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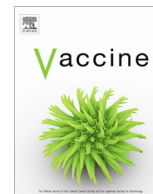
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Repeat pneumococcal polysaccharide vaccine in Indigenous Australian adults is associated with decreased immune responsiveness



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ABSTRACT

Background: Indigenous adults residing in the Northern Territory of Australia experience elevated rates of invasive pneumococcal disease despite the routine use of 23-valent pneumococcal polysaccharide vaccine (23vPPV). We hypothesised that the limited protection from 23vPPV may be due to hyporesponsiveness as a result of vaccine failure from repeated vaccination. To explore this possibility, we evaluated the immune response to a first and second dose of 23vPPV in Indigenous adults and a first dose of 23vPPV in non-Indigenous adults.

Methods: Serotype-specific IgG was measured by ELISA for all 23 vaccine serotypes at baseline and at one month post-vaccination. Individuals were considered to have an adequate immune response if paired sera demonstrated either: a four-fold rise in antibody concentration; a two-fold rise if the post vaccination antibody was $>1.3 \mu\text{g/ml}$ but $<4.0 \mu\text{g/ml}$; or a post-vaccination antibody concentration $>4.0 \mu\text{g/ml}$ for at least half of the serotypes tested (12/23). Our per-protocol analysis included the comparison of outcomes for three groups: Indigenous adults receiving a second 23vPPV dose ($N = 20$) and Indigenous ($N = 60$) and non-Indigenous adults ($N = 25$) receiving their first 23vPPV dose.

Results: All non-Indigenous adults receiving a first dose of 23vPPV mounted an adequate immune response (25/25). There was no significant difference in the proportion of individuals with an adequate response using our definition (primary endpoint), with 88% of Indigenous adults mounted an adequate response following first dose 23vPPV (53/60) compared to 70% having an adequate response following a second dose of 23vPPV (14/20; $p = 0.05$). The risk difference between Indigenous participants receiving first dose compared to non-Indigenous participants receiving first dose was significant when comparing a response threshold of at least 70% (-27% , 95% CI: -43% to -11% ; $p = 0.01$) and 90% (-38% , 95% CI: -60% to -16% ; $p = 0.006$) of serotypes with a positive response.

Conclusion: Indigenous participants demonstrated a poorer response to a first dose 23vPPV compared to their non-Indigenous counterparts, with lower IgG following a second 23vPPV dose. These findings highlight the critical need to evaluate the efficacy of future pneumococcal vaccine programs in the Australian Indigenous populations that recommend repeated doses of 23vPPV.

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1. Introduction

Indigenous adults in the Northern Territory of Australia suffer one of the highest reported rates of pneumococcal disease worldwide, exceeding 10-fold higher than non-Indigenous adults [1]. A

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unique schedule of repeated vaccination with the 23-valent pneumococcal polysaccharide vaccine (23vPPV) is recommended for Indigenous adults residing in the Northern Territory (NT) of Australia owing to their elevated rates of pneumococcal disease [2,3]. Since 1995, 23vPPV has been recommended for all Indigenous Australian adults aged 50 years and over and for all Australians aged 15–49 years with medical risk factors. As a regional initiative in 2000, this was expanded to include all Indigenous adults in the NT aged 15 years and over [4]. Non-Indigenous adults without medical risk factors receive their first dose at 65 years of age. Revaccination was initially recommended every five years, but since 2003, a maximum of three lifetime doses has been recommended for Indigenous adolescents and adults and non-Indigenous adults with medical risk factors: a second dose five years after the first dose and then a third dose ten years after the second, or at age 50 years, whichever is later [5].

This unique 23vPPV regimen has not been associated with a reduction in invasive pneumococcal disease (IPD) incidence within Indigenous adults residing in the NT, although vaccination coverage has been generally low [1]. While there is some evidence of an adequate immunological response to revaccination, no data are available from randomised controlled trials determining clinical efficacy [6–9]. Immune hyporesponsiveness, associated with the production of attenuated serotype-specific IgG responses to vaccine serotypes, has been suggested to result following repeated polysaccharide immunisation and has been demonstrated following 23vPPV in Fijian children [10], although this was no longer apparent by 5 years of age [11]. To investigate the possibility that hyporesponsiveness following repeat vaccination may be one factor contributing to persistently high rates of pneumococcal disease in Indigenous adults, we compared the immune response following a second dose of 23vPPV in Indigenous Australians with the response following a first dose in both Indigenous and non-Indigenous Australians.

2. Ethical approval

The study was approved by the Human Research Ethics Committee of the NT Department of Health and the Menzies School of Health Research (06/57), and includes assessment by the Aboriginal Ethics Sub-Committee.

