EVIDENCE BASED GUIDELINES
FOR
VACCINATION PRACTICE

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A THESIS SUBMITTED TO MEET THE
REQUIREMENTS OF THE DEGREE OF DOCTOR OF
MEDICINE, JUNE 2008
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DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research and except for the work on the ventrogluteal site (dimensions and tissue composition) and the whole cell pertussis vaccine site comparative study which were submitted for a Masters in Family Medicine (Monash) 1998, has not been submitted for a higher degree to any other Institution.

Ian Cook
ACKNOWLEDGEMENTS

To my wife, Barbara, who has shared my vision for best patient care at the point of service and whose support has made this work possible.

I wish to acknowledge the support of colleagues Professor Dimity Pond, University of Newcastle and Professor John Murtagh, Monash University who helped to facilitate the studies reported in this thesis. I also wish to acknowledge the assistance of Dale King and Michelle Williamson, Mayne Health Diagnostic Imaging, Taree, and Bettie Hollis, Mayne/Hampson Pathology, Taree. I would also like to thank Professor Edgar Marcuse, Seattle, for his encouragement.

I would also like to thank Professor Dimity Pond and Dr Parker Magin for providing constructive comments on drafts of this thesis.

Finally, I wish to thank the patients, and parents of patients, who took part in the thesis studies, their willingness to undertake ultrasonography, blood testing and to present for analysis of vaccine adverse effects has allowed for valid conclusions to be drawn from clinical studies.
SUMMARY

Vaccination programs have been so successful that concerns about sequelae of vaccine preventable disease have been replaced by concerns about the safety of vaccines. This context mandates the development and use of the best vaccines and the best vaccination practice (site and route of administration of vaccines).

Evidence based medicine has been championed as a way of improving the quality of medical care.

Assessment of vaccination guidelines from twelve countries, nine states/provinces and two counties reveals that recommendations for vaccination practice are largely based on expert opinion.

In this thesis, clinical studies are presented on:

- The preferred route for administration of vaccines (intramuscular or subcutaneous).
- The needle length required for intramuscular injection.
- The technique for intramuscular injection of vaccines.
- The site for intramuscular injection of vaccines

These studies have resulted in the following publications in refereed journals:

1. Cook IF, Barr I, Hartel G, Pond D, Hampson AW. Reactogenicity and immunogenicity of an inactivated influenza vaccine administered by
intramuscular or subcutaneous injection in elderly adults. Vaccine 2006; 24: 2395-402.


These studies allow evidence based guidelines to be formulated for vaccination practice which should help to maintain public confidence in vaccination programs by minimizing the adverse reactions of vaccines whilst maintaining their efficacy.

It is noteworthy in this context that some recommendations made in this thesis have been translated into Australian Government policy in the 9th edition of “The Australian Immunisation Handbook” 2008, albeit a long time after their publication.

This work also raises questions about contemporary clinical practice and identifies sex as a determinant of immune response and adverse reaction with some vaccines. Further studies in the area of vaccination practice and sex-difference in immune response to vaccines are suggested.
CHAPTER 1: INTRODUCTION

Vaccination is an effective means of eradicating or significantly reducing the incidence and severity of many infectious diseases. However, as the fear of sequelae of vaccine preventable disease has diminished, the relative importance of true and alleged adverse reactions to vaccine recipients’ parents and their health providers has increased. Concerns about the safety and efficacy of vaccination has been exacerbated by the extensive use of the internet by advocacy groups which question the safety of vaccines and the rise in popularity of practitioners of alternative medicine, some denying that vaccination prevents disease. This context mandates the development and use of the best vaccines and the best vaccination practice (site and route of administration of vaccines).

Evidence-based medicine (EBM) has been championed as a way of improving the quality of patient care and is now an integral component of undergraduate medical curricula, post-graduate training and clinical practice. The central thesis of EBM is the putative superiority of scientific evidence over opinion.

The shortcomings of expert opinion are well documented. It results from:

1. selective use of evidence (inadvertently or consciously ignoring studies suggesting another view).
2. biases that stem from personal experience (for example, how one was trained, a particularly bad outcome in a past patient).
3. external influences (for example, patient expectations, medico-legal concerns).
Accepted limitations of EBM are:

1. absence or lack of appropriate evidence in many areas of medicine.
2. data which may lack internal and external validity hampering attempts to extrapolate evidence to individual patients.

Evidence-based medicine (EBM) has been defined as “the conscientious, explicit and judicious use of current best evidence in making decisions regarding the care of individual patients.” The practice of EBM as outlined by Sackett et al comprises five steps:

1. Step 1. – converting the need for information (about prevention, diagnosis, prognosis, therapy, causation, etc) into an answerable question.
2. Step 2. – tracking down the best evidence with which to answer that question.
3. Step 3. – critically appraising that evidence for its validity (closeness to the truth), impact (size of the effect) and applicability (usefulness in our clinical practice).
4. Step 4. – integrating the critical appraisal with our clinical expertise and with our patient’s unique biology, values and circumstances.
5. Step 5. – evaluating our effectiveness and efficacy in executing Steps 1-4 and seeking ways to improve them both for next time.

In Step 3, the data are assigned a ‘strength of evidence’ using one of the six evidence hierarchies currently in use - American College of Chest Physicians (ACCP), Australian National Health and Medical Research Council (ANHMRC), Oxford Centre for Evidence-Based Medicine (OCEBM), Scottish Intercollegiate Guidelines...
The variability of these evidence hierarchies has been addressed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) working group\textsuperscript{16} who rate evidence on a four point scale (\textit{high, moderate, low and very low}) and recommendations as either \textbf{strong (Grade 1)} or \textbf{weak (Grade 2)}.

**Grade\textsuperscript{16} Ratings of Quality of Evidence and Definitions**

<table>
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<tr>
<th>Grade</th>
<th>Definition</th>
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<tr>
<td>High</td>
<td>Further research is very unlikely to change our confidence in the estimate of effect.</td>
</tr>
<tr>
<td>Moderate</td>
<td>Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.</td>
</tr>
<tr>
<td>Low</td>
<td>Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.</td>
</tr>
<tr>
<td>Very Low</td>
<td>Any estimate of effect is very uncertain</td>
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More recently, The American College of Chest Physicians (ACCP) have modified\textsuperscript{17} the GRADE recommendations classifying the quality of evidence as \textbf{high (Grade A)}, \textbf{moderate (Grade B)} or \textbf{low (Grade C)}.

Dissemination of new research findings which improve patient care are achieved\textsuperscript{18} by reviews in clinical journals, clinical guidelines, continuing medical education (CME) courses and conferences. Evidence-based guidelines and protocols have resulted in an improved quality of care in many areas of medicine, critical care\textsuperscript{19}, surgery\textsuperscript{20}, general medicine\textsuperscript{21-23} and paediatrics\textsuperscript{24}. 
A comprehensive review of the evidence base for vaccination practice (route and site of administration of vaccines) was made by searching:

Medline, Embase, Scopus, Biological Abstracts, Science Citation Index, Cochrane Database of Systematic Reviews (CDSR), NHS Database of Abstracts of Reviews of Effects (DARE), using the following search terms and their word variants, “immunization”, “vaccination” and “guidelines”, “protocols”, “manuals”.

Bibliographies of all relevant publications were searched for additional publications.

Guidelines and recommendations on vaccination practice were available from Australia\textsuperscript{25-32} and the Australian state of South Australia\textsuperscript{33-35}, Brazil\textsuperscript{36,37}, Canada\textsuperscript{38-40}, and the Canadian states of British Columbia\textsuperscript{41} and Saskatchewan\textsuperscript{42}, India\textsuperscript{43}, Ireland\textsuperscript{44}, Malaysia\textsuperscript{45}, Mexico\textsuperscript{46,47}, New Zealand\textsuperscript{48-50}, Spain\textsuperscript{51}, Sri Lanka\textsuperscript{52}, United Kingdom\textsuperscript{53-58}, USA\textsuperscript{59-66} and the American states of Colorado\textsuperscript{67}, Georgia\textsuperscript{68}, Kansas\textsuperscript{69}, Minnesota\textsuperscript{70}, New Mexico\textsuperscript{71} and Oregon\textsuperscript{72} and counties of Allegheny\textsuperscript{73}, Los Angeles\textsuperscript{74} and the World Health Organisation\textsuperscript{75-78} (see Appendix 1.).

These guidelines and recommendations are based on expert opinion rather than evidence as:

**Needle**

- There are no data supporting the contention by Mayon-White and Moreton that a 23 gauge needle reduces localized redness and swelling or reduces injection pain (Australian Immunisation Handbook 7\textsuperscript{th} edition\textsuperscript{31}) compared with narrower gauge needles. In fact, in a recent study\textsuperscript{79} it was shown that 23 and 25 gauge needles gave similar rates of injection site reactions.
The use of 25mm compared with 16mm long needles for intramuscular injection is controversial56 (Position statement on injection techniques, UK, 2002).

Injection Technique

- There are no data supporting the use of 45-60° angled needle entry with vaccines in terms of less tissue resistance (Australian Immunisation Handbook 6th30, 7th31 and 8th32 editions; Immunization – The basics, South Australian Immunisation Co-ordination Unit 200434; Immunisation Handbook, New Zealand 199648; or less neural and vascular damage (Australian Immunisation Handbook 6th edition30 and Immunization Handbook, Sri Lanka 200252).

Site

- No sciatic nerve injury has ever been reported80-82 with intramuscular injection of vaccines into the gluteal muscles, yet it is stated as a proscription for buttock injection in a number of guidelines, Immunisation Procedures, Australia 198627; Australian Immunisation Handbook 5th29, 6th30 and 7th31 editions; Indian Academy of Pediatrics, Guidebook on Immunization43 2002, Manual de Vacunacion, Mexico 200546; Manual de Procedimientos Tecnios de Vacunacion Actualizacion, Mexico 200347; Immunisation Handbook, New Zealand 199648, Manual de Vacunas en Pediatria, Spain 200551, Immunisation Against Infectious Disease “The Green Book” UK 200657; Centers for Disease Control and Prevention, Epidemiology and Prevention of Vaccine Preventable Diseases, “The Pink Book” USA 10th edition 200763; General Recommendations on Immunization: Recommendations of the Advisory
Committee on Immunization Practice (ACIP), USA 1994\textsuperscript{64}, 2002\textsuperscript{65}; Immunization Policy, WHO 1996\textsuperscript{78}.

- It has been stated (The Australian Immunisation Handbook, 6\textsuperscript{th}\textsuperscript{30} and 7\textsuperscript{th}\textsuperscript{31} editions) that “as the anterolateral thigh has a larger muscle mass than the gluteal region, some attempted gluteal intramuscular injections result in unintended subcutaneous injection, with more severe local reactions”. However, a review of injection site comparative studies with pertussis vaccines, the most reactogenic of all paediatric vaccines\textsuperscript{83}, does not support this contention. All four published prospective studies\textsuperscript{84-87} comparing buttock with thigh injection reported less injection site reactions with buttock compared with thigh injection. It is of interest that in the four published studies\textsuperscript{84,86,88,89} comparing buttock with deltoid injections, that buttock injection was associated with significantly less injection site reactions compared with the deltoid area in three studies and there was no difference between sites in the other study\textsuperscript{84}.

- It has been stated (Australian Immunisation Procedures 3\textsuperscript{rd} edition 1986\textsuperscript{27}, The Australian Immunisation Handbook 6\textsuperscript{th} edition 1997\textsuperscript{30}, 7\textsuperscript{th} edition 2000\textsuperscript{31}; Norma do Programa de Imunizaceo, Brazil 1998\textsuperscript{36}; Canadian Immunization Guides, 5\textsuperscript{th} edition 1998\textsuperscript{38}, 6\textsuperscript{th} edition 2003\textsuperscript{39}, 7\textsuperscript{th} edition 2006\textsuperscript{40}; Indian Academy of Pedriatrics, Guidebook on Immunization\textsuperscript{43}; Malaysian Immunisation 2001\textsuperscript{45}; New Zealand Immunisation Handbook 1996\textsuperscript{48}; Manual de Vacunas en Pedriatria, Spain 2005\textsuperscript{51}; Immunization Handbook Sri Lanka 2002; Immunsation Against Infectious Disease “The Green Book” UK 2006\textsuperscript{57}) that immune response may be impaired by injection into the gluteal area in infants. The basis of this recommendation is the well documented
difference\textsuperscript{90-97} in immune response with hepatitis B vaccine given by gluteal compared with deltoid injection in adults. This position is challenged in infants by data from a site comparative study and non-site comparative studies with vaccines with accepted serological correlates of protection, in which the same vaccine and vaccination regimen was used. In an open, randomised study\textsuperscript{98} with hepatitis B vaccine administered into the buttock or anterolateral thigh, no difference was observed between the two sites for seroconversion (buttock 99.2%, anterolateral thigh 99.3%). In studies with Haemophilus influenzae type b conjugate vaccines, seroprotection rates of 99%, 100% and 100% were observed in three studies\textsuperscript{99-101} with thigh injection and 100% in a study\textsuperscript{102} with buttock injection. When combined withacellular pertussis vaccine, seroprotection rates of 95.4% – 96.9% were observed\textsuperscript{103} for thigh injection and 94.4% for buttock injection\textsuperscript{104} and 98.6% for deltoid injection\textsuperscript{104} respectively.

The aim of the studies presented in this thesis is to provide high grade evidence for questions pivotal to vaccination practice:

- What is the preferred route for vaccine administration?
- What needle length is required for intramuscular injection?
- What is the best technique for intramuscular injection of vaccines?
- What sites are suitable for intramuscular injection of vaccines?

These, it is hoped, will allow strong recommendations to underpin vaccination guidelines which should help to maintain public confidence in vaccination programs by minimizing the adverse reactions of vaccines whilst maintaining their efficacy.
In keeping with the practice of Evidence Based Medicine (EBM), chapters in this thesis will be headed with a question. Response to these questions will be formatted in a manner similar to that used in the American Family Physician – Cochrane Briefs. In the ‘background evidence’ which is the equivalent of the AFP “Practice Pointers” – a literature review relevant to the clinical question will be presented, including the full text of studies published by the author of this thesis. At the end of each chapter an ‘Evidence based summary and answer’ will be presented.
CHAPTER 2:
WHAT IS THE PREFERRED ROUTE FOR VACCINE ADMINISTRATION?

Background Evidence

Traditional vaccination practice\textsuperscript{106} has been to give all injected vaccines, excluding BCG and smallpox vaccine, by the subcutaneous route. Semple\textsuperscript{106} provided support for this practice in 1910 when he reported a route comparative study (hypodermic versus intramuscular) with typhoid vaccine. Early studies with pertussis vaccine also used the subcutaneous route\textsuperscript{107-111}. However with the observation of increased immunogenicity of aluminium salt adsorbed vaccines by Glenny et al\textsuperscript{112}, it soon became apparent that administration of this type of vaccine by the subcutaneous route gave unacceptable rates of injection site reactions\textsuperscript{113}.

Pneumococcal\textsuperscript{114} and influenza\textsuperscript{115} vaccines have been increasingly endorsed and funded for use in publicly funded health programs for the elderly. However, the optimal route of administration of these vaccines has not been defined with either subcutaneous or intramuscular route being recommended in Australia, Canada, Ireland, New Zealand, Mexico and USA for pneumococcal vaccine and in Australia, Ireland, New Zealand for influenza vaccine.

To determine if route of administration has an impact on the reactogenicity and immunogenicity of vaccines, randomised, observer blind studies were conducted with these vaccines\textsuperscript{116,118} comparing intramuscular with subcutaneous routes of administration.
Reactogenicity and immunogenicity of an inactivated influenza vaccine administered by intramuscular or subcutaneous injection in elderly adults

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Abstract

In many countries there is no clear recommendation regarding the preferred route of administration of inactivated influenza vaccines. In a randomised, observer blind study of 720 elderly subjects, a split, trivalent influenza vaccine was significantly more immunogenic for both A strains (H3N2 and H1N1, p=0.0016 and 0.003, respectively) when given intramuscularly compared to subcutaneously. This difference was due entirely to a gender effect, with females in the intramuscular (IM) group having a significantly greater serological response than females in the subcutaneous (SC) group for both of these strains. Similar results were seen with local adverse effects. These data suggest that vaccination practices that ensure intramuscular injection are required for optimal administration of influenza vaccines in the elderly.

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Keywords: Influenza; Vaccination; Intramuscular; Elderly

1. Introduction

Influenza is recognised as a serious cause of morbidity and mortality in developed countries with temperate climates and more recently there has been the realisation that the burden of illness also extends to countries with tropical climates [1,2].

The World Health Organization estimates that some 3–5 million severe illnesses occur annually with between 250,000 and 500,000 deaths [3] and when influenza pandemics occur the impact is likely to be significantly worse [4].

Protection against influenza relies primarily on annual vaccination with inactivated virus vaccines. Because of residual untoward reactions experienced even with highly purified whole virus influenza vaccines [5,6], the majority of vaccines used today are split virus or sub-unit [7]. It has been estimated that approximately 292 million doses of influenza vaccine are distributed annually, with greatest per capita use in Western Europe, North America, Australasia and Japan [7]. Influenza vaccination programs in these countries are targeted largely to the older adult population [8], however, the effectiveness of vaccination is somewhat reduced in this population. A randomised double-blind placebo-controlled study in subject’s 60 years and older using a combination of clinical and serological criteria, demonstrated that influenza vaccination reduced infection by 38% [9], whereas similar studies in younger healthy adults demonstrate greater levels of protection (e.g. 86% in the study by Bridges et al. [10]).

