booster immunisation with the protein-polysaccharide conjugate vaccine, which was significant in 3 out of the 4 groups tested. Note that the only group (group 3) for which the correlation was not significant was a group with a relatively small sample size. In contrast, there was no correlation when the plain-polysaccharide MenA/C vaccine was used as booster. For variables which a priori are unrelated standard correlation methods can be used, since there is no mathematical coupling present. "Carrier induced epitope suppression" describes the effect of pre-existing immunity to a carrier-protein, on response to a linked

haptenic antigen (in this case the polysaccharide) [14,29]. A variety of mechanisms have been described involving both carrier-protein specific B and T-cells in addition to pre-existing serum antibody [30]. Preformed B cells and T cells specific for the carrier proteins could compete for antigen capture with B cells specific for the polysaccharide, thus inhibiting the immune response to the polysaccharide component of the conjugate vaccine [30–32]. These findings suggest that the level of carrier-specific antibody might correlate with the level of carrier-specific B cells, which may compete with polysaccharide-specific B cells for the capture of the MenCV. An alternative explanation for this could be that antibody to the carrier protein provides more effective steric hindrance of the interaction of vaccine antigen and antigen-specific B-cells than the anti-polysaccharide antibody [30]. This could be due to either of the antigen quality and quantity or that of the antibody. An intriguing possibility is that pre-existing carrier-specific antibody may be a more potent inhibitor of T-cell responses than B-cells. From this point of view it would be useful to look at antibody mediated inhibition due to a vaccine antigen containing only T-dependent constituent antigens. However, another study has reported that high pre-booster concentration of carrier-specific antibody correlated positively with the increase in antibody specific for the polysaccharide antigen following immunisation with a *H. influenzae* type b conjugate vaccine in adults [33]. It was suggested by the investigators of this study that carrier-specific antibodies correlated with the numbers of carrier-specific helper T cells, which in turn could increase the B cell response against the polysaccharide component of the conjugate vaccine. Therefore, the phenomenon of carrier-induced epitope suppression may depend on several factors. For example, the dose of the carrier may be important. It was observed in a murine study that low-dose carrier priming improved subsequent response to polysaccharides conjugated to the same carrier, while high dose priming suppressed the response [34]. Similarly, it was reported that the key factor for the inhibitory effect of maternal antibodies on infant vaccine responses was the ratio of antibody to immunising antigen [4]. In the present study, the concentration of CRM_{197} was quite high for all the vaccines (MenCV and 9vPnC-MenCV), however, the dose of tetanus toxoid used in the Hib-MenC-TT vaccine was quite low.

A recommendation for further research is that the measurements of MenC-IgG and protein-carrier specific-IgG are taken at least twice at a short time interval apart, certainly around baseline and preferably also at follow-up, so that the variance of the measurement error/short term fluctuations in these measurements can be found directly. This would allow Blomqvist's method to be used directly to adjust the correlations between pre- and post- MenC-IgG measurements, and give further insight into the results presented here. It would also allow for adjustments to be made to the correlation coefficients between baseline protein-carrier specific-IgG and changes in MenC-IgG. It would also be desirable in future vaccine trials in general to build repeated measurements of antibodies into the protocol, at least for a sub set of patients, so that these issues can be more fully assessed.

In conclusion, we have demonstrated that understanding the effect of pre-existent antibody on response to immunisation is dependent on using statistical methods that account for mathematical coupling. Using Blomqvist method of adjustment and assuming that there is low error variance, we have shown that the inhibitory effect of pre-existing MenC antibody concentration on the MenC-antibody response to booster immunisation reported previously might be accurate. In contrast, there was a consistent inhibition of the protein carrier-specific antibody pre-booster on the MenC-antibody increase post-booster with the MenCV suggestive of the fact that either (i) serum antibody is inhibitory the effect of immunological memory is excluded or (ii) that carrier protein antibodies are more likely to mediate inhibition through mechanisms such as diversion or alterations in T-cell help. These findings should be further explored as they have implications for the optimal timing of doses of conjugate vaccines and their relationship to booster doses of vaccines containing carrier-protein epitopes.