3. Materials and methods

3.1. Study population

Potential participants were recruited from remote Aboriginal communities in the Top End of the NT, Darwin (the NT capital city), and the Darwin Correctional Centre. Participants were recruited between 10th November 2010–29th November 2011 when the Therapeutic Goods Administration suspended use of 23vPPV owing to reports of increased adverse reactions following repeated doses of 23vPPV. Recruitment then commenced again between 5th December 2012–7th February 2013. The informed consent process included a discussion using a pictorial flip chart and plain language participant information sheet explaining the study requirements, risks and benefits. Potential participants were only recruited when the study staff felt the requirements were understood. Eligible participants were adults aged 15–59 years, due for a first or second dose of 23vPPV as per the recommended NT schedule (Indigenous adults) or for whom the vaccine was not otherwise contraindicated (non-Indigenous adults). Exclusion criteria included pregnancy or known allergy to any component within 23vPPV. Blood was collected immediately prior to and one month after 23vPPV vaccination for the following groups: Group 1; first dose

23vPPV, Indigenous adults; Group 2; second dose 23vPPV, Indigenous adults; and Group 3; first dose 23vPPV, non-Indigenous adults. Life course vaccination status was confirmed with the NT adult vaccination database.

3.2. Measurement of pneumococcal antibody response

Serotype-specific IgG levels for each of the 23 serotypes contained in 23vPPV were measured by a modified WHO ELISA published previously [12,13].

3.3. Definition of adequate response

We defined *a priori* an adequate response to 23vPPV in this population using a combination of internationally standardised criteria developed for evaluation of immune deficiency [14,15] as well as taking into consideration study population characteristics such as age and chronic illness. An ‘adequate’ immune response in paired pre- and post-vaccination sera was defined for each vaccine serotype as either:

- ≥ 4 -fold rise in serotype-specific antibody titre; OR
- ≥ 2 -fold rise in serotype-specific antibody titre if the post vaccination concentration of pneumococcal polysaccharide serotype specific antibody was $\geq 1.3 \mu\text{g/ml}$ and $\leq 4.0 \mu\text{g/ml}$; OR
- a post vaccination pneumococcal polysaccharide serotype specific antibody concentration $> 4.0 \mu\text{g/ml}$ regardless of the fold-rise in titre from pre-vaccination concentration.

An overall positive response to 23vPPV required an adequate response to at least 50% (12 serotypes) of the 23 vaccine serotypes. Comparison of the proportion of participants with an adequate response to $\geq 70\%$ (16 serotypes) of vaccine serotypes was also done according to current AAAAI guidelines for adults [16]. All other responses were defined as an inadequate immune response.

3.4. Statistical analysis

Our primary outcome was the comparison of the immune response between first and second dose Indigenous adult recipients, for which our intended sample size was 120 participants per group. We assumed $\geq 85\%$ of Indigenous adults receiving a first 23vPPV dose would have an adequate immune response thus providing 93% power (alpha 0.05) to detect an adequate immune response in $\leq 65\%$ of second dose recipients. We did not adjust for re-use of the control group (Group 1) in comparisons of the immune response with the non-Indigenous first dose recipients (Group 3).

Pearson's Chi-squared test was used to compare the proportion of participants with an adequate immune response between Indigenous participants receiving first dose and second dose 23vPPV (Groups 1 and 2) as the primary analysis, and between Indigenous and non-Indigenous participants receiving first dose 23vPPV (Groups 1 and 3) as a secondary analysis.

Geometric Mean Concentrations (GMCs) with 95% Confidence Intervals (CIs) for serotype-specific IgG levels were used to compare the pre- and post-vaccination response using a Student's *t*-test for Groups 1 v 2 and Groups 1 v 3. A *p*-value < 0.05 was considered significant.

4. Results

A total of 60 first-dose Indigenous participants, 20 second-dose Indigenous participants and 25 first-dose non-Indigenous participants were included in the final analysis. A further 21 participants

were excluded from the final analysis: six (from Group 1) did not have follow-up blood collected and 15 (13 from Group 1 and two from Group 2) had follow-up blood collected outside the study window of 25–35 days (range 23–97 days). Overall, we did not achieve our intended sample size and were underpowered for the primary analysis.

Table 1 shows the participant characteristics across the groups. Indigenous participants were more likely to be male, and had a wider age range than non-Indigenous participants. Indigenous participants had higher rates of self-reported chronic illness (such as diabetes) (23%) compared to non-Indigenous participants (16%), especially in the second dose group (50%). Self-reported serious medical conditions (such as kidney disease, rheumatic heart disease, bronchiectasis) were similar between Indigenous participants in Groups 1 (12%) and 2 (10%) while non-Indigenous participants had no serious medical condition reported. The mean time elapsed between receiving the first and second dose of 23vPPV was 8 years and 9 months for second dose recipients.

4.1. Poorer adequate response to 23vPPV among Indigenous participants compared to non-Indigenous participants

Adequate immune responses following 23vPPV were found for 88% (n = 53/60) of Indigenous first-dose recipients compared to 70% (n = 14/20) of Indigenous second-dose recipients and 100% (n = 25) of non-Indigenous first-dose recipients (Table 2). Although 18% more Indigenous participants receiving a first dose of 23vPPV met the *a priori* criteria for an adequate immune response compared to a second dose 23vPPV, this was not statistically significant (p = 0.05, 95% CI –3% to 40%). Similarly the –12% risk difference between Indigenous and non-Indigenous first dose participants was not statistically significant (p = 0.07, 95% CI –20% to –4%; Table 3).