There is evidence that this difference may be due to immune senescence and to the influence of underlying health status [11]. Consequently there have been efforts to improve vaccine effectiveness by means such as formulating the vaccines with liposomes or adjuvants [12,13]. Studies to date have shown marginal or no benefit in older adult populations [14–16].

In comparative studies, mostly in children, the route of administration (intramuscular (IM) versus subcutaneous (SC) route) has been shown to influence the reactogenicity of

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a number of inactivated bacterial and viral vaccines [17,18]. Although influenza vaccines have been used for over 50 years little attention has been given to the influence of route of vaccination on either the immune response or rate of adverse reactions. Recommendations regarding route of vaccination vary as do the size of the needles that are either recommended or are supplied with the vaccine. Published trials giving full details of the site, route of administration and needle length used for vaccination are limited [19–22]. In a previous study [23] examining the relationship between body mass index (BMI) in elderly people and SC layer thickness at the deltoid injection site, it was concluded that IM injection would not have been achieved by all vaccinees in these studies. A 25 mm long needle was required to obtain IM in males and females with a BMI of <35 and a 32 mm long needle was needed for women with a BMI ≥35 [23].

We therefore undertook the present study to compare the immunogenicity and reactogenicity of influenza vaccine, in an older adult population, given by practices which ensured SC or IM injection.

2. Methods

2.1. Study design

The study was approved by the University of Newcastle, Australia, Human Research Ethics Committee (approval number H-687-1003). Adults attending a small group practice in Taree, New South Wales from 27 February 2004 to 22 June 2004 were included in the study if they were:

- 65 years of age or older;
- 55 years or older with physician diagnosed chronic respiratory disease (asthma, COPD), cardiovascular or renal disease, diabetes mellitus, malignant neoplasia or were immunocompromised (receiving chemotherapy or immunosuppressive medication to prevent transplant rejection);
- willing to give written informed consent.

Subjects were excluded if they:

- had suspected or proven hypersensitivity to egg or egg protein.

The primary objective of the study was to compare the immunogenicity and reactogenicity of a split, trivalent influenza vaccine given by SC or IM injection. The primary hypothesis was that IM injection would be associated with a lower rate of injection site adverse reactions and better immunogenicity than SC injection. This was based on an earlier study by Ruben and Jackson [24] with a bivalent influenza vaccine in healthy young adults.

Subjects were randomized on a 1:1 basis for either IM (A) or SC injection (B) using a 4-unit block randomisation scheme. At recruitment, the practice nurse recorded the patient’s medical history (history of chronic disease and previous influenza vaccination), measured (BMI) and arranged pre-vaccination blood sampling.

This completed, she then gave the vaccinator (IFC) a sealed envelope containing the random letter code and upon which was written the patient’s BMI. The vaccinator transferred the vaccine from its preformed syringe with fixed 16 mm long needle to a 3 ml syringe. The vaccine was administered to the patient according to the random letter code and their BMI. This was done without the patient or practice nurse observing the procedure.

IM injection was made into the deltoid muscle at the mid point between the acromion and the deltoid tubercle using the World Health Organization injection technique [25] of compression of the skin between the thumb and index finger with the needle entered at 90° to the long axis of the humerus. From our previous study [23], IM injection was made with a 23 gauge 25 mm long needle in males of all BMI and in females with BMI <35. In females with BMI ≥35, IM injection was made with a 23-gauge 32 mm long needle.

SC injection was made with a 23-gauge 25 mm long needle in all subjects. To achieve this, the skin and SC tissue over the deltoid injection site was bunched and the needle fully inserted at an angle of 10–20° with care being taken to deliver the vaccine only into SC tissue, even in very thin subjects.

After vaccination, the vaccinator recorded the patient’s details (name, date of birth, route of vaccine administration) in a document only available to the vaccinator. The subjects were monitored for 30 min post-vaccination for immediate local and/or systemic adverse reactions. The practice nurse obtained vaccine adverse reaction responses via telephone conversation at 7 days and arranged post-vaccination blood testing after 28–31 days. Subjects were instructed to assess the injection site adverse reactions (redness, swelling, tenderness, limited arm movement) and systemic reactions (chills, perceived, headache, muscle ache, decreased appetite, nausea, vomiting, diarrhea and rash). Using a visual analogue scale (VAS) [26], 0 = no reaction, 5 = most severe reaction. Subjects were advised to record the highest score for each symptom/sign over the 7 days and that a score of 1 or 2 represented a mild reaction (present but not bothersome, a nuisance at most), scores of 3 or 4 represented moderate reaction (bothersome, frequent and annoying, somewhat distressing, may require self medication) and score of 5 represented severe reaction (very distressing, interference with the normal function, likely to require medical attention).

2.2. Vaccine

Each 0.5 ml dose of Fluvax® lot numbers 0906 97201 and 0906 98201 (CSL Limited, Melbourne) an inactivated, split, trivalent influenza vaccine contained 15 µg of each of the influenza virus strains recommended by the Australian Influenza Vaccine Committee. For the 2004 season the vaccine strains were A/NewCaledonia/20/99(H1N1)-like strain (actual strain used was A/NewCaledonia/20/99); A/Fujian/411/2002(H3N2)-like strain (the actual strain
used was A/Wyoming/3/2003; B/HongKong/330/2001-like strain (actual strain used was B/Brisbane/32/2002). The vaccine contained no preservatives but may have contained traces of neomycin, polymyxin and gentamicin and was stored at 2–8 °C prior to use.

2.3. Serological assays

Sera were collected prior to vaccination and 28–31 days post-vaccination and stored at −20°C prior to testing.

Antibody titres induced to the viruses were determined using a standard haemagglutination inhibition (HI) assay [27] using the homologous influenza strains to those in the vaccine, except for the A/Wyoming/3/2003 strain where for technical reasons the closely related A/Kumamoto/102/2002 strain was used. Sera were treated with RDE (Sieken, Japan) prior to assay to remove non-specific inhibitors [27]. All viruses used in the HI assay were unmodified isolates (as opposed to high-yield genetic recombinants used for influenza A vaccine manufacture) and were grown in 10–11 day old embryonated eggs. For influenza B/Brisbane, the virus was split by treatment with diethyl ether (Merck, Vic, Australia) to improve the sensitivity of the test. Antibody was measured against four haemagglutinating units of each of the viruses using turkey red blood cells as the indicator. All serological assays were done blinded as to the study group and performed concurrently by two independent operators. Any sample differing by more than two-fold in titre between operators was retested. The results of the two independent HI assays were pooled and averaged for statistical analysis.

2.4. Statistical methods

All analyses were performed with the use of the statistical package JMP (version 5.1.2., © 2005 SAS Institute). The sample size calculation was determined by estimating the number of subjects required to detect a 20% difference in HI responses between the IM and SC groups. Previous HI data showed a standard error of 15% for HI titres as well as for fold-increases (from an analysis of 230 paired sera—not data shown). Based on these parameters a sample size of at least 348 per study arm, for an 80% power and 5% alpha, was calculated. The two groups were compared for differences in age, sex, vaccination history (previously vaccinated or not), immune status, pre-vaccination titre, post-vaccination titre and reactogenicity. Comparisons were made using the criteria used by the Committee for Proprietary Medicinal Products (CPMP) for pre-release assessment of influenza vaccines [28], namely seroprotection (achieving HI titre ≥40), seroconversion (greater than 4-fold titre increase over pre-vaccination titre) and fold increase in geometric mean titre (GMT). HI titres and fold-increases in titres were analysed using analysis of variance and analysis of covariance on the log-transformed results. Geometric mean titres (GMTs) and fold-increases were calculated as anti-logs of the means resulting from the log-transformed analyses. Analyses of the dichotomous end points, e.g. seroprotection and seroconversion, were analysed using Fisher’s exact test and logistic regression likelihood ratio tests. Analyses on the HI titres were first performed as simple comparisons between the SC and IM groups, and then followed up with covariate adjusted multiple regression analyses to confirm the robustness of the results and to investigate the effects of various population differences. The number of males and females reporting local reactions and systemic reactions in each group was compared using Fisher’s exact test and logistic regression.

3. Results

A total of 938 subjects were assessed for eligibility to enter the trial of whom 216 refused to be part of the trial and two were excluded because of an egg allergy. The 720 enrolled subjects were randomized into two groups of 360 to receive vaccination either IM or SC. Following vaccination adverse reaction reports were collected from 356 subjects in the IM group and for 352 subjects in the SC group. Serological analysis was performed on 344 subjects in the IM group and 349 subjects in the SC group (Fig. 1). The study groups were similar in terms of age, gender, previous influenza vaccination and general health status (Table 1).

3.1. Adverse reactions

There were no significant immediate reactions to vaccination and most local and systemic adverse reactions were rated as mild (present but not bothersome, a nuisance at most) and these are summarized in Table 2.

Tenderness, redness and swelling were all significantly greater with SC compared with IM injection (all, p<0.0001) (Table 2). Only three subjects reported limited arm use (SC two, IM one) with the arm that had received the injection. The only statistically significant differences between the two groups for systemic adverse reactions were for muscular ache (p=0.014) and headache (p=0.0489) which were greater for SC compared with IM injection. Females given SC injection reported a significantly greater rate of grouped local adverse reactions (p=0.0001) than those receiving IM injection (Table 3). For males, a higher rate of grouped local adverse reaction was reported with SC injection (17.4%) compared with IM injection (10.5%) but this difference was not statistically significant (p=0.066). No significant gender differences were seen between routes of administration for grouped systemic adverse reactions.

3.2. Immunogenicity

The two vaccination groups showed similar pre-existing antibody distribution and level as illustrated by the GMT val-
Fig. 1. Clinical trial profile of study of two routes (intramuscular and subcutaneous) of administration of influenza vaccine.

Table 1
Baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IM group</th>
<th>SC group</th>
<th>Differences between IM and SC (p)*</th>
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<tbody>
<tr>
<td>Number of subjects</td>
<td>360</td>
<td>360</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female ratio)</td>
<td>172 males/188 females</td>
<td>177 males/183 females</td>
<td>0.709</td>
</tr>
<tr>
<td>Age at vaccination (years)</td>
<td>72.9 ± 8.9</td>
<td>72.4 ± 6.6</td>
<td>0.668</td>
</tr>
<tr>
<td>Previously vaccinated with influenza vaccine in 2003</td>
<td>315 (87.5%)</td>
<td>312 (86.7%)</td>
<td>0.730</td>
</tr>
<tr>
<td>Subjects with possible reduced responses</td>
<td>27 (7.5%)</td>
<td>18 (5.0%)</td>
<td>0.1660</td>
</tr>
</tbody>
</table>

* Fishers exact test.

Table 2
Local and systemic adverse reactions by site of administration

<table>
<thead>
<tr>
<th></th>
<th>IM group (%)</th>
<th>SC group (%)</th>
<th>Differences between IM and SC (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local reactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderness</td>
<td>38 (11.0)</td>
<td>113 (32.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Redness</td>
<td>2 (0.6)</td>
<td>29 (8.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Swelling</td>
<td>5 (1.5)</td>
<td>44 (12.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Limited movement of arm</td>
<td>1 (0.3)</td>
<td>2 (0.6)</td>
<td>&gt;0.99999</td>
</tr>
<tr>
<td>Systemic reactions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chills</td>
<td>3 (0.9)</td>
<td>2 (0.6)</td>
<td>0.6848</td>
</tr>
<tr>
<td>Fever</td>
<td>8 (2.3)</td>
<td>7 (2.0)</td>
<td>0.8005</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (1.7)</td>
<td>16 (4.7)</td>
<td>0.0489</td>
</tr>
<tr>
<td>Muscle ache</td>
<td>8 (2.3)</td>
<td>22 (6.3)</td>
<td>0.0140</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>4 (1.1)</td>
<td>3 (0.9)</td>
<td>0.7238</td>
</tr>
<tr>
<td>Nausea</td>
<td>7 (2.0)</td>
<td>2 (0.6)</td>
<td>0.1054</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3 (0.9)</td>
<td>0 (0)</td>
<td>0.1223</td>
</tr>
</tbody>
</table>

* Fishers exact test.
Table 3
Grouped local and systemic adverse reactions by gender and route of administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Female</th>
<th>Male</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local reactions: n/total, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>21/185, 11.35</td>
<td>18/171, 10.53</td>
<td>39/356, 10.96</td>
</tr>
<tr>
<td>SC</td>
<td>85/175, 48.57</td>
<td>31/178, 17.42</td>
<td>116/353, 32.86</td>
</tr>
<tr>
<td>ρ</td>
<td>0.0001</td>
<td>0.0067</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systemic reactions: n/total, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>15/185, 8.11</td>
<td>6/171, 3.51</td>
<td>21/356, 5.9</td>
</tr>
<tr>
<td>SC</td>
<td>24/175, 13.71</td>
<td>6/178, 3.37</td>
<td>30/353, 8.5</td>
</tr>
<tr>
<td>ρ</td>
<td>0.0027</td>
<td>&gt;0.999</td>
<td>0.1932</td>
</tr>
</tbody>
</table>

* Fisher's exact test.

uses (Table 4). For all criteria assessed, the outcome for the IM group was superior to that for both of the influenza A strains and this was statistically significant. Post-vaccination GMTs were higher for the IM group compared to the SC group and this was statistically significant for the A(H3) (ρ = 0.0016) and A(H1) (ρ = 0.0003) viruses but titres for the B virus were similar and not significantly different (ρ = 0.5973). Similar differences were seen between the two vaccination groups for both the percentage of subjects who had achieved seroprotective titres (percentage with a post-vaccination titre >40) and those who demonstrated seroconversion (percentage with a >4-fold increase in post-vaccination titre). The absolute fold increase, also, was greater for the IM group compared to the SC group and was statistically significant for all three viruses including the B virus (A(H1) ρ = 0.0044, A(H3) ρ = 0.0010, B ρ = 0.0188, Table 4).

It was also noted that post-vaccination titres were positively correlated with the pre-vaccination titres (a high pre-vaccination titre was usually associated with a high post-vaccination titre ρ < 0.0001 for all three viruses) while there were age differences in the pre-vaccination titre for the A(H1) virus but not for the A(H3) or the B virus (data not shown). However, when the post-vaccination data was adjusted to account for these co-variates, there were still significant differences between the IM group and the SC group for both A strains (data not shown).

When the serological responses were analysed by gender (Table 5) the majority of the differences seen between the IM and SC groups was a consequence of the higher responses in the female subjects of the IM group. Both post-vaccination GMTs and fold increases were significantly different between the female subsets of the IM and SC groups for both A viruses (A(H3) GMT ρ = 0.0001, fold increase ρ = 0.0007 and A(H1) GMT ρ = 0.0001, fold increase ρ = 0.0021) but not for the B virus (GMT ρ = 0.1486, fold increase ρ = 0.0649). There was a similar difference between females and males within the IM group (that is significantly higher responses for all three viruses for females compared to males) but this gender difference was not seen in the SC group. There were no significant differences against any of the viruses in post-vaccination sera of males in the IM group compared to males in the SC group based on GMT or fold increase (Table 5).

Table 4
Geometric mean titre of haemagglutination inhibition antibodies pre/post vaccination, fold increase pre/post vaccination, percentage with post-vaccination titre >40, percentage 4 with >4-fold increase post-vaccination by route of administration before and 28–31 days post-vaccination

<table>
<thead>
<tr>
<th></th>
<th>IM group</th>
<th>SC group</th>
<th>p* (IM vs. SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H3</td>
<td>H1</td>
<td>B</td>
</tr>
<tr>
<td>Pre-vaccination GMT</td>
<td>17.8</td>
<td>9.4</td>
<td>14.9</td>
</tr>
<tr>
<td>Post-vaccination GMT</td>
<td>87.1</td>
<td>24.1</td>
<td>42.2</td>
</tr>
<tr>
<td>Fold increase in titre (post/pre vaccination)</td>
<td>4.9</td>
<td>2.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Percentage with post-vaccination titre &gt;40</td>
<td>80.5</td>
<td>37.2</td>
<td>57.0</td>
</tr>
<tr>
<td>Percentage with &gt;4-fold increase post-vaccination titre</td>
<td>38.7</td>
<td>33.1</td>
<td>32.3</td>
</tr>
</tbody>
</table>

* Analysis of variance.

