Comparison and correlation of Neisseria meningitidis serogroup A immunologic assay results following one dose of Meningococcal serogroup A conjugate vaccine in healthy Indian adults

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Introduction

The Meningitis Vaccine Project (MVP) has been created in 2001 by a grant from the Bill and Melinda Gates Foundation as a partnership between WHO and PATH to develop and introduce affordable meningococcal conjugate vaccines for elimination of meningococcal epidemics in sub-Saharan Africa [1].
A serogroup A meningococcal conjugate vaccine using tetanus toxoid as a carrier protein (PsA-TT) has been developed at the Serum Institute of India Ltd., using a new licensed conjugation technique from the Center for Biologics Evaluation and Research/Food and Drug Administration.
Immunogenicity and antibody persistence and safety in healthy volunteers aged 18-35 years was demonstrated in a double-blind randomised controlled phase I study [2].
Kshirsager et al. [2] reported immunogenicity as determined by SBA using baby rabbit complement and standardised ELISA for anticapsular total IgG.

Aim

The aim of the study was to analyse sera by additional immunological assays, in order to investigate the relationship between different serogroup A immunologic assays

Methods

Study group

Healthy adult volunteers (18-35 years) were recruited from three sites within India [2] and randomised in a double-blind fashion to receive intramuscularly in the deltoid region one dose of either:

- PsA-TT conjugate vaccine (Serum Institute of India, Pune, India)
- Meningococcal polysaccharide Vaccine A&CTM (Sanofi Pasteur, Lyon, France) (PsA/C)
- Tetanus Toxoid AdsorbedTM (SIIL, Pune, India)

Blood samples were taken for immunogenicity studies on the day of immunisation and 4, 24 and 48 weeks later.

The immune response to PsA-TT and PsA/C was compared in 6 immunological assays. Data from the Tetanus Toxoid vaccine group is not included in this analysis

Immunological assays

- **Serum bactericidal antibody (SBA) assay**
- SBA assays were performed against the serogroup A target strain, CDC no F8238 (phenotype A:4,21:P1.20,9) [3]
- Complement source used was either
  - serum from 3-4 week old rabbits (Pel-Freez Biologicals, WI) [Performed at HPA, Manchester, UK]
  - pooled human complement (from individual sera with no intrinsic killing of F8238, rSBA titres ≤ 8 and total IgGAM < 30 µg/mL) and showed no intrinsic complement activity against F8238 and showed preservation of complement activity as determined by CH50 titres [performed at FDA, Bethesda, MD, USA]

SBA titres were expressed as the reciprocal serum dilutions yielding ≥ 50% killing after 60 min for rSBA and 90 min for hSBA.

Titres <4 were assigned an arbitrary value of 2 for data analysis

- **Serogroup A-specific IgG**

Serogroup A-specific IgG levels were determined using

- standardised ELISA using CDC 1992 and mouse monoclonal-PAN anti-human IgG Fcγlabelled with horseradish peroxidise [4] [performed at CDC, Atlanta, GA, USA]
- Multiplex bead assay [5] [performed at HPA Manchester]. Briefly, meningococcal serogroup A polysaccharide was attached to poly-L-lysine and then covalently attached to carboxylated microspheres using a two-step carbodiimide reaction. Serum and beads were incubated for 20 min at room temperature of a plate shaker followed by washing and incubation with R-phycoerythrin-conjugated anti-human IgG. The plate was then read on a Bio-plex reader and data analysed using Bio-plex Manager. Data for unknown test sera was generated from a 5-parameter logistic standard curve of CDC 1992 and converted to µg/mL.

- **Opsonophagocytic assay (OPA)**
- The opsonophagocytic activity (performed at the CDC) was measured by incubating serogroup A polysaccharide conjugated to fluorescent beads, with serum dilutions followed by the addition of baby rabbit complement and HL60 cells which had been chemically induced into monocytes [6].
- Titres were reported as the reciprocal of the highest serum dilution yielding ≥ 50% of the maximum phagocytic uptake (calculated from a 5-PL curve of % uptake vs dilution).
- Samples with a maximum phagocytic uptake of <20% were considered negative and were reported to have a titre of 4.
- Opsonophagocytic activity (performed at the NIPH) was measured as the respiratory burst using human complement and live serogroup A strain F8238 [7] The highest reciprocal serum dilution giving ≥ 50% respiratory burst of the PMNs is recorded as the serum titre.
- **Data analysis**
- Results from the 6 assays, at each time point were log transformed and geometric means calculated with 95% confidence intervals (95% CI).
- Differences in GMTs or GMCs between groups were expressed as ratios with 95% CI.
- GMTs and GMCs between time points were significantly different when the confidence intervals did not include 1.0.
- The relationship between the different immunological assays was determined using the Pearson correlation coefficient.