Table 1
Demographic characteristics of study participants.

	Indigenous 1st dose (n = 60)	Indigenous 2nd dose (n = 20)	Non-Indigenous 1st dose (n = 25)
Days post-vaccination mean (range)	29 (25–35)	28 (25–30)	29 (25–35)
Age mean (range)	34 (16–59)	41 (26–59)	32 (16–41)
Proportion male ^a	34 (57%)	18 (90%)	8 (32%)
Chronic illness ^a	8 (13%)	10 (50%)	4 (16%)
Serious medical condition	7 (12%)	2 (10%)	0 (0%)

^a Difference between groups significantly different p < 0.05

Table 2
Proportion of participants achieving adequate response following 23vPPV.

Group	Overall adequate response (any)		≥4-fold rise		≥2-fold rise and [IgG] ≥1.3 and ≤4.0 µg/ml		Post-vaccination ≥4.0 µg/ml	
	≥12 serotypes (%)	Median number of serotypes	≥12 serotypes (%)	Median number of serotypes	≥12 serotypes (%)	Median number of serotypes	≥12 serotypes (%)	Median number of serotypes
Indigenous 1st dose (n = 60)	53 (88%)	18	31 (52%)	12	1 (2%)	5	28 (47%)	11
Indigenous 2nd dose (n = 20)	14 (70%)	16	2 (20%)	12	0 (0%)	3	10 (50%)	6
Non-Indigenous 1st dose (n = 25)	25 (100%)	21	24 (96%)	14	0 (0%)	5	21 (84%)	17
Indigenous 1st vs 2nd dose p-value	18% (–3 to 40%) 0.05		42% (23 to 60%) 0.001		2% (–2 to 5%) 0.56		–3% (–29 to 22%) 0.796	
Indigenous vs non-Indigenous 1st dose p-value	–12% (–20 to –4%) 0.07		–44% (–59 to –30%) 0.0001		2% (–2 to 5%) 0.516		–37% (–56 to –18%) 0.002	

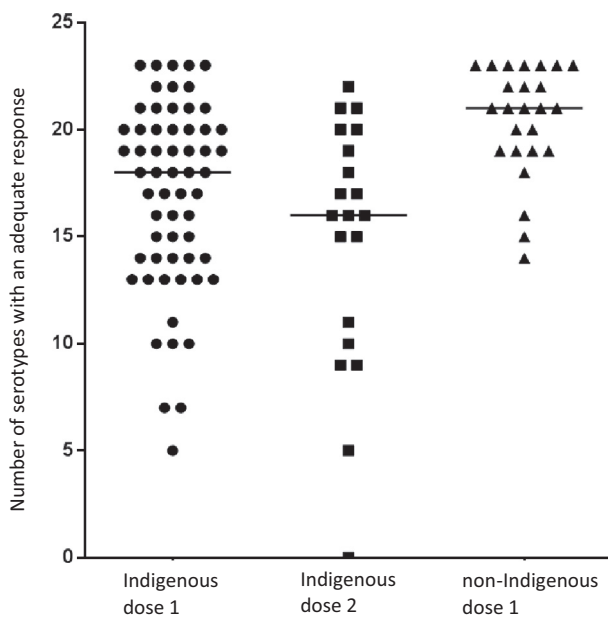
Considering each aspect of the *a priori* criteria for an adequate immune response to ≥12 (50%) serotypes, a 4-fold rise in antibody concentration was found amongst 52% of Indigenous first-dose recipients whilst 47% had post vaccination antibody concentrations beyond the 4.0 µg/ml threshold. Fewer second-dose participants met the criteria of a 4-fold rise (20%) or the 4.0 µg/ml threshold (50%). In contrast, almost all non-Indigenous participants had a 4-fold rise (96%) or a post vaccination titre >4.0 µg/ml (84%). The difference in the proportion of participants with a 4-fold rise in antibody concentration was statistically significant for each study group (p < 0.05). Only one participant (Indigenous first-dose) was classified as having an adequate immune response on the basis of a 2-fold rise when the post vaccination antibody concentration was between 1.3 and 4.0 µg/ml (Table 2). As shown in Fig. 1, non-Indigenous first-dose participants had a median adequate response to 21/23 serotypes, which was significantly higher than for Indigenous first-dose participants response to a median 18/23 serotypes (p = 0.0003). Indigenous second-dose participants responded lowest to 15 serotypes but this was not significantly different than 1st dose Indigenous participants.