Table 5
Geometric mean titre of haemagglutination inhibition antibodies post-vaccination and fold increase post/pre vaccination by gender and route of administration

<table>
<thead>
<tr>
<th></th>
<th>IM group</th>
<th>SC group</th>
<th>p* (IM vs. SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H3</td>
<td>H1</td>
<td>B</td>
</tr>
<tr>
<td>Females, post-Vaccination GMT</td>
<td>10/12</td>
<td>29/47</td>
<td>42.6</td>
</tr>
<tr>
<td>ρ (within groups)</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.2004</td>
</tr>
<tr>
<td>Males, post-Vaccination GMT</td>
<td>67.5</td>
<td>19.3</td>
<td>38.77</td>
</tr>
<tr>
<td>Females, fold Increase in titre (post/pre vaccination)</td>
<td>5.85</td>
<td>3.01</td>
<td>3.06</td>
</tr>
<tr>
<td>ρ (within groups)</td>
<td>0.0045</td>
<td>0.0017</td>
<td>0.1058</td>
</tr>
</tbody>
</table>

* Analysis of variance.
4. Discussion

The recommended route of administration of inactivated influenza vaccines used varies from country to country. In the US and Canada vaccine manufacturers and the US based Advisory Committee on Immunization Practices (ACIP) [29] recommend IM injection usually to be given into the deltoid muscle of the arm. ACIP recommends the use of a 1 in. needle for adults and older children and 7/8-1 in. needle for children under 12 months, in order to get sufficient penetration of the vaccine into the muscle tissue. However, in other countries, for example UK, Germany, Australia and New Zealand, no preferred route for influenza vaccination is recommended with either SC, deep SC injection or IM injection all equally recommended by both national bodies, e.g. the Australian NHMRC [30] and vaccine manufacturers alike. In addition there is generally no recommendation given on needle lengths as most vaccines are now given in pre-filled syringes.

A review by the authors of published studies with split, trivalent influenza vaccines retrieved 83 studies with the use of this type of vaccine in adults. However, only four studies indicated the length of the needle used to administer the vaccine. In all four of these studies, IM injection was given with needles varying in length from 25.4 mm [19], 20 mm [20,21] and 16 mm [22]. From our previous study [23], it was shown that a 25 mm long needle would achieve IM injection in all body mass index males and in females with BMI < 35, if the needle were inserted at 90° angle to the long axis of the humerus. It is thus apparent that, in the study by Jackson et al. [24], routine IM injection would not have been achieved.

Our study shows that it is clinically important to ensure IM injection with influenza vaccine in the elderly, as this route of administration gave significantly better immune response to the A strains of the trivalent vaccine than with the SC route, for elderly female subjects. The basis of the better performance of IM influenza injection in females but not males compared with SC injection is unclear. A gender related difference in immune response has been previously reported with hepatitis B vaccine [31] where a trial in adults (only two subjects were 60 years or older) gave a statistically significantly better seroconversion rate (anti-HBs > 10 IU/L) in females compared with males. This effect was most pronounced in women aged under 40 years of age. However, this study used an intradermal administration and it was suggested that the differences may have been due to oestrogen related increased vascularity of the skin and the stimulation of the reticulo-endothelial system including the Langhans giant cells of the skin. Clearly this was not a factor in our study as the women in our study were post-menopausal and the better response was achieved with the IM injection. Higher seroconversion rates with hepatitis B vaccine have also been observed in females compared with males in healthy adults (18-60 years) [32] and in haemodialysis patients [33] (mean age 53 years) but these differences did not achieve statistical significance.

Adverse reactions with split, trivalent influenza vaccines in subjects 60 years and older have been studied in randomized, placebo controlled, double blind studies with crossover [34] and cohort [35] design. These studies showed that only the local adverse reactions were significantly different than placebo with reaction rates of 20.1% [34] and 17.3% [35]. In our study a similar local adverse reaction rate was observed for males (10.53%) and females (11.35%) given IM injection with a higher rate in males (17.42%) and a significantly higher rate in females (48.57%) given SC injection.

Route and gender effects on the rate of adverse reactions after vaccination have been previously reported with other vaccines in adults. Anthrax vaccine [36], an aluminium hydroxide adjuvanted vaccine, gave significantly higher rates of erythema (p < 0.0001) and induration (p = 0.002) and SC nodules (p < 0.0001) when administered subcutaneously compared with intramuscularly. Anthrax vaccine also gave significantly more erythema, induration and SC nodules in females compared with males (p < 0.001) following SC administration. No reason has been suggested for this difference. The majority of influenza vaccines however, are non-adjuvanted, split or sub-unit vaccines but a number of studies have shown that females have more adverse reactions than males [22,24,37]. The reason for this gender difference is unclear and may relate to gender differences in the awareness of pain perception as suggested by Beyer et al. [37]. Recently an adjuvanted influenza vaccine has become available which demonstrated increased local reaction rates compared with unadjuvanted comparator vaccine, however, no gender-specific data was reported [14].

Due to the reduced immunogenicity to influenza vaccines seen in the elderly there have been ongoing attempts to enhance the immune response generated to influenza vaccines in these older recipients. A vaccine containing an oil-in-water adjuvant based on the metabolizable oil squalene has been registered in a number of countries [14]. In pooled data from trials in over 10,000 elderly recipients increased antibody responses over a comparator vaccine were demonstrated, similar to those obtained by IM injection over SC vaccination in our current study, but in the presence of increased rather than decreased local reactions [16]. Another study in the elderly which compared a trivalent influenza vaccine formulated into virosomes compared to a standard trivalent vaccine, found there were significantly improved responses to the two influenza A components but not to the B component [38] in one trial and significantly better responses to all three components in another trial [39]. The published details of these trials do not provide sufficient detail on route of vaccination or needle lengths to allow speculation regarding their influence on the results.

Our study supports the hypothesis that IM injection is associated with lower rates of adverse reaction and better immunogenicity than SC injection in the elderly, however, this only reached a significant level in female recipients. It is thus preferable that influenza vaccination of older adults should be administered by IM injection. Moreover, the prac-
tice in some countries of-supplying pre-packaged single-dose vaccine with needles of 16 mm length, will fail to provide optimal results in terms of reactogenicity and immunogeneity in many elderly people as IM injection will not be achieved. In practical terms supplying the vaccine with 25 mm long needles (as recommended by ACIP in the USA [29]) will ensure IM injection in all males and females with a BMI < 35 with the needle entered at 90° to the long axis of the humerus [23]. Clearly, for females with BMI ≥ 35, a 32 mm long needle would be preferred, however, this is probably impractical where single-dose vaccines are supplied prepackaged in syringes with fixed needles. We suggest that pre-packaged vaccines have a 25 mm long needle fitted in order to cover the majority of the elderly population’s requirements for receiving influenza vaccine intramuscularly.

Acknowledgments

The authors would like to thank the patients for taking part in the study, Bettie Hollis, Mayne Health, for collection of sera, Rob Shaw, Chris Durrant, Helen Spigren and Aeran Hurt from The Melbourne WHO Collaborating Centre for Reference and Research on Influenza for their technical expertise.

References


In the influenza vaccine study\textsuperscript{116} (Cook et al, page 18), a split trivalent influenza vaccine was administered by techniques which ensured intramuscular injection or subcutaneous injection to 720 elderly subjects in a randomised, observer blind designed trial. Seroprotection was defined\textsuperscript{117} as a haemagglutination inhibition titre $\geq 40$ and seroconversion as a haemagglutination inhibition titre $\geq 4$-fold increase over pre-vaccination titre. A strains (H$_3$N$_2$ and H$_1$N$_1$) but not the B strain were significantly more immunogenic when given by the intramuscular compared with the subcutaneous route. This is shown by the number needed to treat (NNT) in Table 1.

**Table 1. Seroprotection and Seroconversion by Strain and Route of Administration**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Route Comparison</th>
<th>NNT Post Vaccination Titre $\geq 40$</th>
<th>NNT Percentage $\geq 4$-Fold Increase Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_3$N$_2$</td>
<td>IM vs SC</td>
<td>10.6</td>
<td>6.7</td>
</tr>
<tr>
<td>H$_1$N$_1$</td>
<td>IM vs SC</td>
<td>9.7</td>
<td>7.6</td>
</tr>
<tr>
<td>B</td>
<td>IM vs SC</td>
<td>19.6</td>
<td>15.4</td>
</tr>
</tbody>
</table>

For seroconversion, single digit numbers were obtained for the A strains NNT, ie less than 10 subjects were needed to be given intramuscular injection to achieve seroconversion in one additional subject compared with subcutaneous injection.

Injection site reactions were significantly less with intramuscular compared with subcutaneous injection. This is shown by the number needed to harm (NNH) data in Table 2.
Table 2. Injection Site Reaction by Route of Administration of Influenza Vaccine

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Comparison</th>
<th>NNH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenderness</td>
<td>SC vs IM</td>
<td>4.5</td>
</tr>
<tr>
<td>Redness</td>
<td>SC vs IM</td>
<td>13.0</td>
</tr>
<tr>
<td>Swelling</td>
<td>SC vs IM</td>
<td>9.0</td>
</tr>
</tbody>
</table>

For tenderness, for every 4.5 subjects given subcutaneous injection one additional subject would report tenderness as an injection site reaction compared with intramuscular injection.

A similarly designed study\textsuperscript{118} was conducted with a 23-valent pneumococcal vaccine which was given to 254 elderly subjects.
Comparative reactogenicity and immunogenicity of 23 valent pneumococcal vaccine administered by intramuscular or subcutaneous injection in elderly adults

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Abstract

23 Valant pneumococcal vaccine is provided to the elderly through public health programs in many countries. However there is no clear recommendation regarding its route of administration (subcutaneous or intramuscular).

In a randomised, observer blind study of 254 elderly subjects, the immunogenicity of a 23 valant pneumococcal vaccine was not influenced by its route of administration. A low rate of systemic adverse reactions was observed with the vaccine (subcutaneous and intramuscular both 6.3%). Local adverse reaction rates were: intramuscular 7.1% and subcutaneous 18.9% and these were predicted by:

- Pre-vaccination antibody titres >1 μg/ml, odds ratio 22.4 (8.06–74.84) compared with pre-vaccination antibody titre <1 μg/ml.
- Female gender, odds ratio 5.0 (1.83–14.83) compared with male gender.
- Subcutaneous injection route, odds ratio 3.20 (1.13–9.13) compared with intramuscular injection route.
- Female gender subcutaneous injection route, odds ratio 2.99 (1.10–8.70) compared with female gender intramuscular injection route.

These data support the intramuscular injection of 23 valent pneumococcal vaccine, especially in elderly females.

Keywords: 23 Valant pneumococcal vaccine; Elderly; Intramuscular; Subcutaneous

1. Introduction

Infection with Streptococcus pneumoniae is responsible for significant morbidity and mortality worldwide, especially in the very young, the elderly and those with predisposing risk factors [1,2]. The magnitude of this disease has prompted an interest in its control through vaccination. Successful quadrivalent and pentavalent vaccines were prepared in the 1940s [3,4] but these were withdrawn by 1949 due to the advent of penicillin. However, the emergence of penicillin-resistant pneumococci [5] has shifted the focus of disease management again to the prevention of infection through vaccination.

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A 14 valent polysaccharide vaccine was licensed in 1977 [6] and this was replaced by a 23 valent vaccine in 1983. The latter vaccine covers at least 90% of serotypes responsible for invasive pneumococcal disease in developed and developing countries [7].

Prospective clinical trials [8,9] with 23 valent pneumococcal vaccine have failed to demonstrate its efficacy with this being attributed to inadequately sized study populations [10]. However observational studies using case control, indirect and retrospective cohort designs have observed a 50–80% vaccine effectiveness [11] with pneumococcal bacteremia as the outcome measure.

Limitations of this vaccine have not been overcome by conjugating the polysaccharide antigens with carrier proteins [12], as has occurred with Haemophilus influenzae type b [13]. Future pneumococcal vaccines will probably

To optimise the immunogenicity and minimize the reac-togenicity of the 23 valent pneumococcal vaccine currently recommended for adult vaccination, the preferred route of administration (intramuscular or subcutaneous) needs to be defined. This has not been done with regulatory authorities in many countries recommending either route of administration [2,15,16].

The study presented here examines the comparative reactogenicity and immunogenicity of a 23 valent pneumococcal vaccine administered to elderly subjects by practices which ensure subcutaneous or intramuscular injection in all vaccin-ees.

2. Methods

2.1. Study

Adults attending a solo practice in Taree, New South Wales from 11 June 2003 to 9 September 2003 were included in the study if they were:

- 60 years of age or older.
- 55 years or older with physician diagnosed chronic respiratory disease (asthma, COPD), cardiovascular or renal disease, diabetes mellitus, malignant neoplasia or were immunocompromised (receiving chemotherapy or immunosuppressive medication to prevent transplant rejection).

Patients were excluded if they:

- Had previous vaccination with pneumococcal vaccine.
- Were unwilling to give written informed consent to partici-pate in the study.

The study was approved by the University of Newcastle, Australia, Human Research Ethics Committee, Approval Number H-H-564-0403.

The primary objective of the study was to compare the reactogenicity and immunogenicity of a 23 valent pneumococcal vaccine given by subcutaneous or intramuscular injection. The primary hypothesis was that intramuscular injection would be preferable to subcutaneous injection in these terms as it has been endorsed by a prominent worker in this area [7]. Both routes are, however, recommended by manufacturers of the vaccine and the US based Advisory Committee on Immunization Practices (ACIP) [2].

Subjects were randomised on a 1:1 basis for either intramuscular (A) or subcutaneous (B) injection using a 4 unit block randomisation scheme. At recruitment, the practice nurse recorded the patient’s medical history (history of chronic disease and previous pneumococcal vaccination), measured body mass index (BMI) and arranged pre-vaccination blood sampling. This completed, she then gave the vaccinator (IFC) a sealed envelope containing the random letter code and upon which was written the patient’s BMI. The vaccine was administered to the patient according to the random letter code and their BMI. This was done without the patient or practice nurse observing the procedure.

Intramuscular injection was made into the deltoid muscle at the mid point between the acromion and the deltoid tuber-cle using the World Health Organisation injection technique [17] of compression of the skin between the thumb and index finger with the needle entered at 90° to the long axis of the humerus. From our previous study [18], intramuscular injection was made with a 23 gauge 25 mm long needle in males of all BMI and in females with BMI <35. In females with a BMI ≥35, intramuscular injection was made with a 23 gauge 32 mm long needle.

Subcutaneous injection was made with a 23 gauge 25 mm long needle in all subjects. To achieve this, the skin and subcutaneous tissue over the deltoid injection site was bunched and the needle fully inserted at an angle of 10–20° with care being made to deliver the vaccine only into the subcutaneous tissue even in very thin subjects.

After vaccination, the vaccinator recorded the patient’s details (name, date of birth, route of vaccine administration) in a document only available to the vaccinator. The subjects were monitored for 30 min post-vaccination for immediate local and/or systemic adverse reactions. The practice nurse reviewed vaccine adverse reactions via telephone conversa-tion after 7 days and organized a post-vaccination blood test after 28–31 days. Subjects were instructed to assess the injection site for adverse reactions (redness, swelling/tenderness and limited arm movement) and to observe systemic reactions (chills, perceived fever, headache, muscle ache, decreased appetite, nausea, vomiting, diarrhoea and rash). A visual analogue scale (VAS) [19], 0 = no reaction, 5 = most severe reaction was to be used to score these adverse reactions. Subjects were advised to record the highest score for each symptom/sign over the 7 days and that a score of 1 or 2 repre-sented a mild reaction (present but not bothersome, a nuisance at most), scores of 3 or 4 represented moderate reaction (bothersome, frequent and annoying, somewhat distressing, may require self medication) and a score of 5 represented severe reaction (very distressing, interference with the normal func-tion, likely to require medical attention).

2.2. Vaccine

Each 0.5 ml dose of Pneumovax 23 Lot Number R0664-1 (MSD, Australia) contained 25 mg of each polysaccharide type (Danish nomenclature) 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17C, 18C, 19F, 19A, 20, 22F, 23F and 33F dissolved in isotonic saline solution containing phenol 0.25% as preservative.

2.3. Serological assays

Sera were collected prior to vaccination and 28–31 days post-vaccination and stored at −20°C prior to testing.
Antibody to serotype 3, 4 and 6B (PPS3, 4 and 6B) were determined by ELISA using a method based on that described by Konradsen [20] but with the use of Polysorp® plates (NUNC, Rochester, NY, USA) with PABST-EDTA buffer and phosphorylecholine rather than e-poly saccharide for pre-adsorption of serum to eliminate the effect of non-specific antibodies to pneumococcal cell wall polysaccharide.

Samples, standards and controls were tested in triplicate. Every plate contained pooled human serum samples as in-house quality controls and eight dilutions of a pooled human sera standard calibrated to the international standard, 89-SF (kindly provided by Dr Carl E Frasch, Centre for Biologies Evaluation and Research, FDA, Bethesda, MD). Anti-PPS1gG concentrations were calculated using a four-parameter curve fit program, Kinetica® software. The results were expressed as micrograms per millilitre calculated on the basis of the officially assigned IgG values for 89-S reference serum [21].

2.4. Statistical methods

The primary analysis compared ELISA IgG antibody concentrates between the two treatment groups. IgG antibody titres were log transformed prior to analysis to make the distribution more symmetric and closer to normal. Resulting estimates and confidence intervals are anti-logged for presentation, yielding geometric mean concentrations (GMCs) for means and geometric fold increases for differences.

Baseline titres and demographic data, including age, sex and BMI are summarized by treatment group and compared using t-tests and Fisher’s Exact tests. The number of subjects experiencing local and systemic reactions is summarized by treatment groups and compared using Fisher’s Exact test. A greater than two-fold increase in titre post-vaccination was considered a response.