Conflict of interests

AJP has conducted clinical trials on behalf of Oxford University, sponsored by Wyeth Vaccines, GlaxoSmithKline Vaccines, Sanofi Pasteur, Sanofi Pasteur MSD and Novartis Vaccines, but does not accept any personal payments from vaccine manufacturers. Industry-sourced honoraria for lecturing, writing or consultancy and travel expenses for attendance at scientific meetings are paid to an educational/administrative fund held by the Department of Paediatrics, University of Oxford. MDS has received assistance to attend scientific meetings from Wyeth Vaccines, Novartis Vaccines and GlaxoSmithKline Vaccines and has had travel and accommodation expenses paid by Novartis Vaccines while working in collaboration with Novartis Vaccines in Siena, Italy.

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References

[1] Heyman B. Regulation of antibody responses via antibodies, complement, and Fc receptors. Annual Review of Immunology 2000;18:709–37.
[2] Heyman B. Feedback regulation by IgG antibodies. Immunology Letters 2003;88(2):157–61 (August 5).
[3] Hjelm F, Carlsson F, Getahun A, Heyman B. Antibody-mediated regulation of the immune response. Scandinavian Journal of Immunology 2006;64(September (3)):177–84.
[4] Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. Vaccine 2003;21(24):3406–12 (July 28).
[5] Siegrist CA, Barrios C, Martinez X, Brandt C, Berney M, Cordova M, et al. Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. European Journal of Immunology 1998;28(December (12)):4138–48.
[6] Danilova E, Shiryayev A, Kristoffersen EK, Sjursen H. Attenuated immune response to tetanus toxoid in young healthy men protected against tetanus. Vaccine 2005;23(42):4980–3 (October 10).
[7] Danilova E, Shiryayev A, Skogen V, Kristoffersen EK, Sjursen H. Short-term booster effect of diphtheria toxoid in initially long-term protected individuals. Vaccine 2005;23(12):1446–50 (February 10).
[8] Ronne T, Valentelis R, Tarum S, Griskevica A, Wachmann CH, Aggerbeck H, et al. Immune response to diphtheria booster vaccine in the Baltic states. The Journal of Infectious Diseases 2000;181(February (Suppl. 1)):S213–9.
[9] Nicolay U, Girgsdies OE, Banzhoff A, Hundt E, Jilg W. Diphtheria booster vaccination: one or two injections? Vaccine 1999;17(18):2223–8 (May 4).
[10] Blanchard Rohner G, Snape MD, Kelly DF, John T, Morant A, Yu LM, et al. The magnitude of the antibody and memory B cell responses during priming with a protein–polysaccharide conjugate vaccine in human infants is associated with the persistence of antibody and the intensity of booster response. The Journal of Immunology 2008;180(4):2165–73 (Feb 15).
[11] Hayes RJ. Methods for assessing whether change depends on initial value. Statistics in Medicine 1988;7(September (9)):915–27.
[12] Tu YK, Gilthorpe MS. Revisiting the relation between change and initial value: a review and evaluation. Statistics in Medicine 2007;26(2):443–57 (January 30).
[13] Oldham PD. A note on the analysis of repeated measurements of the same subjects. Journal of Chronic Diseases 1962;October (15):969–77.