We also considered the proportion of participants in each group with an adequate response to varying ‘thresholds’ using three cut-offs for the number of serotypes: ≥12 (50% of serotypes), ≥16 (70%) and ≥21 (90%) as a post hoc analysis (Table 3). These results show a similar pattern, where an adequate immune response was highest among non-Indigenous first-dose participants for all thresholds, with 60% producing an adequate response to ≥21 serotypes, followed by Indigenous first-dose and Indigenous second-dose participants (22% and 15%, respectively). Using these higher thresholds, the absolute difference in the proportions of adequate responders was 5% for Indigenous first- versus second-dose participants for ≥16 serotypes (95% CI –20% to 30%, p = 0.69) and 7% for ≥21 serotypes (95% CI –12% to 25%, p = 0.52). In contrast, the differential was substantially higher when Indigenous first-dose participants were compared with non-Indigenous first-dose participants: absolute difference was –27% for ≥16 serotypes (95% CI –43% to –11%, p = 0.01) and 38% for ≥21 serotypes (95% CI –60% to –16%, p = 0.006). This suggests that while repeat 23vPPV among Indigenous participants did not appear to significantly reduce pneumococcal antibody levels, Indigenous participants had lower responses to a single 23vPPV dose than non-Indigenous participants.

We conducted secondary post hoc analyses where we included participants who had initially been excluded because their post-vaccination blood collection was outside the study window. This increased the number of participants to: 73 Indigenous adults receiving their first 23vPPV dose (increase = 13); 22 Indigenous adults receiving a second 23vPPV dose (increase = 2); and 25

Table 3
Participants with an adequate immune response according to various thresholds.

Group	Participants with adequate response to		
	≥12 serotypes	≥16 serotypes	≥21 serotypes
Indigenous 1st dose (n = 60)	53 (88%)	39 (65%)	13 (22%)
Indigenous 2nd dose (n = 20)	14 (70%)	12 (60%)	3 (15%)
Non-Indigenous 1st dose (n = 25)	25 (100%)	23 (92%)	15 (60%)
<i>Indigenous 1st vs 2nd dose</i>			
Risk difference (95% CI)	18% (–3 to 40%)	5% (–20% to 30%)	7% (–12% to 25%)
p-value	0.05	0.69	0.52
<i>Indigenous vs non-Indigenous 1st dose</i>			
Risk difference (95% CI)	–12% (–20 to –4%)	–27% (–43% to –11%)	–38% (–60% to –16%)
p-value	0.07	0.01	0.006

**Fig. 1.** Number of serotypes with an adequate immune response for Indigenous 1st or 2nd dose 23vPPV recipients and non-Indigenous 1st dose recipients. Each data point represents one member of the study group; the solid line represents the median.

non-Indigenous adults receiving their first 23vPPV dose (no change). Under this scenario, the results remained similar with no significant difference in the proportion of individuals with an adequate response using our primary endpoint: 89% of Indigenous adults mounted an adequate response following first dose 23vPPV (65/73) compared to 73% having an adequate response following a second dose of 23vPPV (16/22; $p = 0.058$).

4.2. Effect of age and gender on response to 23vPPV

We stratified the groups based on age and gender, including the 15 participants who had delayed post-vaccination blood collection. No difference in the proportion of individuals with an adequate response using any of the criteria was observed when comparing Indigenous and non-Indigenous participants receiving a first dose of 23vPPV, or between Indigenous participants given a first or second dose of 23vPPV (Table 4). However, for participants ≥ 41 years of age (oldest age in the non-Indigenous group), there was a significant difference in the median number of serotypes responded to between first-dose Indigenous (18 serotypes) and non-Indigenous participants only (21 serotypes, $p = 0.0001$; Fig. 3). For participants >41 years old, Indigenous first dose recipients responded to a significantly higher number of serotypes (20) compared to Indigenous second dose recipients (15; $p < 0.0019$). There were no non-

Table 4

Proportion of participants achieving adequate response following 23vPPV; by age and gender (including participants with delayed blood collection, $n = 120$).

Group	Overall adequate response (any)	
	≥12 serotypes (%)	Median number of serotypes
Age		
Indigenous 1st dose		
≤41 years (N = 58)	52 (90)	17
>41 years (N = 15)	13 (87)	18
Indigenous 2nd dose		
≤41 years (N = 12)	10 (83)	18
>41 years (N = 10)	6 (60)	12
Non-Indigenous 1st dose		
≤41 years (N = 25)	25 (100)	20
>41 years (N = 0)	–	–
<i>Indigenous 1st vs 2nd dose p-value</i>		
≤41 years	0.53	
>41 years	0.13	
<i>Indigenous vs non-Indigenous 1st dose p-value</i>		
≤41 years	0.095	
>41 years	NA	
Gender		
Indigenous 1st dose		
Male (N = 40)	38 (95)	18
Female (N = 33)	27 (82)	16
Indigenous 2nd dose		
Male (N = 19)	13 (68)	15
Female (N = 3)	3 (100)	18
Non-Indigenous 1st dose		
Male (N = 7)	7 (100)	20
Female (N = 18)	18 (100)	21
<i>Indigenous 1st vs 2nd dose p-value</i>		
Male	0.005	
0.42		
Female		
<i>Indigenous vs non-Indigenous 1st dose p-value</i>		
Male	0.545	
Female	0.054	

Indigenous participants >41 years of age so we could not compare with the other Indigenous groups.