Comparison of categorical variables was made using Fisher’s Exact test. Significant level was set at $\alpha = 0.05$ for all tests. Data analyses were performed using the SAS system (SAS Version 9.1.3SP3 and JMP Version 6.0.2, both SAS Institute, Cary, NC). A sample size calculation of 242 adults was based on the assumption of 60% of adults achieving a $>2$-fold seroresponse to serotype 6 [22]. If this level of sero-response was obtained with IM injection and SC injection gave only 40% $>2$-fold increase then the study would have 85% power with a 5% alpha.

![Clinical trial profile](image)

Fig. 1. Clinical trial profile of study of two different routes (subcutaneous and intramuscular) of administration of a 23 valent pneumococcal vaccine.
Table 1
Baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IM group</th>
<th>SC group</th>
<th>Differences between IM and SC p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>66 M/61 F</td>
<td>59 M/68 F</td>
<td>0.4515a</td>
</tr>
<tr>
<td>Gender (male/female ratio)</td>
<td>74.2 (61.0–94.5)</td>
<td>73.8 (61.3–94.0)</td>
<td>0.8368b</td>
</tr>
<tr>
<td>Age at vaccination (years) mean (min–max)</td>
<td>73.8 (61.0–94.5)</td>
<td>73.1 (62.6–92.7)</td>
<td>0.6653c</td>
</tr>
<tr>
<td>Female mean (min–max)</td>
<td>27.1</td>
<td>27.3</td>
<td>0.7090d</td>
</tr>
</tbody>
</table>

a Two-sided Fisher’s Exact test.
b Pooled t-test.
c Two-way Anova.

3. Results

A total of 498 subjects were assessed for eligibility to enter the trial of whom 197 had been previously vaccinated and 47 refused vaccination. The 254 enrolled subjects were randomised into two groups of 127 to receive vaccination either by intramuscular or subcutaneous route. Following vaccination, adverse reaction reports were collected for all subjects.

Serological analysis was performed on paired sera from 124 subjects in the IM group and 121 subjects in the SC group (Fig. 1). The study groups were similar in terms of age, gender and body mass index (Table 1).

3.1. Immunogenicity

The two vaccination groups had similar pre-existing geometric mean concentrations (GMC) for the 3 serotypes tested (Manova F3,346 = 1.66, p = 0.1765). There were minor differences in pre-vaccination GMCs for PPS 3 (SC GMC = 0.37 versus IM GMC = 0.58, p = 0.0384 without adjustment for multiple testing). There was no difference between vaccination groups in the number of individuals with pre-existing PPS >1.0 (SC 34.4% versus IM 39.2%, Fisher’s exact, p = 0.5121). Nonetheless, covariant adjustment for pre-existing titres will be included in all immunogenicity analysis.

Table 2
Pre- and post-vaccination Antibody titre GMCs by serogroup and gender

<table>
<thead>
<tr>
<th>Route</th>
<th>Sex</th>
<th>GMCa</th>
<th>95% Confidence intervala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>SC</td>
<td>0.34</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.40</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>0.68</td>
<td>0.46</td>
</tr>
<tr>
<td>PPS 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>SC</td>
<td>1.61</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.53</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.69</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2.03</td>
<td>1.32</td>
</tr>
<tr>
<td>Post</td>
<td>SC</td>
<td>1.07</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>1.32</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>1.40</td>
<td>0.94</td>
</tr>
<tr>
<td>PPS 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>SC</td>
<td>2.52</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3.20</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>4.39</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3.65</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>4.40</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.82</td>
<td>4.62</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>6.06</td>
<td>4.12</td>
</tr>
<tr>
<td>PPS 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>SC</td>
<td>7.15</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12.27</td>
<td>8.15</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>13.69</td>
<td>8.97</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>14.28</td>
<td>9.38</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>10.25</td>
<td>6.87</td>
</tr>
</tbody>
</table>

a GMC is geometric mean concentration.
b 95% confidence interval based on log-normal distribution of antibody titres.
Females tended to have slightly lower baseline GMCs than males (Table 2). None of the pre-vaccination titres were statistically significantly different (p > 0.05) and all were within about a two-fold difference (Table 2).

Post-vaccination antibody concentrations and fold increases were not significantly different for the two groups and the GMCs were within a two-fold difference for each of the three serotypes tested. Females had slightly lower post-vaccination GMCs than males (Table 2).

Response defined as a >2-fold increase in GMC was greatest with serotype 3 (57%) and least with serotype 6 (38%). However, protective antibody level defined as >1 μg/ml was least with serotype 3 (48%) and greater with serotype 6 (64%) (Table 3).

3.2. Adverse effects

Local adverse reactions (redness, swelling/tenderness) were significantly more frequent (both p < 0.005) with SC than IM injection (Table 4a). No significant difference was seen between the two groups for the systemic adverse reactions (perceived fever, chills, muscle ache/.headache and tiredness, cold-like symptoms, decreased appetite, nausea, vomiting diarrhea and rash) (Table 4b).

Almost all subjects experiencing local reactions (27 of 33) had pre-existing antibody titres of PPS3, PPS4, and PPS6 >1 μg/ml, and among those, most were females vaccinated via the SC route (16 of 27) (Table 5). Subjects who had pre-vaccination titres >1 μg/ml for all three serotypes, had 22 times the odds of experiencing a local reaction than subjects who had a pre-vaccination titre of <1 μg/ml for one of the three serotypes (OR = 22.4, CI 8.06 to 74.84). Females had 5 times the odds of males and subjects given SC injection had 3.2 times the odds of those given IM injection. The gender by route interaction was statistically significant (LR χ² = 4.45, p = 0.0348), with females given SC injection having 3 times the odds of a local reaction as females given IM injection (OR = 2.99, 95% CI 1.10, 8.70). The interactions of pre-existing titres with gender or route of administration were not statistically significant, and their inclusion did not appreciably alter the logistic model.

4. Discussion

Pneumococcal vaccine has been increasingly endorsed and funded for use in publicly funded health programs for the elderly [23]. However, the optimal route for administration (subcutaneous or intramuscular) has not been defined.

In our study, immune response to serotypes 3, 4 and 6 was measured as these serotypes are a major cause of invasive pneumococcal disease in patients >65 years of age in Australia [24]. Route of administration did not affect the immune response to these serotypes.

Serotype 3 gave a greater percentage of responders, defined as a greater than two-fold increase in post-vaccination
Table 4a
Number of subjects with local reactions by sex and route of administration

<table>
<thead>
<tr>
<th>Local reactions</th>
<th>IM group N = 127</th>
<th>SC group N = 127</th>
<th>p-Valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any local reaction</td>
<td>9 (7.1%)</td>
<td>24 (18.9%)</td>
<td>0.0032</td>
</tr>
<tr>
<td>Male</td>
<td>4 (6.1%)</td>
<td>3 (2.3%)</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>Female</td>
<td>5 (8.2%)</td>
<td>21 (30.9%)</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Logistic modelb: route p = 0.1411; sex p = 0.0077; sex x route p = 0.0561

- Redness: route 6 (4.7%); sex 3 (4.6%); sex x route 3 (4.9%)
- Male: route 23 (18.1%); sex 3 (5.1%); sex x route 20 (29.4%)
- Female: route 0; sex 0; sex x route 0

Logistic modell: route p = 0.0376; sex p = 0.0439; sex x route p = 0.00527

- Tenderness/swelling: route 7 (5.5%); sex 3 (4.6%); sex x route 4 (5.6%)
- Male: route 22 (17.3%); sex 2 (3.4%); sex x route 0
- Female: route 0; sex 0; sex x route 0

Logistic modelm: route p = 0.0376; sex p = 0.0439; sex x route p = 0.00527

Any limited movement of arm 0

* Fisher's Exact Test comparing IM and SC groups.

b Likelihood ratio logistic model of number of subjects with local reaction versus sex and route of administration and sex x route interaction.

titre, (37%) than serotype 4 (42%) and serotype 6 (38%). However, the percentage of subjects deemed to have a protective IgG antibody level (>10 μg/ml) [25] was greater with serotype 6 (64%) compared with serotype 4 (56%) and serotype 3 (48%).

These results contrast with those of Simonsen et al. [26] in elderly Brazilian patients where serotype 3 elicited the lowest fold increase and absolute IgG concentrations compared with serotypes 1, 5, 6B, 8 and 14. The difference is due to a lower pre-vaccination GMC for serotype 3 in our study (male 0.53 μg/ml, female 0.41 μg/ml) compared with that of Simonsen (2.60 μg/ml). Serotype 3 is the weakest immunogen in our study. As Sorensen et al. [27] have observed, fold increase in antibody concentration is an inappropriate way of interpreting immune response without concomitant consideration of level of antibody concentration reached.

In our study, antibody concentrations tended to be higher in elderly men than women before and after vaccination, in agreement with other studies [28–30]. Smoking habit, past and present, a suggested basis for this difference [30] does not apply in our study as subjects with tobacco use and/or tobacco related illnesses had previously vaccinated, apart from two females and one male.

Significant systemic adverse reactions were not seen in our study. A wide range of local reaction rates has been previously reported [2] in the elderly with 23 valent pneumococcal vaccination; 0 [31] to 21% redness and 51% tenderness [32]. The latter authors administered the vaccine with a 16 mm long needle and our ultrasound study with elderly subjects [18] demonstrated that needles of this length would result in subcutaneous injection in most subjects. In accord with this, the observed rate of redness following subcutaneous injection was 18.9% in our study and this is comparable with the rate of redness observed by these authors (21%). Administration of this vaccine, albeit re-vaccination, with a 25.4 mm long needle was reported [33] to give a local adverse reaction rate of 11.3% which is comparable with that observed in our intramuscular injection group (7.1%).

In our study, local adverse reaction was predicted by pre-vaccination titre, gender and route of administration in that order.

The risk of local adverse reactions has been strongly correlated with pre-vaccination antibody concentrations [34]. This presumably reflects an Arthus-type reaction (type 3 hypersensitivity reaction) caused by the formation of antigen-antibody complexes at the injection site. Sanklamp et al. [34] noted higher GMCs for 6 serotypes in subjects with post-vaccination pain compared with those without post-vaccination pain. This difference was statistically significant for serotype 14 which is found in high pre-vaccination titres in

Table 4b
Number of subjects with systemic reactions by sex and route of administration

<table>
<thead>
<tr>
<th>Systemic reactions</th>
<th>IM group N = 127</th>
<th>SC group N = 127</th>
<th>p-Valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any systemic reaction</td>
<td>8 (6.3%)</td>
<td>8 (6.3%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>2 (3.0%)</td>
<td>2 (3.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>6 (9.8%)</td>
<td>6 (9.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Perceived fever/flulike</td>
<td>1 (0.8%)</td>
<td>2 (1.6%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>1 (1.5%)</td>
<td>1 (1.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Muscle aches/headaches</td>
<td>4 (3.2%)</td>
<td>3 (2.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>2 (3.0%)</td>
<td>1 (1.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>2 (3.0%)</td>
<td>2 (2.9%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cold-like symptoms</td>
<td>3 (2.4%)</td>
<td>2 (1.6%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>1 (1.5%)</td>
<td>1 (1.4%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>2 (3.2%)</td>
<td>1 (1.5%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>GIT upset</td>
<td>2 (1.6%)</td>
<td>1 (0.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>2 (3.0%)</td>
<td>1 (1.5%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>1 (0.8%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Fisher's Exact Test comparing IM and SC groups, n.s. means p-value >0.05.
Table 5
Number of subjects with local reactions by pre-vaccination titre, route of administration and gender

<table>
<thead>
<tr>
<th>Antibody status</th>
<th>Route</th>
<th>Sex</th>
<th>N</th>
<th>N (%) subjects with one or more local reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with at least one of PPS3, PPS4 or PPS6 pre-vaccination titre &gt; 1 μg</td>
<td>IM</td>
<td>M</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>M</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>44</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td></td>
<td>166</td>
<td>6</td>
</tr>
<tr>
<td>Subjects with all three PPS3, PPS4 and PPS6 pre-vaccination titre &gt; 1 μg</td>
<td>IM</td>
<td>M</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>M</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td></td>
<td>84</td>
<td>27</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td>250</td>
<td>33</td>
</tr>
</tbody>
</table>

Odds ratio\(^a\) 95% Confidence interval

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PPS 3, 4 and 6 &gt; 1</td>
<td>22.40</td>
<td>8.06–74.84</td>
</tr>
<tr>
<td>Female gender</td>
<td>5.00</td>
<td>1.85–14.83</td>
</tr>
<tr>
<td>Subcutaneous route of administration</td>
<td>3.20</td>
<td>1.13–9.13</td>
</tr>
<tr>
<td>Female gender x subcutaneous route</td>
<td>2.99</td>
<td>1.10–8.70</td>
</tr>
</tbody>
</table>

Logistic model of local reaction by pre-vaccination titres (≥ 1) to PPS3, 4, and 6, gender and route of administration.

\(^a\) Odds ratios of subjects experiencing one or more local reactions predicted by pre-vaccination titres to PPS3 PPS4 and PPS6 being positive, gender, route of administration and 2-way interaction from Logistic model.

the elderly [26,35]. Similarly, Jackson et al. [32] observed that the risk of local reaction was strongly correlated with higher pre-vaccination concentrations of serotypes 4, 14, 23F in subjects vaccinated for the first time and previously vaccinated ≥ 5 years before.

Female gender was associated with an increased risk of local adverse reaction in our study. Socan et al. [36] have reported an increased rate of both systemic and local adverse reactions in females compared with males following pneumococcal vaccination, although the magnitude of this effect is obscured by the pooling of adverse reaction data from subjects receiving pneumococcal and influenza vaccines with those receiving pneumococcal vaccine alone. This gender effect has also been observed with influenza [37] and anthrax [38] vaccines in adults and a diphtheria-tetanus booster in adolescents [39]. The basis of this effect is unclear but Beyer et al. [40], in a meta-analysis of fourteen independent studies with activated influenza vaccine, concluded that gender is a predictor of influenza vaccine reactions.

Injection of the pneumococcal vaccine into subcutaneous tissue was associated with a greater rate of local reaction than intramuscular injection. This has not been previously observed with pneumococcal vaccine but has been observed with influenza [37] and anthrax [38] in adults, a DT booster in adolescents [39] and Hib-tetanus toxoid conjugate vaccine in infants [41]. This effect has been explained [42] on the basis of poorer drainage channels in subcutaneous tissue with the retention of vaccine antigens in this tissue rendering subcutaneous fat susceptible to antigen mediated adverse reactions.

Our study supports the hypothesis that intramuscular injection is the preferred route of administration of pneumococcal vaccine in the elderly, especially females.

Acknowledgements

The authors would like to thank the patients who took part in this study, Dr Melanie Wong and the staff at Westmead Hospital for their technical expertise, and Mayne Health Laboratory, Tarne for blood sampling.

References


Immune response to serotypes 3, 4 and 6, those responsible for most invasive pneumococcal disease in patients > 65 years of age in Australia\textsuperscript{119}, were not influenced by the route of administration, although serotype 4 approached significance with IgG ≥ 1µg/ml. This is shown by the number needed to treat data in Table 3.

Table 3. ≥ 2-Fold Increase in IgG and ≥ 1µg/ml by Serotype and Route of Administration

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Comparison</th>
<th>NNT ≥ 2-Fold Increase in IgG</th>
<th>NNT ≥ 1µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype 3</td>
<td>IM vs SC</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Serotype 4</td>
<td>IM vs SC</td>
<td>20</td>
<td>11.1</td>
</tr>
<tr>
<td>Serotype 6</td>
<td>IM vs SC</td>
<td>50</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Injection site reaction was significantly less with intramuscular compared with subcutaneous injection, IM vs SC, NNH 8.5.

A review\textsuperscript{120} (Cook, page 39) of route comparative studies (subcutaneous vs intramuscular) of vaccines through the assessment of published clinical data and manufacturers’ websites showed that intramuscular injection was associated with better immune response and a lower rate of injection site reactions than subcutaneous injection. These observations were made with aluminium-adjuvanted vaccines (diphtheria and tetanus toxoid containing vaccines, hepatitis A and B and anthrax vaccines), non-adjuvanted subunit and whole cell vaccines (influenza, pneumococcal and meningococcal and \textit{Haemophilus influenzae} type b polysaccharide vaccines and a leptospirosis vaccine) and live attenuated vaccines (yellow fever, varicella, measles and measles-mumps-rubella vaccines).
Cook IF. Evidence based route of administration of vaccines. *Human Vaccines* 2008; 4: 63-73
Evidence based route of administration of vaccines

I.F. Cook

University of Newcastle; School of Medical Practice and Population Health; Callaghan, New South Wales, Australia

Key words: vaccine administration, subcutaneous, intramuscular, injection site reaction, immunogenicity

Vaccination is a proven public health initiative, however it is imperative in the context of increasing concerns about vaccine induced adverse reactions and a decreasing incidence of diseases they prevent that the optimal route for their administration is defined.

Traditionally all vaccines were given by subcutaneous injection until it was recognized that adjuvanted vaccines given via this route induced an unacceptable rate of injection site reaction.