[14] Buttery JP, Riddell A, McVernon J, Chantler T, Lane L, Bowen-Morris J, et al. Immunogenicity and safety of a combination pneumococcal-meningococcal vaccine in infants: a randomized controlled trial. Journal of the American Medical Association 2005;293(14):1751–8 (April 13).
[15] Riddell A, Buttery JP, McVernon J, Chantler T, Lane L, Bowen-Morris J, et al. A randomized study comparing the safety and immunogenicity of a conjugate vaccine combination containing meningococcal group C and pneumococcal capsular polysaccharide–CRM197 with a meningococcal group C conjugate vaccine in healthy infants: challenge phase. Vaccine 2007;25(19):3906–12 (May 10).
[16] MacLennan JM, Shackley F, Heath PT, Deeks JJ, Flamank C, Herbert M, et al. Safety, immunogenicity, and induction of immunologic memory by a serogroup C meningococcal conjugate vaccine in infants: a randomized controlled trial. Journal of the American Medical Association 2000;283(21):2795–801 (June 7).
[17] English M, MacLennan JM, Bowen-Morris JM, Deeks J, Boardman M, Brown K, et al. A randomised, double-blind, controlled trial of the immunogenicity and tolerability of a meningococcal group C conjugate vaccine in young British infants. Vaccine 2000;19(9–10):1232–8 (December 8).
[18] Snape MD, Maclennan JM, Lockhart S, English M, Yu LM, Moxon RE, et al. Demonstration of immunologic memory using serogroup C meningococcal glycoconjugate vaccine. The Pediatric Infectious Disease Journal 2009;28(February (2)):92–7.
[19] Pace D, Snape M, Westcar S, Oluwalana C, Yu LM, Begg N, et al. A novel combined Hib-MenC-TT glycoconjugate vaccine as a booster dose for toddlers: a phase 3 open randomised controlled trial. Archives of Disease in Childhood 2008;93(November (11)):963–70.
[20] Pace D, Snape M, Westcar S, Hamaluba M, Yu LM, Begg N, et al. A new combination haemophilus influenzae type B and Neisseria meningitidis serogroup C-tetanus toxoid conjugate vaccine for primary immunization of infants. The Pediatric Infectious Disease Journal 2007;26(November (11)): 1057–9.
[21] Blance A, Tu YK, Gilthorpe MS. A multilevel modelling solution to mathematical coupling. Statistical Methods in Medical Research 2005;14(December (6)):553–65.
[22] Peltola H. Meningococcal vaccines: current status and future possibilities. Drugs 1998;55(March (3)):347–66.
[23] Gold R, Lepow ML, Goldschneider I, Draper TF, Gotshlich EC. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. The Journal of Infectious Diseases 1979;140(November (5)):690–7.
[24] Tu YK, Maddick IH, Griffiths GS, Gilthorpe MS. Mathematical coupling can undermine the statistical assessment of clinical research: illustration from the treatment of guided tissue regeneration. Journal of Dentistry 2004;32(February (2)):133–42.
[25] Tu YK, Gilthorpe MS, Griffiths GS. Is reduction of pocket probing depth correlated with the baseline value or is it mathematical coupling? Journal of Dental Research 2002;81(October (10)):722–6.
[26] Gheesling LL, Carlone GM, Pais LB, Holder PF, Maslanka SE, Plikaytis BD, et al. Multicenter comparison of Neisseria meningitidis serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. Journal of Clinical Microbiology 1994;32(June (6)):1475–82.
[27] Elie CM, Holder PK, Romero-Steiner S, Carlone GM. Assignment of additional anticapsular antibody concentrations to the Neisseria meningitidis group A, C, Y, and W-135 meningococcal standard reference serum CDC1992. Clinical and Diagnostic Laboratory Immunology 2002;9(May (3)):725–6.
[28] Granoff DM, Maslanka SE, Carlone GM, Plikaytis BD, Santos GF, Mokatrin A, et al. A modified enzyme-linked immunosorbent assay for measurement of antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. Clinical and Diagnostic Laboratory Immunology 1998;5(July (4)):479–85.
[29] Dagan R, Eskola J, Leclerc C, Leroy O. Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants. Infection and Immunity 1998;66(May (5)):2093–8.
[30] Dagan R, Poolman J, Siegrist CA. Glycoconjugate vaccines and immune interference: a review. Vaccine 2010;28(34):5513–23.
[31] Barington T, Skettrup M, Juul L, Heilmann C. Non-epitope-specific suppression of the antibody response to *Haemophilus influenzae* type b conjugate vaccines by preimmunization with vaccine components. Infection and Immunity 1993;61(February (2)):432–8.
[32] Insel RA. Potential alterations in immunogenicity by combining or simultaneously administering vaccine components. Annals of the New York Academy of Sciences 1995;754:35–47 (May 31).
[33] Barington T, Kristensen K, Henrichsen J, Heilmann C. Influence of prevaccination immunity on the human B-lymphocyte response to a *Haemophilus influenzae* type b conjugate vaccine. Infection and Immunity 1991;59(March (3)):1057–64.
[34] Daum RS, Zenko CE, Given GZ, Ballanco GA, Parikh H, Germino K. Magnitude of interference after diphtheria-tetanus toxoids-acellular pertussis/*Haemophilus influenzae* type b capsular polysaccharide-tetanus vaccination is related to the number of doses administered. The Journal of Infectious Diseases 2001;184(10):1293–9 (November 15).