A significantly lower proportion of males had an adequate response in the second dose Indigenous group (68%) compared to first dose Indigenous recipients (95%, $p = 0.0053$; Table 4). No differences were found for males or females for all other comparisons. Similarly, male Indigenous second dose recipients responded with a significantly lower median number of serotypes (16) compared to Indigenous first dose recipients (19; $p = 0.037$; Fig. 3). For female participants, Indigenous first dose recipients responded to a significantly lower median number of serotypes (18) compared to non-Indigenous participants (21; $p = 0.0001$).

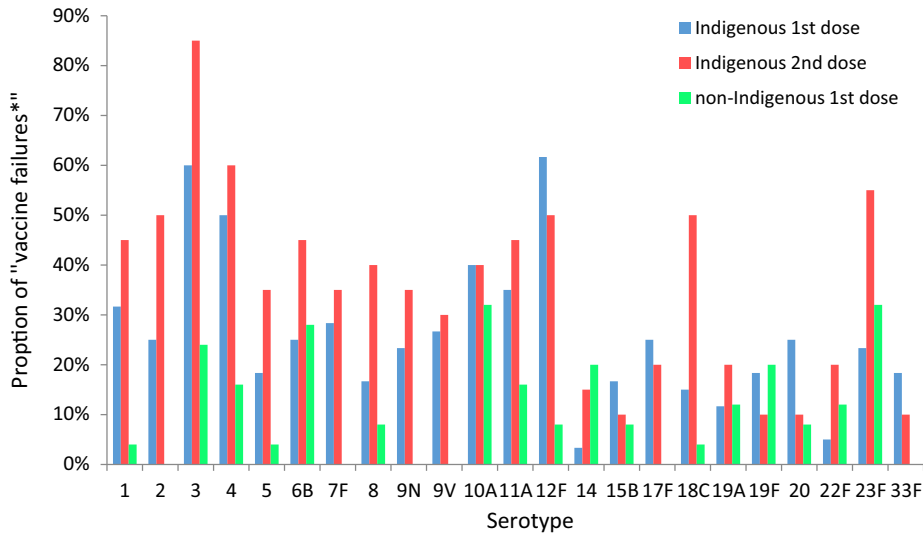


Fig. 2. Proportion of participants in each group who failed to adequately respond to each serotype contained in the 23vPPV (i.e. vaccine failure). Vaccine failure defined as participants who failed to meet the definition of an adequate immune response for the specific serotype.

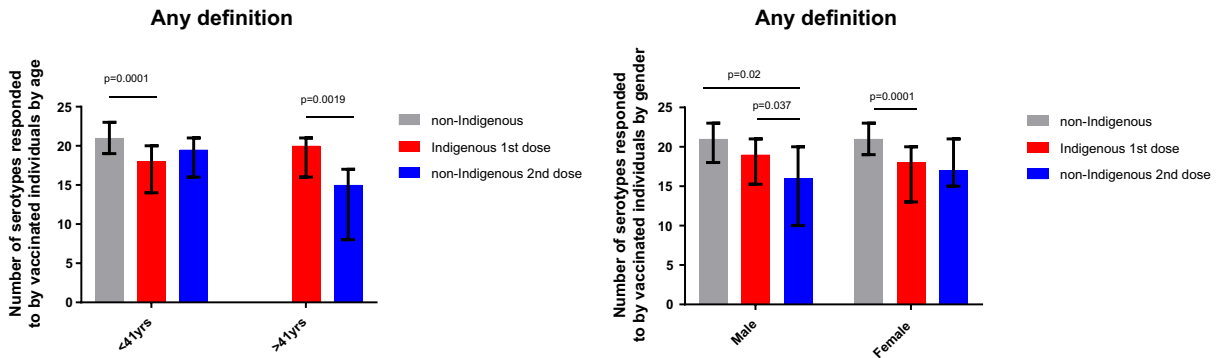


Fig. 3. Number of serotypes with an adequate immune response for Indigenous 1st or 2nd dose 23vPPV recipients and non-Indigenous 1st dose recipients. Each data point represents one member of the study group; the solid line represents the median. (A) By age. (B) By gender.

4.3. Vaccine failures

We classified inadequate vaccine response by serotype as a secondary analysis, showing the proportion of participants who failed to meet the definition of an adequate immune response for the specific serotype, referred to here as “vaccine failure”. Whilst noting that numbers were small, and acknowledging that failure to achieve the pre-specified immune response may not mean that the vaccine will fail to protect an individual from disease, we found the proportion of vaccine failures amongst Indigenous first-dose recipients compared to Indigenous second-dose participants was higher for all but seven of the 23 serotypes (Fig. 2). Serotype 3 was the most common serotype for which Indigenous participants failed to mount an adequate immune response (60% and 85% of participants in Groups 1 and 2, respectively) while for non-Indigenous participants, vaccine failures were highest for serotypes 14 and 19F (both 20%) compared to Indigenous 1st dose (5% and 14%, respectively) and 2nd dose (19% and 9%, respectively) recipients.