Evidence-based medicine has been championed as a way of improving the quality of patient care. Application of this methodology to the route of administration of vaccines demonstrates that vaccines should be given by intramuscular injection in preference to subcutaneous injection as the intramuscular route is associated with better immune response and a lower rate of injection site reaction. The basis of this superiority is discussed.

Evidence Based Route of Administration of Vaccines

Evidence based medicine has been championed as a way of improving the quality of patient care through the stepwise process of formulating the question to be answered, collating and appraising relevant data and developing the best practice solution to the clinical question.

Review of the evidence base for route of administration of vaccines (subcutaneous or intramuscular injection) through the assessment of published clinical data and manufacturers’ websites reveals practice based on tradition rather than clinical data.

Strengthening the evidence base for route of administration of vaccines has the potential to simplify vaccination practice, whilst maximizing the immunogenicity and minimizing the reactogenicity of vaccines.

In this commentary, clinical trial data on the reactogenicity and immunogenicity of vaccines administered by subcutaneous or intramuscular injection are presented. The methodology of these studies is variable in terms of site of injection, needle parameters (needle length and gauge) and technique of vaccine injection. These data, where available, are presented in the tabulated summaries.

Traditional vaccination practice has been to give all vaccines, excluding BCG, by the subcutaneous route, with the study by Semple in 1910 with typhoid vaccine seeming to support this position.

However, with the observation of increased immunogenicity of aluminum salt adsored vaccines by Glenny, it soon became apparent that administration of this type of vaccine by the subcutaneous route gave an unacceptable rate of injection site reaction.

Aluminum-Adjuvanted Vaccines

It is currently recommended that all aluminum-adjuvanted vaccines be given by intramuscular injection except anthrax vaccine.

The twelve studies comparing subcutaneous with intramuscular administration of aluminum-adjuvanted vaccines, presented in Table 1, support this recommendation.

Injection site reaction was greater with subcutaneous compared with intramuscular injection in the two studies in which needle parameters and injection technique were specified. It was also greater in four of the other five studies in which adverse reaction data were presented including the study with anthrax vaccine. Immunogenicity was also greater with intramuscular compared with subcutaneous injection in six of the studies.

Live Attenuated Virus Vaccines

Live virus vaccines have traditionally been given by subcutaneous injection as it is asserted that it may be less painful and associated with a lower risk of bleeding. It had also been maintained that "any vaccination using less than the standard dose or a non-standard route or site of administration should not be counted, and the person should be revaccinated according to age." Although this recommendation has been rescinded, it is demonstrably invalid for live virus vaccines (Table 2).

The immunogenicity of yellow fever and varicella vaccines was greater with intramuscular compared with subcutaneous injection. Whilst measles/mumps/rubella and varicella vaccines gave good immune responses when administered by intramuscular injection. Injection site reaction was greater with subcutaneous compared with intramuscular injection with varicella and measles/mumps/rubella vaccines.
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Patients</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlson et al.</td>
<td>Open, randomized, prospective study</td>
<td>Swedish infants n = 287</td>
<td>Diptheria toxoid; Diptheria/Tetanus toxoid; Inactivated Polio (IPV/DT) reconstituted with Hib [Tet/Hib] given at 3, 5, 12 months with defined injection technique. SC - 30° angle, 25 mm long needle, thigh IM - 90° angle, 25 mm long needle, thigh.</td>
<td>SC injection caused more injection site reaction than IM injection, but did not affect immune response to any antigens.</td>
</tr>
<tr>
<td>Mark et al.</td>
<td>Open, randomized, prospective study</td>
<td>Swedish school students, 10 years of age n = 222</td>
<td>DT Vaccine [DTP-Avax A2] SC n = 127, IM n = 123. SC - 30° angle, IM - 90° angle, deltoid 25 mm long needle, deltoid.</td>
<td>SC injection caused more injection site reaction than IM injection, but did not affect immune response to any antigens.</td>
</tr>
<tr>
<td>Holt &amp; Bousfield</td>
<td>Prospective study</td>
<td>English children, age not clearly defined n = 805</td>
<td>PAP with varying amounts of magnesium, aluminium, phosphate. SC n = 339, IM n = 556.</td>
<td>IM gave significantly greater Schick conversion rate than SC injection. Difference thought to be due to &quot;fibrous encapsulation of much of the material injected&quot;.</td>
</tr>
<tr>
<td>Rothstein et al.</td>
<td>Double blind, comparative study</td>
<td>American infants aged 2,4,6 months n = 80</td>
<td>DTaP-US [Carnivorous], formaldehyde-inactivated PT and FHA with their currently licensed diphtheria and tetanus toxoids, SC n = 40, IM n = 40. Subcutaneous injections given with 25 gauge 16 mm needle. Intramuscular injections with 25 gauge 16 mm needle at 2 months of age and 25 gauge 25 mm needle at 4 and 6 months of age, injections into the anterolateral thigh.</td>
<td>No difference in immune response between SC and IM injections. SC &gt; IM for Erythema &lt;2.5 cm at 4,6 months. Induration at 6 months - any local reaction at 4 and 6 months.</td>
</tr>
<tr>
<td>CERTIVAR® [DTP-Avax product information</td>
<td>a) Data Troilfors et al. N Engl J Med 1995, 333: 1045-50. Randomized double blind placebo controlled study; b) Data on file Cerovax at North American Vaccines Inc.</td>
<td>(a) Swedish infants Aged 3 to 12 months, n = 3450 (b) American infants, aged 2 to 15 months n = 2480</td>
<td>(a) DTP n = 1724 DT n = 1726 Vaccine given by SC injection anterolateral thigh at 3, 5 and 12 months (b) DTP n=2480. Vaccine given by IM injection anterolateral thigh at 2, 4, 6 and 15 months.</td>
<td>DTP Any redness dose 1 22.2% dose 2 50.9% dose 3 67.6% any swelling dose 1 10.8% dose 2 34.7% dose 3 45.9% (b) DTP Any redness dose 1 4.4% dose 2 7.7% dose 3 10.9% dose 4 21.0% Any swelling dose 1 3.6% dose 2 5.4% dose 3 7.9% dose 4 12.7%.</td>
</tr>
<tr>
<td>Ragni et al.</td>
<td>Open, non randomized, prospective study</td>
<td>American children aged 2-8 yrs, 45 with haemophilus, 41 siblings</td>
<td>Hepatitis A Vaccine [Havrix 720] administered at 0 and 6 months by SC injection to haemophilus and IM to siblings.</td>
<td>IM injection gave greater GMT's than SC at 1 and 8 months. No differences in injection site reaction between routes of administration.</td>
</tr>
<tr>
<td>Fisch et al.</td>
<td>Open, randomized, prospective study</td>
<td>French adults aged 19.2 to 46.8 years n = 147</td>
<td>Inactivated HAV absorbed onto aluminium hydroxide. Injections given with injector device or SC or IM with needle. IM n = 50, SC n = 49, injector device n = 48 Delcoid.</td>
<td>Injection site reaction greater with both primary and booster dose for SC compared with IM injection. Seroconversion IM ≥ SC at week 4, GMT IM &gt; SC at 4 &amp; 28 weeks.</td>
</tr>
<tr>
<td>Parent du Chatel et al.</td>
<td>Open, randomized, prospective study</td>
<td>French adults 18 years - 60 years n = 147</td>
<td>Hepatitis A Vaccine [AVAXIM] SC n = 48, IM n = 49.</td>
<td>GMT at 4 weeks, 1mule 305 mI/mL, 1mule 211 mI/mL SC 116 mI/mL seroconversion at 4 weeks 1mule 100% IM 100% SC 97.5%.</td>
</tr>
<tr>
<td>Fessard et al.</td>
<td>Prospective study</td>
<td>French adults, no age given, who failed to seroconvert (&gt;10 IU/I) after primary course of subcutaneous injections of HEVAC n = 85</td>
<td>Hepatitis B vaccine [HEVAC] SC n = 43, IM n = 42. SC given into supraspacular area, IM given into deltoid area.</td>
<td>SC &amp; IM equal rates of seroconversion.</td>
</tr>
</tbody>
</table>

Continued
Table 1  Aluminum-adjuvanted vaccines—Intramuscular/subcutaneous administration (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Patients</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Lolla et al.</td>
<td>Open, randomized,</td>
<td>Italian adults, 299 aged mean 26.3 to 28 years, n = 299</td>
<td>MSD Hepatitis B vaccine IM buttock n = 71; SC arm n = 76, IM arm n = 75; Pasteur Hepatitis B Vaccine SC arm n = 77.</td>
<td>Seroconversion with MSD vaccine IM arm &gt; SC arm. SC arm &gt; IM buttock. MSD vaccine, SC and IM arm better than Pasteur vaccine SC arm but Pasteur vaccine SC arm &gt; MSD IM buttock. Seroconversion at 7 months IM 98%, SC 97%. GMT IM 791 IU/L SC 168 IU/L SC more injection site reaction than IM injection. No difference in immune response between routes of administration.</td>
</tr>
<tr>
<td>Yamamoto et al.</td>
<td>Open, randomized, prospective study</td>
<td>Japanese adults n = 172</td>
<td>Recombinant Hepatitis B Vaccine (HBK-SC) SC and IM n = 62. 10 μg given as 3 dose regimen 0.1, 1, 6 months, 25 gauge, 25mm needle.</td>
<td></td>
</tr>
<tr>
<td>Pitman et al.</td>
<td>Open, randomized, prospective study</td>
<td>American adults aged 18 to 64 years, n = 173</td>
<td>Anthrax vaccine (AVA) was administered via seven different protocols 0.25 SC, n = 28 0 SC, n = 25 0 IM, n = 25 0.25 SC, n = 25 0.2 IM, n = 25 0.4 SC, n = 23 0.4 IM, n = 22</td>
<td></td>
</tr>
</tbody>
</table>

Non-adjuvanted Subunit / Whole cell vaccines

Induction about the optimal route of administration of these vaccines is clear:

1. AcHib (Hib-TT) is recommended by its manufacturer to be given by intramuscular injection but it has been given by subcutaneous injection in studies in Chile, France, Niger and Sweden.

2. Subunit, non-conjugated polysaccharide vaccines for Salmonella typhimurium, Neisseria meningitidis and Streptococcus pneumoniae have been given by intramuscular and subcutaneous injection.

3. Whole cell inactivated plague, influenza and polio have also been administered by both intramuscular and subcutaneous injection.

However, route comparative studies favor intramuscular over subcutaneous injection in terms of injection site reaction and immune response (Table 3). Injection site reaction was greater with subcutaneous compared with intramuscular injection in the two studies in which needle parameters and injection technique were specified. In another seven studies injection site reaction data were presented:

- Subcutaneous injection was associated with a greater rate of local adverse reaction than intramuscular injection in five studies and a small study with influenza vaccine.

- Pain at time of injection was greater with intramuscular compared with subcutaneous injection in one study.

- No difference in rates of injection site reaction was seen in a small study with influenza vaccine.

Intramuscular injection gave a better immune response than subcutaneous injection in three studies where these data were presented. In a study with an inactivated whole cell leptospirosis vaccine and an influenza vaccine, no difference in immune response was noted between the two routes of administration. Frayha et al. observed reduced anti PRP antibody levels when PRP-D was administered subcutaneously compared with other studies where this vaccine was given by intramuscular injection.

Vaccines failures, associated with death, have been observed with rabies vaccine given by injection into the subcutaneous fat of the gluteal area rather than by intramuscular injection into the deltoid area.

Clearly, for all vaccine groups which induce active immunity (subunit, toxoid, live attenuated and inactivated whole cell), intramuscular injection was associated with better immune response and a reduced rate of injection site reaction compared with subcutaneous injection.

Pathogenesis of the increased injection site reaction and impaired immune response with subcutaneous compared with intramuscular vaccinations

Two theories have been advanced for the pathogenesis of the observed increased rate of injection site reaction with subcutaneous compared with intramuscular injection of vaccines.

Lindblad has suggested that, “immunizing by the subcutaneous route (sc) the vaccine inoculum is introduced into a compartment with numerous sensory neurons (in contrast to the intramuscular compartment).” This is an unlikely explanation of the observed difference as:

- Although it is generally assumed that innervation density decreases in the order skin, muscle and visera, this is unknown.

- It can not be assumed, even if this gradient exists, that subcutaneous tissue as compared with skin, has greater innervation density than muscle.

- Inflammation from muscle and cutaneous inoculation is processed differently in the spinal cord with the former subject to stronger descending inhibition than the latter.

Laurensie et al. has suggested that injection site reaction, “could be explained by participation of the immune system and the inflammatory cells located in the skin and deep dermis.”

www.lindihbsciences.com  Human Vaccines
Table 2  Live virus vaccines—Intramuscular/subcutaneous administration

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Patients</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Fox et al.21   | Quasi-randomized, prospective study | Brazilian adults 15–40 years old, Numbers uncertain | Yellow fever vaccine - 17D administered:  
IM - 22 gauge, 1.5 inch needle  
SC - 25 gauge, 1/2 inch needle  
ID  
Dosage given:  
ID - 0.1 ml  
IM - 0.5 ml  
SC - 0.1 ml  
SC - 0.5 ml | Vaccine more immunogenic  
IM > SC  
As minimum immunizing dose for mice:  
1.15 - intradermal (ID)  
1.60 - IM  
2.5 - SC 0.5 ml  
4.16 - SC 0.1 ml concluded  
"the reduced susceptibility by the subcutaneous route may have had a more or less mechanical basis.  
"Absorption of virus from the subcutaneous tissue, which is apparently somewhat more difficult than absorption of virus placed intramuscularly or intradermally." |
| Denney et al.18 | Open, randomized, prospective study | American children 1–10 years old, n = 166 | Varicella vaccine(Oka/Merck), SC and IM n = 83 each  
SC - 26 gauge, 5/8 inch needle  
IM - 25 gauge, 1 inch needle  
deltoid injection. | Seroconversion greater  
IM > SC  
100%/97%  
Injection site reaction significantly greater SC vs IM.  
Measles seerocconversion percentages by age of initial immunization:  
7–8 months 88%  
9–10 months 90%  
11–12 months 88% |
| McGrow22       | Open, randomized, prospective study | American children aged 7–12 months, n = 127 | Experimental group, n = 97 received measles vaccine (MSD) at study entry and MMR at aged 15–18 months. Control group n = 30 received only MMR(MSD) at aged 15–18 months Intramuscular injection | | |
| Luleber et al.23 | Open, randomized, prospective study | Dutch infants aged 14 months n = 67 | MMR vaccine.  
Measles (Moraten strain), mumps (Jeryl Lynn strain), rubella (RA27/3 strain)  
n = 33 IM, n = 34 SC | Pain at time of injection greater with SC than IM injection. No difference for other injection site or systemic adverse effects. Immune response not significantly different for measles, mumps, rubella antigens, but levels somewhat higher with SC injection than IM injection. Concluded inadvertent intramuscular injection of MMR vaccine is no reason for revaccination. |
| Dunlop et al.24 | Open, prospective study | English infants aged 15 months, n = 335 | MMR vaccine measles (Schwartz strain), mumps (Jeryl Lynn strain), rubella (RA27/3 strain)  
n = 319  
Measles(Schwartz strain) n = 16  
Vaccine given by IM or SC injection into gluteal region. | Seroconversion rates:  
Measles vaccine-masles 100%  
MMR vaccine - Measles 95.6%  
Mumps 96.9%  
Rubella 100% |
| Barzaga et al.25 | Open, prospective study | Thai subjects aged 9 months to 60 years, n = 246 | Varicella vaccine (Oka strain) 0.5 ml intramuscular injection, right deltoid. | Seroconversion in seronegative patients:  
<7 years 96.6%  
7–12 years 100%  
213 years 86.1% |

This thesis is supported by animal and human data. In the cat model, the observed greater tissue reaction in subcutaneous tissue compared with muscle can be attributed to delayed absorption of substances from the subcutaneous injection site. In humans, immunoglobulin is more rapidly absorbed after intramuscular compared with subcutaneous injection. The relative retention of injected antigens in the subcutaneous tissue compared with muscle results in a greater degree of processing by antigen presenting cells (e.g. dendritic cells) in the subcutaneous tissue with consequent greater inflammatory reaction in this tissue. Trapping of antigens in the subcutaneous tissue has been suggested as the basis of the poorer immune response with subcutaneous compared with intramuscular injection; Holt and Bousfield (Table 1) and Fox et al.21 (Table 2).