Results

Table 1. SBA

	PsA-TT	PsA/C
rSBA GMT (95%CI)		
Pre-vaccination	228 (84-617)	422 (191-929)
4 weeks post-vaccination	8192 (5221-12885)*	5257 (2798-9878)*
24 weeks post-vaccination	6339 (4973-8023)	4096 (2798-5997)
48 weeks post-vaccination	4211 (3517-5078)	2226 (1552-3191)
hSBA GMT (95%CI)		
Pre-vaccination	4.56 (3.3-6.3)	6.73 (4.2-10.7)
4 Weeks post-vaccination	73.94 (38.5-141.9)*	19.17 (9.9-37.1)*
Proportion of subjects with 4-fold rise pre- to 4 weeks post-vaccination		
hSBA	18/21 (86%)	12/22 (54%)
rSBA	20/24 (83%)	18/25 (73%)

- 4 weeks post-vaccination there was a significant difference between the hSBA GMTs of the 2 vaccine groups (p<0.005) which was not seen with the rSBA
- 1 year post-vaccination there was a significant difference between the rSBA GMTs (p<0.005) (data not available at 1 year for hSBA)

* Significant increase pre- to 4 weeks post-vaccination (p<0.005)

Number of subjects demonstrating a four-fold rise in SBA titre pre- to 4-weeks post-vaccination

PsA-TT

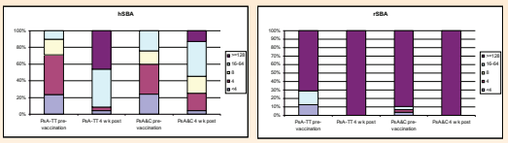
	4-fold rise in hSBA	No 4-fold rise in hSBA
4-fold rise in rSBA	15	3
No 4-fold rise in rSBA	3	0

PsA&C

	4-fold rise in hSBA	No 4-fold rise in hSBA
4-fold rise in rSBA	11	5
No 4-fold rise in rSBA	1	5

There was a good correlation between subjects demonstrating a four-fold rise in the hSBA and rSBA for both groups (71.4% for PsA-TT and 72.7% for PsA&C).

Figure 1. Percentage of subjects before and 4 weeks following vaccination with either PsA-TT or PsA/C at serum bactericidal titre cutoffs of <4, 4, 8, >8, 128 and >128



The proportion of subjects with putatively protective titres (rSBA ≥ 8; hSBA ≥ 4) 4 weeks post-vaccination determined by both SBA assays were similar regardless of the vaccine administered.

In each vaccine group there was one subject which had a rSBA ≥ 8 and a hSBA < 4.

Post-vaccination 96% of subjects in both vaccine groups had hSBA titres ≥ 4, but a higher proportion in the PsA-TT group achieved higher hSBA threshold cut-offs than in the PsA&C group.

Table 2. Serogroup A-specific IgG

	PsA-TT	PsA/C
ELISA		
Pre-vaccination	4 (3-7)	5 (3-8)
4 weeks post-vaccination	110 (68-174)*	44 (26-71)*
24 weeks post-vaccination	72 (42-123)	39 (22-66)
48 weeks post-vaccination	52 (30-93)	34 (21-54)
Multiplex bead assay		
Pre-vaccination	1.8 (1-3)	1.3 (0.81-2.2)
4 weeks post-vaccination	76 (47-123)*	19 (10-34)*
24 weeks post-vaccination	50 (28-89)	19 (10-35)
48 weeks post-vaccination	32 (18-56)	15 (9-28)

- The serogroup A-specific IgG determined by both assays showed a strong correlation (figure 2 & table 4) although overall the multiplex bead assay GMCs are lower.
- 4 weeks post-vaccination the GMC of the PsA-TT group was significantly higher than the GMC of the PsA/C group (p<0.005) for both assays.