4.4. Absolute levels of serotype-specific IgG were similar across all groups

Baseline serotype-specific IgG geometric mean concentrations (GMCs) were similar across Indigenous and non-Indigenous participants who had not yet received 23vPPV (Table 5). As expected,

Indigenous second-dose participants had higher baseline IgG levels for most 23vPPV serotypes. Following 23vPPV, both Indigenous and non-Indigenous groups receiving their first dose had higher post-vaccination serotype-specific IgG levels for a majority of serotypes than participants in the second-dose group. Serotype-specific IgG was significantly higher in Indigenous first-dose compared to Indigenous second-dose groups only for serotype 8 ($p = 0.02$). Non-Indigenous first-dose participants achieved higher serotype-specific IgG than Indigenous first-dose participants for most serotypes, although Indigenous first-dose participants had significantly higher IgG levels for serotypes 19A ($p = 0.003$), 22F ($p = 0.002$) and 23F ($p = 0.009$). Moreover, Indigenous participants receiving their second dose had the smallest post-vaccination fold-difference across all serotypes (except serotype 12F; Table 5), most likely due to the higher baseline levels of serotype-specific IgG. Non-Indigenous first dose participants had the highest fold-difference across all serotypes apart from 19F and 33F, where Indigenous first-dose participants had a higher fold rise.

5. Discussion

This is the first study to compare the immune response of Australian Indigenous adults receiving a first or second dose of 23vPPV with non-Indigenous Australians. Despite our small sample size, our study highlights a serious concern in relation to the immune response to 23vPPV among Indigenous Australians. Our findings

Table 5

Serotype-specific IgG geometric mean concentration (95% confidence intervals) pre and post 23vPPV vaccination, by participant group.

Serotype	Indigenous 1st dose (n = 60)			Indigenous 2nd dose (n = 20)			Non-Indigenous 1st dose (n = 25)		
	Pre	Post	FR ^a	Pre	Post	FR	Pre	Post	FR
1	0.47 (0.37,0.60)	2.57 (1.85,3.56)	10.2	1.21 (0.74,2.00)	2.63 (1.46,4.71)	3.4	0.37 (0.27,0.52)	8.39 (5.06,13.92)	38.1
2	0.65 (0.50,0.85)	4.46 (3.12,6.36)	11.5	1.56 (1.16,2.09)	3.43 (2.11,5.56)	4.7	0.95 (0.65,1.40)	14.79 (8.53,25.63)	25.3
3	0.66 (0.53,0.82)	1.50 (1.14,1.97)	3.1	0.63 (0.42,0.93)	1.14 (0.71,1.83)	2.3	0.71 (0.47,1.08)	3.14 (2.04,4.84)	7.3
4	0.30 (0.23,0.38)	1.08 (0.79,1.49)	5.6	0.81 (0.46,1.45)	1.53 (0.81,2.90)	2.5	0.24 (0.15,0.38)	2.10 (1.43,3.08)	14.7
5	0.77 (0.61,0.96)	4.63 (3.49,6.15)	12.4	1.33 (0.77,2.33)	3.55 (1.74,7.24)	4.6	0.55 (0.40,0.75)	5.83 (3.58,9.51)	16.0
6B	0.86 (0.65,1.13)	4.19 (2.89,6.09)	9.3	1.43 (0.81,2.54)	2.98 (1.60,5.57)	3.5	0.42 (0.31,0.58)	2.82 (1.71,4.67)	12.2
7F	1.00 (0.80,1.25)	3.82 (2.89,5.03)	8.2	1.21 (0.80,1.85)	3.44 (2.14,5.53)	3.7	0.50 (0.33,0.76)	9.47 (6.43,13.94)	26.4
8	0.74 (0.54,1.01)	4.74 (3.54,6.34)	14.9	1.10 (0.75,1.63)	2.56 (1.49,4.40)	2.7	0.50 (0.32,0.79)	10.87 (6.35,18.61)	36.4
9N	0.74 (0.57,0.96)	4.19 (3.13,5.61)	10.8	1.83 (1.12,2.99)	4.93 (2.75,8.82)	3.8	0.47 (0.29,0.74)	6.81 (4.75,9.76)	25.3
9V	0.78 (0.62,0.98)	3.42 (2.60,4.51)	7.5	1.19 (0.67,2.10)	3.01 (1.63,5.59)	4.8	0.61 (0.44,0.86)	5.02 (3.58,7.03)	11.8
10A	1.19 (0.96,1.49)	3.05 (2.35,3.94)	3.7	1.36 (0.81,2.28)	3.01 (1.69,5.36)	3.2	0.67 (0.48,0.94)	2.85 (1.90,4.28)	5.6
11A	0.97 (0.75,1.26)	3.14 (2.45,4.02)	6.9	0.86 (0.57,1.29)	2.39 (1.46,3.92)	4.5	1.17 (0.67,2.06)	5.03 (3.25,7.79)	8.7
12F	0.25 (0.20,0.31)	0.83 (0.62,1.13)	7.9	0.36 (0.23,0.56)	1.13 (0.66,1.94)	8.5	0.13 (0.09,0.18)	1.60 (0.99,2.59)	18.4
14	2.52 (1.69,3.76)	14.02 (10.11,19.44)	13.2	9.17 (5.29,15.89)	16.07 (9.47,27.26)	11.2	1.00 (0.54,1.86)	7.64 (3.75,15.56)	13.3
15B	1.70 (1.29,2.24)	8.02 (5.88,10.95)	7.6	4.03 (2.46,6.60)	10.07 (6.12,16.56)	5.1	1.25 (0.76,2.07)	10.01 (6.43,15.57)	12.9
17F	0.59 (0.47,0.74)	3.36 (2.40,4.72)	11.4	1.51 (1.01,2.26)	4.70 (3.29,6.70)	5.0	0.45 (0.32,0.63)	8.20 (5.78,11.65)	24.0
18C	0.92 (0.69,1.22)	4.60 (3.50,6.06)	8.1	1.14 (0.68,1.90)	2.91 (1.65,5.13)	4.5	0.59 (0.39,0.89)	7.76 (5.30,11.37)	20.5
19A	7.31 (5.87,9.10)	10.51 (8.30,13.31)	2.0	5.35 (3.22,8.89)	7.92 (4.75,13.22)	1.9	2.71 (1.94,3.79)	6.86 (4.69,10.05)	3.7
19F	2.16 (1.70,2.75)	7.89 (5.85,10.63)	7.1	4.83 (3.03,7.68)	9.00 (5.41,14.95)	2.7	4.79 (0.66,1.33)	4.79 (2.98,7.70)	6.6
20	0.76 (0.61,0.95)	3.81 (2.75,5.26)	8.1	2.42 (1.34,4.38)	6.56 (3.54,12.17)	3.5	1.36 (0.91,2.03)	9.39 (6.25,14.11)	9.2
22F	5.42 (3.75,7.83)	13.20 (10.03,17.35)	4.3	5.41 (2.92,10.02)	10.42 (5.36,20.24)	3.6	1.06 (0.78,1.46)	6.27 (4.29,9.17)	11.1
23F	0.84 (0.65,1.07)	3.71 (2.89,4.77)	9.0	1.54 (1.00,2.35)	2.98 (1.68,5.27)	2.8	0.33 (0.22,0.51)	1.89 (1.00,3.59)	11.4
33F	1.39 (1.06,1.82)	8.24 (5.74,11.83)	12.5	2.79 (1.73,4.49)	7.89 (4.73,13.15)	5.3	1.78 (1.17,2.71)	16.18 (11.43,22.91)	12.3