Conclusion

Although the data presented came from studies with varying methodological standard, route of administration (subcutaneous or intramuscular) does affect the immune response and injection site reaction rate of vaccines.
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Patients</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cook et al. (^{39})</td>
<td>Observer blind, randomized, prospective study</td>
<td>Australian adults 65 years and older - well adults, 55 years and older with chronic disease, n = 720</td>
<td>Split trivalent influenza vaccine, 2A strains, 1 B strain (Fluvax, CSL). IM and SC n = 360 each. Administered: SC - 23 gauge 25mm needle, technique - 10:20° to skin’s surface. IM - 23 gauge 25mm needle, technique - needle introduced at 90° to skin’s surface, deltoid.</td>
<td>Immunogenicity IM &gt; SC, for both A strains but not B strain. Injection site reaction SC &gt; IM.</td>
</tr>
<tr>
<td>Cook et al. (^{40})</td>
<td>Observer blind, randomized, prospective study</td>
<td>Australian adults, 65 years and older - well patients. 55 years and older with chronic disease. n = 254</td>
<td>Pneumovax 23 (MSD) vaccine IM and SC n = 127 each. SC - 23 gauge 25mm needle inserted at 10:20° to skin’s surface. IM - 23 gauge 25mm needle inserted at 90° to skin’s surface, deltoid.</td>
<td>No difference in immunogenicity for serotypes 2, 4, 6. Injection site reaction SC &gt; IM.</td>
</tr>
<tr>
<td>Ruben and Jackson (^{41})</td>
<td>Open, randomized, prospective study</td>
<td>American adults aged 18-25 years n = 67.</td>
<td>Influenza vaccines: - Subunit vaccine prepared with tri-n-butylphosphosphate (TNP) (Wyeth) AQ/Alchi/BIMass n = 10, IM, n = 15, SC.  - (Sharples - Wyeth conventional) n = 10, SC.  - (Zanola - ultracentrifuged MSD) n = 10, SC.  - Subunit ether (Parke-Davis) n = 13, IM.</td>
<td>The three vaccines given SC (Sharples, Zanola and TNP-subunit) all caused maximal pain responses graded higher than 2. The vaccines given IM (other-subunit and TNP-subunit) had lower maximal pain responses. Erythema and induration at the local site, which averaged from 4 to more than 5 cm in diameter with vaccines given SC, was hardly measurable in the groups vaccinated IM. Systemic adverse reactions were not different for the two routes of administration.</td>
</tr>
<tr>
<td>Skafteli et al. (^{42})</td>
<td>Non randomized, prospective study</td>
<td>Canadian children aged 4 to 6 years n = 101.</td>
<td>Meningococcal quadrivalent vaccine (Connaught) SC n = 53, IM n = 48.</td>
<td>Redness and swelling but not tenderness were greater with SC compared with IM injection.</td>
</tr>
<tr>
<td>Ruben et al. (^{43})</td>
<td>Open, randomized, prospective study</td>
<td>American adults IM = 21.9 years SC = 20.6 years n = 141.</td>
<td>Meningococcal polysaccharide vaccine, A,C,Y and W(_{135}) (Menomune, Aventis Pasteur) SC n = 66, IM n = 67 completed protocol. SC - administered into patient’s arm. IM - administered into lateral deltoid. Both injections with 25 gauge, 5/8 inch needle.</td>
<td>Immunogenicity: IM injection gave higher GMTs for serogroup A and C than SC injection. Reactogenicity: Erythema &lt; 1 inch at injection site significantly greater for SC compared with IM injection. Headache at day 1 and 2 also significantly greater for SC compared with IM injection.</td>
</tr>
<tr>
<td>Leung et al. (^{44})</td>
<td>Quasi randomized, not blinded study</td>
<td>Canadian children aged 18 months to 5 years n = 498.</td>
<td>Haemophilus influenzae type b - non conjugated (PRP) (Praxis Biologics). Equal numbers in each group SC 27 gauge 1/2 inch needle IM 25 gauge 1 inch needle, upper outer quadrant of buttoc.</td>
<td>Pain manifest as crying. IM more common than SC. Incomplete data; 194 subjects from each study group.</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Patients</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frayha et al.45</td>
<td>Randomized, prospective study</td>
<td>Canadian children aged 15-17 months n = 101</td>
<td>Haemophilus influenzae type b vaccine - non-conjugated (PRP) (Praxis Biologica) n = 50</td>
<td>Lower immunogenicity of PRP-D compared with three other studies reviewed in this publication in which this vaccine was given intramuscularly. The lower immune response in this study may have been due to other factors such as vaccine lot, study group characteristics or inter-laboratory variation in measurement of anti PRP antibody.</td>
</tr>
<tr>
<td>JAMA 200646</td>
<td>Retrospective study</td>
<td>Subjects inadvertently given subcutaneous injection Age of subjects not stated, n = 101</td>
<td>Meningococcal capsular polysaccharide A,C,Y, W135 conjugated with diphtheria toxin toxoid MC54</td>
<td>GMT significantly lower for SC injection for serogroups A,C,Y compared with IM injection, but not for W135.</td>
</tr>
<tr>
<td>Delafuente et al.47</td>
<td>Observer-blind, randomized, prospective study</td>
<td>Elderly American adults aged 61-81 years, mean 68years n = 26</td>
<td>influenza vaccine, A/Taiwan A/Beijing B/panama (Wyeth/Ayerst 1991-2) SC and IM n = 13 each.</td>
<td>No difference in immune response or adverse reaction between both sites of vaccination. Both jet injectors gave significantly more injection site reactions than IM injection. No difference in immune response with different techniques of administration.</td>
</tr>
</tbody>
</table>
| Jackson et al.48  | Randomized, dose but not device blind, study | American adults aged 18-45 years n = 304 | Split virus influenza vaccine (Fluzone) 1998/1999. Vaccine contained 15 μg of antigen per 0.5 ml dose from each of the following strains: A/Beijing A/Sydney and B/Belling.
- Viject®
- Bioject®
- Conventional needle/syringe. 54 gauge 14 mm with doses of 0.2, 0.3 and 0.5 ml | No difference in immune response with SC vs IM vaccination site. Injection site reaction significantly more frequent after SC compared with IM route. Suggested this difference due to local inflammation could be explained by participation of the immune system and the inflammatory cells located in the skin and deep dermis. Alternatively, local reactions may also occur in muscle, but are more frequently clinically silent because of the depth. |
| Larchesse et al.49 | Double blind, placebo controlled, randomized study | French adults aged 18-40 years n = 84 | Leptospirosis vaccine 2 x 10^6 leptospires from icterohaemorrhagiae serogroup, inactivated with formaldehyde. SC vaccine n = 30, IM vaccine n = 30 SC placebo n = 12 IM placebo n = 12 Given by deltoid injection. | No difference in immune response with SC vs IM vaccination site. Injection site reaction significantly more frequent after SC compared with IM route. Suggested this difference due to local inflammation could be explained by participation of the immune system and the inflammatory cells located in the skin and deep dermis. Alternatively, local reactions may also occur in muscle, but are more frequently clinically silent because of the depth. |

Route of administration is a poorly developed area of vaccinology with Potier et al.60 observing a review of 83 vaccine trials that 59% described the anatomic injection site. 24% utilizing intramuscularly administered vaccines recorded needle length and only 10% described the injection technique used.

As intramuscular injection is the preferred route of administration compared with subcutaneous injection, for vaccines where route comparative data exist, it behooves editors of publications which accept vaccine trials to routinely report needle length and injection techniques which ensure intramuscular injection.

This standardization will allow better inter-trial comparison of vaccines, maximize their immunogenicity and minimize their injection site reaction rates.

References
Sex differences in humoral immunity and adverse reactions with human vaccines.

In the influenza study\textsuperscript{116} (Cook et al, page 18) analysis of serological responses by sex showed that the majority of the difference seen between the IM and SC groups was a consequence of the higher responses in the female subjects of the IM group.

Table 4. Geometric Mean Titres of Haemagglutination Inhibition Antibodies Post-Vaccination and Fold Increase Post/Pre Vaccination by Gender and Route of Administration.

<table>
<thead>
<tr>
<th></th>
<th>IM Group</th>
<th>SC Group</th>
<th>(p^a) (IM vs SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H3</td>
<td>H1</td>
<td>B</td>
</tr>
<tr>
<td>Females, post Vaccination GMT</td>
<td>110.7</td>
<td>29.7</td>
<td>45.6</td>
</tr>
<tr>
<td>Males post-Vaccination GMT</td>
<td>67.5</td>
<td>19.3</td>
<td>38.77</td>
</tr>
<tr>
<td>(p) (within groups)</td>
<td>0.0002</td>
<td>0.0002</td>
<td>0.2004</td>
</tr>
<tr>
<td>Females, Fold Increase in Titre (post/pre vaccination)</td>
<td>5.85</td>
<td>3.01</td>
<td>3.06</td>
</tr>
<tr>
<td>Males, Fold Increase in Titre (post/pre vaccination)</td>
<td>4.05</td>
<td>2.19</td>
<td>2.60</td>
</tr>
<tr>
<td>(p) (within groups)</td>
<td>0.0048</td>
<td>0.0017</td>
<td>0.1058</td>
</tr>
</tbody>
</table>

In both this study and the pneumococcal vaccine study\textsuperscript{118} (Cook et al, page 29), injection site reactions were significantly more common in female compared with male subjects with subcutaneous injection.

Influenza study, SC route female vs male NNH 3.2, IM route female vs male NNH 122; pneumococcal study, SC route, female vs male NNH 3.9, IM route female vs male NNH 47.6.
(i) Sex-differences in humoral immunity with human vaccines.

The influence of sex on the humoral immune response to vaccines in humans has been said to be “difficult to identify” (Whitacres et al121) and “very scant” (Birx122).

However, in a review123 (Cook, page 49), 97 studies were found by searches made of Medline, Embase, Scopus, Biological Abstracts, Science Citation Index, Cochrane Database of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL), NHS Database of Abstracts of Reviews of Effects (DARE) using the following search terms and their word variants “sex-difference”, “gender-difference”, “sexual dimorphism” and “humoral response”, “serological response” and “vaccines”, “immunisation”. Bibliographies of all relevant articles were searched for additional studies.
Cook IF. Sexual dimorphism of humoral immunity with human vaccines.
Vaccine 2008; 26: 3551-5123.
Review

Sexual dimorphism of humoral immunity with human vaccines

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ABSTRACT

It has been contended that limited data exist on sex-difference in immune response with vaccines in humans.

However, a comprehensive search of the literature retrieved 67 studies with 14 vaccines influenza (7 studies), hepatitis A (15 studies), hepatitis B (50 studies), pneumococcal polysaccharide (4 studies), diphtheria (4 studies), rabies (3 studies), measles (2 studies), yellow fever (3 studies), meningococcal A (1 study), meningococcal C (1 study), tetanus (1 study), brucella (1 study), Venezuelan equine encephalitis (1 study) and rabies (4 studies), with sex-difference in humoral (antibody) response. These differences are associated with sex-difference in the clinical efficacy of influenza, hepatitis A, hepatitis B, pneumococcal polysaccharide and diphtheria vaccines and significant adverse reactions with rabies, measles and yellow fever vaccines.

The genesis of these differences is uncertain but not entirely related to gonadal hormones (differences are seen in pre-pubertal and post-menopausal subjects not on hormone replacement therapy) or female sex (males had greater serological response for pneumococcal, diphtheria, yellow fever, Venezuelan encephalitis and in some studies with rabies vaccine).

As sex-difference in humoral immune response was seen with most vaccines which cover the spectrum of mechanisms by which infectious agents cause disease (mucosal replication, viral viremia, bacterial bacteremia, toxin production and neuronal invasion), it is mandatory that vaccine trialists recruit a representative sample of females and males to be able to assess sex-differences which may have clinical implications.

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Infectious diseases are a major cause of morbidity and mortality worldwide [1], and vertebrates are endowed with two defence systems [2] to protect the host against infection. In innate immunity, the first line of defence, is mediated via pattern-recognition receptors, one class of which are the TLR-like receptors which have the ability to recognise pathogens or pathogen-derived products and signal events leading to the activation of this immunity. Adaptive immunity, the second line of defence, is a more sophisticated system possessing properties of clonal expansion and memory. Vaccination, whose goal is immunological memory [3], is thus dependent on adaptive immunity.

As immunity has been observed [4,5] to be sexually dimorphic in both animals and humans and it might be expected that sex-differences in immune responses would be observed with vaccines in humans. However, a number of authors [4–7] have commented on the paucity of such data. Walshere et al. [4] noted that such data are “difficult to identify”, Lockshin offers very limited data. whilst Bilh, in 2005, concluded that “the literature is very scant”.

In this review, sex-difference of humoral immunity with vaccines in humans, and their clinical implications, is discussed. Antibody (humoral) response to vaccines has been chosen as the means of assessing these differences as serological levels of protection are available [8] for many vaccines and they offer a convenient, objective means of assessing these differences. Searches were made of medline, Embase, Scopus, Biological Abstracts, Science Citation Index, Cochrane Database of Systematic Reviews (CDSR), Science Citation Index, Cochrane Central Register of Controlled Trials (CENTRAL), and NHS Database of Abstracts of Reviews of Effects (DARE) using the following search terms and their work variants, “sex-difference”, “gender difference”, “sexual dimorphism” and “immune response”, “serological response”, “antibody response” and “vaccines”, “immunisation”. Bibliographies of all relevant articles were searched for additional study.

Vaccines were grouped according to the disease mechanism they prevent [9]: Sex-difference in antibody response was seen with all vaccine groups (see Table 1).

Sex-difference in antibody response, female greater than male, was seen with the following vaccines: influenza (elderly adults, young adults with acute stress and eccentric exercises).
Table 1
Sex differences in humoral response with vaccines by mechanism of the disease they prevent and clinically significant differences in vaccinated populations or individuals

<table>
<thead>
<tr>
<th>Sex difference in humoral response</th>
<th>Vaccine</th>
<th>Mechanism of disease</th>
<th>Clinical significant sex-difference in vaccinated population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F= M [10–13]</strong> elderly subjects</td>
<td>Influenza</td>
<td>Macrophage replication</td>
<td>Lower rates of hospitalization and mortality [14–17] in females compared with males in studies with elderly subjects.</td>
</tr>
<tr>
<td><strong>F= M [18,19]</strong> young adults, acute stress and exercise</td>
<td>Influenza</td>
<td>Macrophage replication</td>
<td></td>
</tr>
<tr>
<td><strong>M&gt; F [20]</strong> young adults, life events and social support</td>
<td>Hepatitis A</td>
<td>Hepatitis B</td>
<td>Viral viremia</td>
</tr>
<tr>
<td><strong>M&gt; F [21–35]</strong> living in alcoholics</td>
<td>Pneumococcal polysaccharide</td>
<td>Bacterial bacteremia</td>
<td>In a study of geriatric patients in Austria, males [90] had a lower risk of pneumonia than females and non-vaccinated males had a lower risk of pneumonia than non-vaccinated females.</td>
</tr>
<tr>
<td><strong>M&gt; F [26–44]</strong> in undernourished children</td>
<td>Tuberculosis</td>
<td>Tuberculosis</td>
<td>In the experimemt epidemic in the Russian Federation, cases occurred more commonly in people greater than 15 years of age and in females more than males [81–84].</td>
</tr>
<tr>
<td><strong>F= M [25–30]</strong> Rubella virus</td>
<td>Rubella</td>
<td>Viral viremia</td>
<td>Musculoskeletal syndromes, transient or chronic arthritis are more frequently observed [100] in adult females than males of pre-pubertal children or prepubescent children. The pathogenesis of this syndrome is uncertain but it has been suggested that the virus itself or the viral antigenic response in females may allow the increased viremia with escape of the virus antigen to the lymphatic or lymphoid tissues such as the synovial</td>
</tr>
<tr>
<td><strong>M&gt; F [101]</strong> pre-pubertal children</td>
<td>Measles</td>
<td>Viral viremia</td>
<td>Higher titre measles vaccine has been observed [101] to increase immunity in females 1–2 years after vaccination compared with standard titre vaccine. A greater rate of post-vaccination [102] encephalitis was reported in males compared with females in an early yellow fever vaccine.</td>
</tr>
<tr>
<td><strong>M&gt; F [105]</strong> adults</td>
<td>Yellow fever</td>
<td>Viral viremia</td>
<td></td>
</tr>
<tr>
<td><strong>M&gt; F [107]</strong> young adults, acute stress</td>
<td>Meningococcal A</td>
<td>Bacterial bacteremia</td>
<td></td>
</tr>
<tr>
<td><strong>F= M [109]</strong> for most age groups</td>
<td>Meningococcal C</td>
<td>Bacterial bacteremia</td>
<td></td>
</tr>
<tr>
<td><strong>F= M [110]</strong></td>
<td>Tetanus</td>
<td>Toxoid production</td>
<td></td>
</tr>
<tr>
<td><strong>F= M [111]</strong></td>
<td>Venezuelan equine encephalitis</td>
<td>Viral viremia</td>
<td></td>
</tr>
<tr>
<td><strong>F= M [112]</strong> infant, intramuscular</td>
<td>Rubella</td>
<td>Neuronal invasion</td>
<td></td>
</tr>
<tr>
<td><strong>F= M [113]</strong> adult, intradermal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>M= F [114]</strong> adult, intramuscular</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

hepatitis A, hepatitis B, rubella, measles (adults), diphtheria (undernourished infants, primary vaccination), tetanus, bronchitis, and rubella, with male greater than female with the following vaccines; influenza (young adults, life events and social support effects), pneumococcal polysaccharide (healty and alcoholic adults), diphtheria (booster dosing), measles (pre-pubertal), yellow fever, meningococcal A (adults, acute stress), meningococcal C (most age groups), Venezuelan equine encephalitis vaccines and rubelles.