Significant increase pre- to 4 weeks post-vaccination (p<0.005)

Figure 2. Correlation of serogroup A-specific IgG determined by ELISA and multiplex bead assay (both vaccine groups and all visits combined)

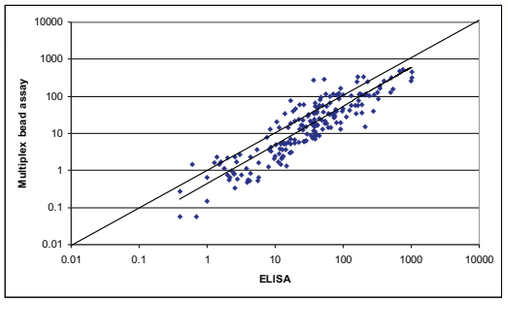
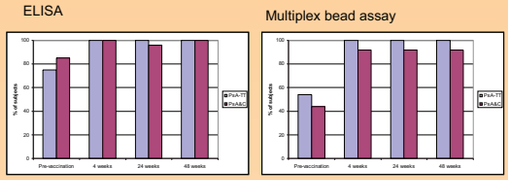


Figure 3. Proportion of subjects with serogroup A-specific IgG ≥ 2 µg/mL



Pre-vaccination the proportion of subjects with serogroup A-specific IgG ≥ 2 µg/mL differed but at all other time points the proportions were similar.

Table 3. OPA

For both assays for both vaccine groups:

	PsA-TT	PsA/C
CDC OPA		
Pre-vaccination	443 (232-845)	820 (526-1277)
4 weeks post-vaccination	2656 (1713-4118)	2165 (1268-3695)
24 weeks post-vaccination	813 (571-1156)	556 (350-883)
48 weeks post-vaccination	271 (163-452)	147 (81-268)
NIPH OPA		
Pre-vaccination	11 (6-20)	10 (6-15)
4 weeks post-vaccination	50 (31-78)	22 (13-37)
24 weeks post-vaccination	11 (6-20)	6 (4-9)
48 weeks post-vaccination	10 (6-18)	5 (3-8)

- significant rise pre to 4 weeks post-vaccination (p<0.001)
- significant decline 4-weeks post-vaccination to 24 weeks post-vaccination (p<0.001)
- significant decline 24 weeks post-vaccination to 48 weeks post-vaccination (p<0.001)
- significant decline 4 weeks post-vaccination to 48 weeks post-vaccination (p<0.001)

The CDC OPA assay showed a significant decline pre- to 48 weeks post-vaccination (p<0.01)

Four weeks post-vaccination there was a significant difference between the NIPH OPA GMT of the two vaccine groups (p=0.02) which was not seen for the CDC OPA assay.

Table 4. Pearson correlation coefficients for the PsA-TT (white shading) and PsA&C (values shaded grey) vaccine groups

	ELISA	MULTIPLEX	hSBA	OPA (CDC)	OPA (NIPH)	rSBA
ELISA		0.90 (n=96)	0.82 (n=45)	0.25 (n=96)	0.10 (n=96)	0.54 (n=96)
MULTIPLEX	0.9 (n=98)		0.78 (n=45)	0.29 (n=96)	0.19 (n=96)	0.56 (n=96)
hSBA	0.45 (n=47)	0.5 (n=46)		0.42 (n=45)	0.48 (n=45)	0.57 (n=45)
OPA (CDC)	0.09 (n=100)	0.08 (n=98)	0.45 (n=47)		0.36 (n=96)	0.37 (n=96)
OPA (NIPH)	-0.07 (n=100)	-0.05 (n=98)	0.42 (n=47)	0.21 (n=100)		0.37 (n=96)
rSBA	0.55 (n=100)	0.53 (n=98)	0.36 (n=47)	0.32 (n=100)	0.11 (n=100)	

Correlations between immunological assays were calculated for all visits combined therefore covering a whole range of antibody levels.

There was a strong correlation between serogroup A-specific IgG (by either method) and the hSBA in the PsA-TT group

The two OPA methods correlated weakly in both vaccine groups
For both OPA methods there was low or no correlation to any other immunoassays

Conclusions

- The proportion of subjects determined to be putatively protected by hSBA (titres ≥ 4) and rSBA (titres ≥ 8) and also ≥ 4 fold rises were similar.
- Four weeks post-vaccination the vaccine groups differed by hSBA four fold rise, proportion achieving a titre of ≥ 8 and GMT, IgG GMC, and NIPH OPA GMT. At 48 weeks post-immunisation, the vaccine groups differed by rSBA GMT and IgG GMC (hSBA not available).
- Four weeks post-vaccination a difference between the vaccine groups was seen by hSBA and serogroup A-specific IgG concentration (by either assay).
- Serogroup A-specific IgG concentrations determined by ELISA and the multiplex bead assay are very comparable.
- OPA assays shows a rapid decline in antibody in the year post-vaccination whereas both the rSBA titres and serogroup A-specific IgG concentrations show antibody persistence.
- Both ELISA and multiplex bead assay showed a good correlation with hSBA.

References

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