^a FR = fold rise from pre to post-vaccination.

suggest that Indigenous participants have a poorer response to 23vPPV compared to their non-Indigenous counterparts, while Indigenous adults receiving a second dose of 23vPPV produced a poorer immune response when compared to Indigenous adults receiving the vaccine for the first time. As this is the first study to be conducted in this setting, we may have been overly conservative in our *a priori* definition that an adequate response should include at least 50% of the serotypes contained within the 23vPPV. As a post hoc analysis, we found that setting the benchmark of an adequate response to 70% (≥ 16) of the 23vPPV serotypes (as per AAAAI guidelines for immunodeficiency) or 90% (≥ 21) both showed Indigenous first-dose recipients had poorer immunological responses than non-Indigenous first dose 23vPPV recipients.

The effect of 23vPPV on the immune response in children and adults, especially following a repeat doses, has yielded conflicting data [6–8,17]. Some of these studies used pooled immune response data within participant groups for comparison rather than individual level data, making the results difficult to compare. In the Alaskan study, high rates of chronic illness were also observed but with much lower rates of invasive pneumococcal disease than our study population [6]. Lower antibody concentrations in our population [18] suggest that it may be the failure to produce adequate antibody following vaccination as the key factor in the continued susceptibility to disease (and failure to observe a reduction in rates of invasive pneumococcal disease) compared with those in the Alaskan study.

Furthermore, the non-randomised nature for some of these studies make interpretation of the results difficult. In a recent study in Fiji, we found that while hyporesponsiveness was detected in 23vPPV-vaccinated children after a small challenge dose of 23vPPV (20% dose) at 18 months of age, their immune status had normalised in response to a PCV13 booster dose by 5 years of age [11]. In the current study, despite the mean time since previous 23vPPV among Indigenous participants being 8 years and nine months, there was still a trend towards reduced responsiveness, although not significant ($p = 0.05$), suggesting a residual impact of the first 23vPPV dose. Although repeat 23vPPV among Indigenous participants yielded a slightly poorer response compared to first time vaccinees, the difference in response was greatest between Indigenous and non-Indigenous participants.

Nasopharyngeal carriage of pneumococcus at the time of vaccination is known to induce poorer antibody and OPA levels, and is typically higher among Indigenous compared to non-Indigenous Australians. A study in Indigenous adults in the Northern Territory of Australia found pneumococcal nasopharyngeal carriage in 26% [19] of adults, and may be one reason for the reduced response level among Indigenous adults. Furthermore, 23vPPV has little impact on pneumococcal vaccine-type carriage [20], likely contributing to the suboptimal impact on IPD in this population.