Consistent with the sex differences are sex-differences in disease attack rates following vaccination, with females having less clinical disease than males with influenza [14–17], hepatitis A [36–38] and hepatitis B [85] vaccines and males having less clinical disease than females with pneumococcal [90] vaccine. Disease occurrence with these vaccines probably reflects primary vaccine failure (failure to achieve a protective antibody response). The increased rate of diphtheria infection in males compared with females in patients >14 years of age (many years after primary vaccination) in the 1990s epidemic in the Russian Federation [99,100] probably reflects secondary vaccine failure (wanning of the antibody level below that which is protective). It is supported by the observation that females have lower levels of immunity to diphtheria than males in zero-surveys in the United Kingdom [115], northern Norway/north-western Russia [116] and Israel [117], not accounted for by booster immunization of men upon entering military service.

Whether this is due to poorer anti-toxin antibody response in females compared with males as seen in booster studies in adolescents [92] and adults [93,94] or poorer persistence of anti-toxin antibody levels despite better initial response [91] in females compared with males is uncertain.

Sex-difference in clinically significant adverse reactions are seen with rubella [100], measles [103] and yellow fever [105] vaccines. The genesis of the sex-difference in antibody response with vaccines is uncertain.

It has been suggested [5] that an observed relative increase [118] in CD4+ T cells in males compared with females may offer "partial explanation" for the more vigorous antibody response in the former group compared with the latter group forrogens antigens. Other studies [119–121] have observed greater CD4+ T cell counts in females compared with males but it is apparent that function (TH1 or TH2) rather than number is likely to be important.

Functional differences in CD4+ T cells have been observed [122] in females compared with males, with an in vitro study of peripheral blood mononuclear cells (PBMC) showing that stimulation of CD8+ T cells with a polyclonal activator, phyto-haemagglutinin, gave a greater TH1 response in females compared with males.

Consonal hormones have a stimulatory effect on the immune function of females which becomes evident after menarche and diminishes after menopause [123]. The influence of sex hormones on the TH1/TH2 balance is seen in pregnancy [124,125] and with the use of exogenous hormones [126].
Successful pregnancy is favoured [124] by a Th1/Th2 balance that favours a Th1 response with Th1-type cytokines at both the maternal–foetal interface and PBMCs being higher in women with unexplained habitual abortions. Similarly, in an in vivo study [125] of transsexual men and women, testosterone titrated the Th1/Th2 balance of CD4+ T cells differentiation of PBMC with polyclonal stimulation with phytohaemagglutinin to Th1 response whilst with oestrogen the balance remained titrated in favour of Th2 response.

The sex-difference in humoral immune response seen with vaccines in this review are not related entirely to gonadal hormones (differences are seen in pre-pubertal and post-menopausal subjects not on hormone replacement therapy) or female sex (males had greater serological response for pneumococcal, diphtheria, yellow fever and Venezuelan equine encephalitis) but probably reflects an antigen specific interaction with the immune system via mechanism as yet to be defined.

As sex-differences in humoral immune response is seen with vaccines which cover the spectrum of mechanisms by which infectious agents cause disease (mucosal replication, viral viremia, bacterial bacteremia, toxin production and neuronal invasion) it is mandatory that vaccine clinical trials recruit a representative sample of females and males to be able to assess sex-differences which may have clinical implications.

References


The genesis of sex-difference in antibody response with vaccines which can have clinical implications is uncertain.

It has been suggested\textsuperscript{124} that an observed\textsuperscript{125} relative increase in CD\textsubscript{4}\textsuperscript{+} T cells in females compared with males may offer partial explanation for the more vigorous antibody response in the former group compared with the latter group for exogens antigens like vaccines. Certainly other studies\textsuperscript{126-128} have also observed greater CD\textsubscript{4}\textsuperscript{+} T cell counts in females compared with males but function is more likely than number to be important with naive CD\textsubscript{4}\textsuperscript{+} T cell differentiating under the influence of cytokines and antigen stimulation into T helper 1 (TH\textsubscript{1}) and T helper 2 (TH\textsubscript{2}) cells, the former mediating cellular immunity and the latter humoral (antibody) immunity.

Functional differences in CD\textsubscript{4}\textsuperscript{+} T cells have been observed\textsuperscript{129} in females compared with males, with an in vitro study of peripheral blood mononuclear cells (PBMC) showing that stimulation of these cells with a polyclonal activator, phytohaemagglutin gave a greater TH\textsubscript{2} response in females compared with males. Gonadal hormones have a stimulatory effect on the immune function of females which becomes evident after menarche and diminishes after menopause\textsuperscript{130}. The influence of sex hormones on the TH\textsubscript{1}/TH\textsubscript{2} balance is seen in pregnancy\textsuperscript{131,132} and with the use of exogenous hormones\textsuperscript{133}.

Successful pregnancy is favoured\textsuperscript{132} by a TH\textsubscript{1}/TH\textsubscript{2} balance that favours a TH\textsubscript{2} response with TH\textsubscript{1} type cytokines at both the maternal-foetal interface and in PBMCs stimulated with polyclonal activators, being higher in women with unexplained
habitual abortions. Similarly, in an in vivo study\textsuperscript{133} of transsexual men and women, testosterone tilted the TH$_1$/TH$_2$ balance of CD$_{4}^{+}$ T cells differentiation of PBMC with polyclonal stimulation with phyto-haemagglutinin to TH$_1$ cells whilst with oestrogen, the balance was tilted in favour of TH$_2$ cells.

The sex-difference in antibody response seen with vaccines is not related entirely to gonadal hormones (differences are seen in pre-pubertal and post menopausal subjects not on hormone replacement therapy) or female sex (males had greater serological response for pneumococcal, diphtheria (booster doses), yellow fever and Venezuelan equine encephalitis) but probably reflects an antigen specific interaction with the immune system via mechanism as yet to be defined.

**(ii) Sex difference in adverse reactions with human vaccines.**

A database search, like that made for sex differences and immune response and vaccines, for sex differences and adverse reactions with vaccines also yielded a substantial amount of data.

Adverse reactions, as in the influenza and pneumococcal vaccine trials reported in this thesis were reported more commonly in females than males (hepatitis B\textsuperscript{134}, influenza\textsuperscript{135-146}, pneumococcal\textsuperscript{147}, rubella and rubella containing vaccines \textsuperscript{148-151}, diphtheria/tetanus toxoid vaccines and toxoid containing vaccines\textsuperscript{152-155}, BCG\textsuperscript{156}, bioagents – anthrax\textsuperscript{157-160} and botulinum toxoid\textsuperscript{161,162}).

The basis of the sex-differences in pain perception by females compared with males following vaccination could be explained\textsuperscript{163} in terms of either sex-difference in
biochemistry or behavioural responses to pain, with in all probability both playing a causal role.

The greater rate of other injection site reactions (erythema, swelling and induration) in females compared with males may reflect the slower clearance of vaccine antigens from the injection site as seen with hepatitis B immunoglobulin\textsuperscript{164}. The persistence of vaccine antigen could result in inflammatory sequelae due to the antigen’s interaction with antigen presenting cells (APCs) like dendritic cells at the injection site. This mechanism is presumably the genesis of the greater rate of local adverse reactions with subcutaneous compared with intramuscular injection of vaccines, as seen with influenza vaccine\textsuperscript{116} (Cook et al, page 18), pneumococcal vaccine\textsuperscript{118} (Cook et al, page 29) and in a review\textsuperscript{120} (Cook, page 39) of route comparative studies (subcutaneous vs intramuscular) with vaccines.

**Evidence Based Summary and Answer**

Using the modified GRADE recommendation classifying the quality of evidence, there is high (Grade A) evidence that vaccines should be given into muscle rather than subcutaneous tissue. Using GRADE classification of recommendations, strong (Grade 1) recommendation can be made about the route of administration of vaccines, intramuscular in preference to the subcutaneous route.
CHAPTER 3:
WHAT LENGTH NEEDLE IS REQUIRED FOR INTRAMUSCULAR INJECTION?

Background Evidence

Intramuscular injection has been defined\textsuperscript{165} as penetration of the muscle layer by 5mm or more.

Direct measurement of the maximal length of needles required to satisfy this definition has been made in infants by ultrasonography and in adults by ultrasonography, computerised tomography and autopsy studies.

Clinical trials comparing injection site reaction rates with vaccines administered with needles of different length offer an indirect measure of the adequacy of intramuscular injection. The basis of this is the documented lower rate of injection site reactions with vaccines administered by intramuscular injection compared with subcutaneous injection (see Chapter 2, page 39).

Infants

1. Anterolateral Thigh

Four ultrasonographic studies have been made of the tissue composition of the anterolateral thigh, (Table 5).
Table 5: Ultrasonographic Studies of the Anterolateral Thigh in Infants

<table>
<thead>
<tr>
<th>Author</th>
<th>Country/Age of Children</th>
<th>Number of Subjects</th>
<th>Subcutaneous Layer (SCL) Thickness (mm)</th>
<th>Muscle Layer (ML) Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hick et al\textsuperscript{166} 1989</td>
<td>USA 4 months</td>
<td>13M, 11F</td>
<td>Males 14 ± 2.4 Females 13 ± 2.8</td>
<td>18.2 ± 2.1 15.5 ± 1.9</td>
</tr>
<tr>
<td>Chugh et al\textsuperscript{167} 1993</td>
<td>India 6-12 weeks</td>
<td>52</td>
<td>10.3 ± 2.3</td>
<td>8.3 ± 1.7 11.3 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>13-18 weeks</td>
<td>58</td>
<td>10.4 ± 2.1</td>
<td>11.2 ± 2.9 12.1 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>19-24 weeks</td>
<td>63</td>
<td>9.5 ± 1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 months ≤ 1 month</td>
<td>42</td>
<td>10.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Groswasser et al\textsuperscript{168} 1997</td>
<td>Belgium 9-27 weeks</td>
<td>40</td>
<td>Right: 8.0 ± 0.3 range 4.8 - 12.0</td>
<td>Right 9.2 ± 0.3 range 6.3 – 13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left 8.1 ± 0.3 range 5.1 – 11.7</td>
<td>Left 9.3 ± 0.3 range 6.8 – 13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right 7.5 ± 0.4 range 4.8 - 11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Left 7.6 ± 0.4 range 5.1 – 11.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>68-88 weeks</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cook and Murtagh\textsuperscript{169} 2002</td>
<td>Australia 2 months</td>
<td>14</td>
<td>8.6 ± 3.0 range 6.0 – 15.1</td>
<td>10.5 ± 2.4 range 6.2 – 14.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.2 ± 2.0 range 9.6 – 15.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.8 ± 2.0 range 10.2 – 17.1</td>
</tr>
<tr>
<td></td>
<td>4 months</td>
<td>13</td>
<td>9.4 ± 2.0 range 6.5 – 13.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>18</td>
<td>10.2 ± 2.1 range 6.7 – 13.5</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 months</td>
<td>12</td>
<td>8.1 ± 1.7 range 5.2 – 14.4</td>
<td>16.5 ± 4.6 range 9.5 – 22.5</td>
</tr>
</tbody>
</table>

As the study by Hick et al\textsuperscript{166} was the first of these chronologically it was instrumental in the recommendation of a 25mm long, 23 gauge needle for intramuscular injection.
in the anterolateral thigh of infants. The studies by Chugh et al and Groswasser et al lack external validity for North American, Western European and Australian infants, as Indian infants would be expected to have lower birth weight and poor nutritional status compared with infants in these areas and the children in the Belgian study were underweight (10-50\textsuperscript{th} weight percentile).

In the study by Cook and Murtagh\textsuperscript{169} (page 61) all the children were above the 50\textsuperscript{th} weight percentile, it was concluded that a 16mm long needle would routinely give intramuscular injection if the WHO injection technique was employed\textsuperscript{75}. 
Cook IF, Murtagh J. Needle length required for intramuscular vaccination of infants and toddlers: an ultrasonographic study. Aust Fam Phys 2002; 31: 1-3169
Needle length required for intramuscular vaccination of infants and toddlers
An ultrasonographic study

Ian F Cook, John Murtagh

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BACKGROUND To be maximally effective and to induce less adverse reactions, injection with many vaccine antigens must penetrate muscle rather than subcutaneous tissue.

AIM To determine the length of needle needed to penetrate muscle at the anterolateral thigh vaccination site in children aged two, four, six and 18 months.

METHOD Ultrasound measurements were made of the subcutaneous and muscle layer thickness of children aged two, four, six and 18 months at the junction of the upper and middle thirds of the anterolateral thigh with the probe applied parallel to the long axis of the leg and at 45° to the vertical and at 90° to the skin’s plane.

RESULTS Subcutaneous tissue (SCT) and muscle layer (ML) thickness were measured in 57 children (2 months, n=14; 4 months, n=13; 6 months, n=18; 18 months, n=12) with mean SCT thickness of: 8.6 ± 3.0 mm at 2 months; 9.4 ± 2.0 mm at 4 months; 10.2 ± 2.1 mm at 6 months; and 8.1 ± 1.7 mm at 18 months.

Muscle layer thickness in these children was: 10.5 ± 2.4 mm at 2 months; 12.2 ± 2.0 mm at 4 months; 14.8 ± 2.0 mm at 6 months, and 16.3 ± 4.6 mm at 18 months.

CONCLUSION The optimal needle length to routinely penetrate muscle of the anterolateral thigh in children aged two, four, six and 18 months depends on the technique employed. A 16 mm long needle is suitable with the WI-IO technique (injecting at 90° to skin’s surface) and 25 mm long needle with the NH&MRC and American techniques (injecting at 45° to skin’s surface).

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Intramuscular injection of aluminium adsorbed antigens is preferred to subcutaneous injection because of their reduced incidence of local adverse reactions. Better immune response also has been observed for diphtheria and hepatitis B antigens when injected intramuscularly rather than subcutaneously.

Three injection techniques are currently recommended for anterolateral thigh injection.

• In America, the needle is inserted into the upper lateral quadrant of the thigh at an angle of 45° with the long axis of the leg and posteriorly at a 45° angle to the table top with the baby supine. The thigh muscle is bunched at the injection site to increase muscle mass and to minimise the chance of striking bone. An ultrasound study to estimate the depths of structures, with only 24 children, led to a recommendation for vaccination using a 25 mm needle length for children of all ages.

• The technique recommended by the National Health and Medical Research Council (NH&MRC), (drawn from New Zealand guidelines), involves penetration of the junction of the upper and middle thirds of the vastus lateralis with the needle angled at 45° to 60° to the skin, with the angle pointing down towards the knee. No
ultrasonographic study has been made to validate this technique.

- The World Health Organization technique requires stretching the skin of the anterolateral thigh flat between the index finger and thumb followed by injecting the needle at 90° to the skin. This technique should satisfactorily accommodate needles only 16 mm long, according to an ultrasonographic study of Belgian children.

We attempted to estimate the depth of structures of different aged Australian children and the consequential suitable needle length for vaccination.

**Method**

Infants and toddlers aged two, four, six, and 18 months of age were recruited before vaccination in one general practice in Tarneit, NSW for an ultrasonographic study if:

- they were in apparent good health
- had no history of lower limb injury or neurological or muscle disease, and
- had written informed consent from their parent/guardian to participate.

Ethical approval was provided by the Monash University Ethics Committee. Participation was dependent on the availability of the ultrasonographer, and the willingness of the parent/guardian to make their child available at the times offered, so this represents a convenience sample.

Anterolateral thigh measurements were made at the junction of the upper third and lower middle thirds of the muscle mass with the ultrasound probe applied at 45° to the vertical, at right angles to the skin's plane and parallel to the long axis of the leg, with the child gently restrained with his or her pelvis flat on the examination couch (Figure 1).

Subcutaneous tissue (SCT) and muscle layer (ML) thickness were measured using a high resolution real time ultrasonograph (ACUSON X 14) with a 4 cm footprint, and 7 MHz linear transducer. The transducer was applied lightly to the skin to avoid tissue compression. Measurements were made on both thighs with data being pooled for analysis due to lack of significant difference between thigh measurements.

**Table 1. Demographic characteristics of subjects**

<table>
<thead>
<tr>
<th>Age of children (months)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>10/4</td>
<td>6/7</td>
<td>7/11</td>
<td>8/4</td>
</tr>
<tr>
<td>Mean weight (kg)</td>
<td>5.3</td>
<td>7.1</td>
<td>8.3</td>
<td>11.0</td>
</tr>
<tr>
<td>Standard deviation (kg)</td>
<td>0.7</td>
<td>0.9</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Range (kg)</td>
<td>4.4-6.6</td>
<td>5.7-8.8</td>
<td>6.6-10.5</td>
<td>9.3-15.3</td>
</tr>
<tr>
<td>Mean weight percentile*</td>
<td>58</td>
<td>74</td>
<td>73</td>
<td>60</td>
</tr>
<tr>
<td>Range (%)</td>
<td>10 to &gt;97</td>
<td>40 to &gt;97</td>
<td>10 to &gt;97</td>
<td>10 to &gt;97</td>
</tr>
</tbody>
</table>

* compared to Australian children (Personal Health Record, "Blue Book", NSW Health Department).