Non-response to some pneumococcal serotypes within individuals [21,22] has been documented previously. Differential immune responses across ethnic groups has also been demonstrated, as well as across serotypes [23]. Furthermore, individuals with chronic illness (which were over-represented in our second dose Indigenous participants) are unlikely to achieve the same level of protective efficacy of 23vPPV as do healthy adults [18]. We were unable to adjust for the level of chronic illness in our study due to small sample size. Nevertheless, our data supports these findings and may provide some explanation as to the failure in demonstrating a reduction in the high rates of pneumococcal disease experienced throughout life in our study population despite increasing coverage with 23vPPV [1]. Our results suggest that intrinsic genetic differences between Indigenous and non-Indigenous populations may affect immune responsiveness to 23vPPV, although this needs to be explored in more detail. It is known that the response to influenza vaccine is lower among Indigenous adults due to differential expression in certain HLA genes [24]. While we were unable to rule out other immune response defects in our study population, measurement of functional immunity by opsonophagocytosis and numbers of serotype-specific memory B cells may provide further insights into the mechanisms for the reduced response among Indigenous Australians adults.

Response to vaccines is known to be influenced by age and gender. In our study, non-Indigenous participants of young age (≤ 41 years of age) and being female had the highest response to 23vPPV compared to Indigenous first dose recipients while older (>41 years) male Indigenous first dose participants had higher responses compared to their second dose counterparts. This is likely due to the overall increased co-morbidities observed in

second dose Indigenous participants. However, we did not observe any effect of gender on the response of each group to 23vPPV, although for both males and females, non-Indigenous participants responded highest followed by Indigenous first dose and second recipients.

It is possible that our *a priori* requirement for an adequate response against 12 serotypes may have been too conservative. Given that the 23vPPV covers 74% of known serotypes in our Indigenous Australian population [1] with an estimated vaccine efficacy of ~74% against IPD, a response to a larger number of serotypes such as 16 or 21 may be more appropriate. A more stringent requirement would be in line with the AAAAI definition of an adequate response to 70% or 16 serotypes in adults [25]. Based on these definitions, indigenous second dose recipients responded the lowest to 23vPPV. Our rationale for setting an *a priori* definition of an adequate immune response considered several factors given that the protective threshold of serotype-specific IgG in adults, or following 23vPPV, is unknown. A number of criteria have been described for normal response to 23vPPV [26–28]. Typically, immune response to 23vPPV is based on a two-four-fold rise in serotype-specific IgG and/or a post-immunisation titre $\geq 1.3 \mu\text{g/ml}$ [29]. Indeed, serotype-specific IgG levels across all groups in this study were above $1.3 \mu\text{g/ml}$. Therefore, analysis based purely on group IgG GMCs and not on defined criteria to respond to 23vPPV may potentially mask groups of individuals with an inadequate response [30]. Our definition therefore required both an adequate rise in post-vaccination antibody titre and a post-vaccination antibody titre to be above a critical threshold for a given number of serotypes, based on data from a limited number of adult studies [21,31] and expert opinion [16].

A number of limitations were associated with our study. The sample size was lower than expected, particularly for the second dose 23vPPV group. Recruitment for this group was stalled for 9 months due to an interim recommendation by the Therapeutic Goods Administration of Australia advising against the administration of second or subsequent vaccine doses whilst an investigation into elevated rates of significant local reactions was undertaken [32]. The primary definition used in this study was based on AAAAI guidelines for infants less than 5 years of age (i.e. response to $\geq 50\%$ of serotypes) and was not as sensitive to detect inadequate response, and so may have masked any true hyporesponsiveness in this study population.

In light of these findings, the current recommendation of 23vPPV immunisation for Indigenous adults may need reconsidering. Furthermore, strategies to improve 23vPPV uptake within this population should also be a priority. The licensure of new conjugate vaccines (PCV13) for use in older adults may be one way to improve the magnitude and duration of immunity in this population. In the Northern Territory, PCV13 has since replaced 23vPPV for Indigenous infants at 18 months of age, and should provide more effective boosting of immunity as well as impact on carriage. The current issues faced with the use of 23vPPV, especially in high-risk populations such as those individuals in our study, highlights the need for a robust vaccination programme in these settings.

6. Conclusion

Taken together, our data has demonstrated a poorer response to a first dose 23vPPV in Indigenous adults compared to non-Indigenous adults in Australia, as well as a reduced immune response for those receiving a second dose 23vPPV compared to their first dose. Confirmation of the immunogenicity of future pneumococcal vaccine programs is important, particularly for Indigenous Australian adults. We propose that in the context of the current 23vPPV vaccination program in this setting, standardized

and well-recognised definitions of an adequate immune response such as those by AAAAI be used in conjunction with serotype-specific IgG GMCs to determine 23vPPV responsiveness in high-risk settings.

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