**Table 2. Subcutaneous and muscle layer thickness of anterolateral thigh in children aged 2–18 months old**

<table>
<thead>
<tr>
<th>Age of children (months)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous tissue (SCT) Mean (mm)</td>
<td>8.6</td>
<td>9.4</td>
<td>10.2</td>
<td>8.1</td>
</tr>
<tr>
<td>Standard deviation (mm)</td>
<td>3.0</td>
<td>2.0</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6-15.1</td>
<td>6.5-13.5</td>
<td>6.7-13.5</td>
<td>5.2-14.4</td>
</tr>
<tr>
<td>Muscle layer (ML) Mean (mm)</td>
<td>10.5</td>
<td>12.2</td>
<td>14.8</td>
<td>16.5</td>
</tr>
<tr>
<td>Standard deviation (mm)</td>
<td>2.4</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.2-14.3</td>
<td>9.6-15.3</td>
<td>10.1-17.1</td>
<td>9.5-22.5</td>
</tr>
</tbody>
</table>

**Results**

Fifty-seven patients were recruited for the study (Table 1). The morphometric characteristics of the children in the study placed them above the 50th weight percentile for normal Australian children. No child had a subcutaneous layer thickness greater than 15.1 mm (Table 2). Mean muscle layer thickness showed a progressive increase with age. There was no significant increase in muscle layer thickness in children aged 18 months (who might be expected to be walking) compared with younger children.

**Discussion**

We conclude that, because children greater than the 50th weight percentile had at four months and 18 months, maximal subcutaneous tissue thickness (mean plus added three standard deviations to accommodate large children) of
the thigh of 15.6 mm; and 13.2 mm, respectively:

- a 16 mm long needle would penetrate muscle in these children if entered at 90° to the skin's plane with the WHO technique, which produces compression of the subcutaneous layer, because the skin is stretched taut between the index finger and thumb.

- a 25 mm long needle — but not a 16 mm long one — would be needed with the NH&MRC or American techniques in these children as the subcutaneous tissue track length at four months would be 15.6 x \( \sqrt{2} \) = 22.0 mm and at 18 months 13.2 x \( \sqrt{2} \) = 18.7 mm due to the needle being angled at 45° to the skin's plane.

The disparity of our results to the other two studies may relate to differences in weight. The children in the Belgian study had a significantly lower body weight (10–50th weight percentile range) than the children in our study. No weight percentiles were given in the Hick et al study. Different positioning of the ultrasound probe on the leg was offered as a possible explanation of the discordance of the results in the other two studies.

The method of assessing suitable needle length (based on maximal subcutaneous tissue thickness derived from the mean subcutaneous tissue thickness plus three standard deviations), might result in overestimates if the compression of the leg occurs, as with the WHO technique. Our data shows 16 mm long needles will penetrate the vastus lateralis muscle with the WHO injection technique, as will 25 mm long needles even angled at 45° to the skin's plane with the NH&MRC and American injection techniques.

Acknowledgments
Thanks to Dale King for performing the ultrasounds, and the staff of the Manning Valley Medical Imaging for reporting them.

References

Implications of this study for the GP

- The 16 mm (25 gauge) orange hub needle should be suitable for intramuscular vaccination of infants and toddlers using the WHO injection technique.
- The 25 mm (23 gauge) blue hub needle should be suitable for intramuscular vaccination of infants and toddlers using the NH&MRC and American techniques.

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The World Health Organisation technique causes significant compression of the subcutaneous layer because the skin is stretched flat between the index finger and thumb.

Support for the use of 25mm compared with 16mm long needles for intramuscular vaccination of infants and toddlers has been drawn from studies\textsuperscript{79,170,171} with whole cell pertussis containing vaccines.

In a small study (n=119) involving 4 month old infants, Diggle and Deeks\textsuperscript{170} reported that parents recorded a significantly lower rate of injection site reaction with a 25mm compared with a 16mm long needle. This result was replicated in a larger study\textsuperscript{79} (n = 693) of infants aged 2, 3 and 4 months by these authors. The latter study reported no difference in injection site reactions for 25 gauge compared with 23 gauge needles and needle length and gauge did not affect the immune responses of any vaccines.

Ipp et al\textsuperscript{171} also reported a greater parent observed injection site reaction rate with 16mm compared with 25mm long needles. They also reported that children vaccinated in the thigh had significantly decreased movement compared with those vaccinated in the deltoid muscle and that two thirds of the former limped for 24 to 48 hours.

However, by contrast Scheifele et al\textsuperscript{172}, using a more defined methodology to retrieve adverse reaction data from parents in a similar study with a whole cell pertussis containing vaccine in children of the same age as those in the Ipp et al study\textsuperscript{171}, reported that limp was seldom reported by parents whose children received vaccine in
the thigh. A number of explanations\textsuperscript{172} were offered to explain this significant
discrepancy, differences between vaccines (DTP-PRP/D and DTP-IPV), the parents’
ability to accurately assess local reactions and how the post vaccination assessment
was conducted.

Girard\textsuperscript{173} in response to the study by Diggle et al\textsuperscript{79} commenting on the reliability of
drug safety data had difficulty reconciling the extremely high rates of adverse
reactions they reported following intramuscular injection of vaccines (combined
vaccine 61\% (local reaction), meningococcal vaccine up to 42\% (local reaction), with
2\% of subjects withdrawn due to severe local or general reactions) with the 0.4\%
injection site reaction rate reported by Zuckerman\textsuperscript{174} for intramuscular injection.
Certainly in studies with meningococcal sero-group C-CRM-197 conjugate vaccines
given to infants aged 2 and 4 months\textsuperscript{*}, parents reported widely varying rates of
injection site reactions (Table 6).

<table>
<thead>
<tr>
<th>Trialist/Country /Vaccine</th>
<th>No. Of Infants</th>
<th>Redness 2 mths</th>
<th>Redness 4 mths</th>
<th>Swelling 2 mths</th>
<th>Swelling 4 mths</th>
<th>Tenderness 2 mths</th>
<th>Tenderness 4 mths</th>
<th>Any Reaction 2 mths</th>
<th>Any Reaction 4 mths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diggle et al\textsuperscript{79} UK / Meningitec Menjugate</td>
<td>696</td>
<td>42.0%</td>
<td>39.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bramley et al\textsuperscript{175} UK / Meningitec</td>
<td>322</td>
<td>3.5%</td>
<td>7.4%</td>
<td>2.9%</td>
<td>5.3%</td>
<td>6.4%</td>
<td>4.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>English et al\textsuperscript{176} UK / Menjugate</td>
<td>122</td>
<td>40.7%</td>
<td>6.3%</td>
<td>13.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halperin et al\textsuperscript{177} Canada/ Menjugate</td>
<td>175</td>
<td>6.3%</td>
<td>15.0%</td>
<td>4.0%</td>
<td>14.5%</td>
<td>11.4%</td>
<td>7.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buttery et al\textsuperscript{178} UK / Meningitec</td>
<td>119</td>
<td>1.7%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>2.5%</td>
<td>1.7%</td>
<td>6.7%</td>
<td>8.4%</td>
</tr>
</tbody>
</table>

\*Not all meningococcal vaccine was given as a 2, 3, 4month schedule.
Having vaccination response assessed by a trained, treatment blinded observer, as was done in paediatric vaccine trials reported later in this thesis, may reduce the great variability of adverse reaction rates seen with meningococcal serotype C-CRM\textsubscript{197} vaccines and prevent the contradictory results seen with whole cell pertussis containing vaccines reported by Ipp et al\textsuperscript{171} and Scheifele et al\textsuperscript{172}.

The development of case definitions for adverse reactions and guidelines for collection, analysis and presentation of these data, as undertaken by the Brighton Collaboration\textsuperscript{179} will result in more consistent adverse reaction data, allowing for better inter-study comparisons.

A review of all published vaccination studies (64) in infants (≤ 12 months of age) where the length of needle used for intramuscular injection was reported, revealed that trialists used similar numbers of 16mm and 25mm long needles (29 and 25 respectively), see Table7.
Table 7: Needle Length/Gauge used for Intramuscular Injection of Infants in Published Clinical Trials by Country of Trials

<table>
<thead>
<tr>
<th>Needle Gauge/Length</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>25gauge/12mm</td>
<td>USA(^{180})</td>
</tr>
<tr>
<td>No gauge/16mm</td>
<td>Argentina(^{181}), Belgium/Germany(^{182}), Belgium/Turkey(^{183}), Germany(^{184}), Israel(^{185})</td>
</tr>
<tr>
<td>26gauge/16mm</td>
<td>Sweden(^{186}), Turkey(^{187}), USA(^{188})</td>
</tr>
<tr>
<td>25gauge/16mm</td>
<td>Australia(^{189,190}), Canada(^{191,192}), Chile(^{193}), France(^{194-196}), Israel(^{197}), Spain(^{198,199}) (2 months old), Turkey(^{200}), UK(^{201-203}), USA(^{204-207}), USA(^{208}) (2 months old)</td>
</tr>
<tr>
<td>23gauge/16mm</td>
<td>UK(^{209})</td>
</tr>
<tr>
<td>24gauge/19mm</td>
<td>Brazil(^{210})</td>
</tr>
<tr>
<td>23gauge/20mm</td>
<td>Indonesia(^{211})</td>
</tr>
<tr>
<td>25gauge/22mm</td>
<td>Canada(^{212-215})</td>
</tr>
<tr>
<td>No gauge/25mm</td>
<td>Spain(^{216}), Taiwan(^{217}), USA(^{218-221})</td>
</tr>
<tr>
<td>25gauge/25mm</td>
<td>Canada(^{222-224}), Taiwan(^{225}), Spain(^{226}), Sweden(^{227}), UK(^{228}), USA(^{229-231})</td>
</tr>
<tr>
<td>23gauge/25mm</td>
<td>Australia(^{232}), Israel(^{233}), New Zealand(^{234}), Turkey(^{235}), UK(^{236,237}), USA(^{238,239}), Vietnam(^{240})</td>
</tr>
<tr>
<td>23gauge/30mm</td>
<td>Brazil(^{98}), UK(^{241,242})</td>
</tr>
</tbody>
</table>

A similar division of opinion on length of needle used for infant vaccination was seen in a study of general practitioners\(^{243}\) (Cook & Murtagh, page 68) and general practice registrars\(^{244}\).
Paediatric vaccination practice in a Division of General Practice

Ian F Cook, John Murtagh

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John Murtagh, AM, MBBS, MD, BSc, BEd, FRACGP, is Adjunct Professor of General Practice, Monash University, Victoria.

BACKGROUND Currently the National Health and Medical Research Council (NH&MRC) recommend the use of a 23 gauge, 25 mm long needle inserted 45°-60° into the anterolateral thigh for paediatric vaccination.

AIM To assess the compliance of general practitioners (GPs) in a rural practice division with vaccination practice (site and needle size and gauge) prescribed for infants and toddlers by the NH&MRC.

METHOD In 1999, a questionnaire survey was sent by the divisional office to all 150 GPs in the Hunter Rural Division of General Practice. The questionnaire collected demographic data (age, gender, university of graduation, number of paediatric vaccines administered per week) and elicited responses about the site of vaccination and the size and gauge of needle to be used for children 2–18 months and 18 months and older.

RESULTS Completed questionnaires were available from 112 GPs (74.6% completion rate). There was a high level of compliance with the NH&MRC prescription of buttock vaccination with only 4.3% and 4.1% of responses to the question of vaccination site at 2–18 months and 18 months and older respectively nominating this site. The anterolateral thigh was the favoured site for vaccination in children 2–18 months old (77.5% of responses) with the deltoid being the favoured site in children 18 months and older (59.2% of responses). There was a very low level of compliance with the NH&MRC recommended standard needles (23 gauge, 75 mm long, blue hub needle) (2.3% of responses). The orange hub needle (25 gauge, 16 mm long needle) was most favoured (48.7% of responses) with additional strong support for the 25 gauge, 25 mm long needle (40.2% of responses).

CONCLUSION In the Hunter Rural Division of General Practice there was good compliance with the NH&MRC’s recommendations for site of vaccination, but not needle size and gauge to be used in infants and small children. Imprecise wording of these recommendations has created apparent uncertainty about the site of vaccination of children at 18 months of age.

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The first clear statement of paediatric vaccination practice (site, injection technique, needle size and gauge to be used) was made by Sauer in 1950 for plain and alum precipitated pertussis vaccines. It involved the deep subcutaneous or intramuscular injection into the lateral gluteal area with a 25 gauge, 1/2 or 3/8 inch long needle with each dose being terminated with 0.2 cu of air. The gluteal site has subsequently been proscribed for childhood vaccination on the basis of tangential safety and immunological data.

Vaccination site

Neurovascular complications
Gilles and Matson1 and Gilles and French2 have observed that gluteal injection has been associated with neurovascular injury in infants, especially sciatic nerve palsy. A comprehensive review3 of adverse reactions associated with gluteal injection showed that significant neurovascular complications (sciatic neuropathy and transverse myelitis) have been observed following injection with viscous/irritant solutions, particularly penicillin and paraldehyde. No such neurovascular complications have been reported after administration of any vaccine to this area. The only reported adverse reaction following gluteal vaccination being a painful buttock lump in a 26 year old man following injection of tetanus toxoid.4

Australian Family Physician Vol. 30, No. 12, December 2001 • 1185
Neurovascular complications have been reported following intramuscular injection into the two now preferred vaccination sites (deltoid and anterolateral thigh sites). Vascular injury to the leg has been reported with pencyclacin injection into the lateral thigh, with the publication by Talbert et al. providing the basis for the current USA, New Zealand and Australian recommendation for thigh vaccination technique. Radial nerve injury has been reported after deltoid injection, most often with pencyclacin. A single case of vaccine induced radial nerve injury has been reported, a 47 year old Chinese man given tetanus toxoid into the left arm. No neurological injury to the leg has been reported following injection of any vaccine.

Immunogenicity

Concern about the immunogenicity of paediatric vaccines administered to the gluteal area has arisen from adult studies with hepatitis B and rabies vaccines in which lower seroconversion and antibody titres were observed when these vaccines were given into the gluteal site compared with the deltoid region. The most recent transformation of vaccination practice followed the work of Hick et al. in defining the optimum needle length for diphtheria, tetanus, pertussis vaccination. In a small study of 24 children (13 male and 11 female), age 3.5–4.5 months of age, no growth percentiles given, the thickness of the subcutaneous tissue (SCT) was 14 mm ± 2.4 mm (males) and 13 mm ± 2.8 mm (females) with muscle layer (MC) thickness 18 mm ± 2.1 mm (males) and 15 mm ± 1.9 mm (females). These authors note that if a 58 inch (16 mm) long needle is inserted at an angle of 45° into the lateral thigh then 'the muscle layer would have been penetrated in only 21% of the 24 patients'.

NH&MRC recommendations

Currently, the National Health and Medical Research Council (NH&MRC) recommend the use of 25 gauge, 25 mm long needle inserted at 45–60° into the anterolateral thigh at the junction of the upper and middle thirds of the muscle for paediatric vaccination.

A pilot study, with a questionnaire designed to assess GP practice with respect to needle size and gauge used for paediatric vaccination and site of injection of infants and toddlers, had been distributed to GPs in the Hunter Urban and Tamworth Divisions of General Practice by two medical representatives during July 1997.

This numerically small study (64 GPs) had doubtful external validity due to obvious design defects including selection biases (small sample of practitioners in these divisions and participation on the basis of willingness to complete the questionnaire). It did highlight the good compliance with the prescription of buttok injection (≤4% of responses), the uncertainty of the vaccination site of the child 18 months of age and older and the poor compliance with the NH&MRC recommended 23 gauge 25 mm long needle (5.6% responses), most responses (66.7%) being for the 25 gauge 16 mm long needle.

Method

A questionnaire that had been previously validated as a research instrument for assessing GP practice with respect to site and needle gauge/length used for paediatric vaccination was sent to all GPs in the Hunter Rural Division of General Practice by the divisional office. Completed questionnaires were collected by the divisional immunisation nurse and sent for collation to the authors.

Demographic data collected on the respondents were:
- age
- gender
- year of graduation
- university of graduation and
- number of childhood vaccinations given or supervised each week.

Site of injection of 'triple antigen' (diphtheria-tetanus-pertussis) vaccine in children 2–18 months of age and 18 months or older was sought with one or more choices being available from:
- buttock
- anterolateral thigh
- medial thigh
- deltoid and
- other site.

Needle gauge/length used for childhood vaccination was sought with one choice being available from:
- 25 gauge, 16 mm long
- 25 gauge, 25 mm long
- 23 gauge, 25 mm long
- 23 gauge, 32 mm long and
- not sure.

Response to questions about site of injection in children 2–18 months and 18 months and older, and needle gauge/length were analysed by gender, age group (19–49 years and 50–79 years), Australian/overseas initial medical qualification and number of vaccinations given or supervised weekly (0–5 and 6–11) by chi-square analysis with Yates' correction.

Results

Completed questionnaire surveys were received for 112 respondents (150 GPs were identified in the division, 74.6% responder rate). The demographics of the respondents are shown in Table 1. The demographic of respondents was comparable to the national demographic for rural GPs in terms of women practitioners (study group 21.4%, national demographic 25–27.5%). The age distribution of the divisional respondents was different to the NSW workforce, with fewer younger and older practitioners (20–29 years age group, study group 3.9%, NSW workforce 4.8%; 60–69 years age group, study group 7.8%, NSW work-
Table 1. GP demographics

<table>
<thead>
<tr>
<th>Gender</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-29 years</td>
<td>4 (3.9%)</td>
<td>24</td>
</tr>
<tr>
<td>30-39 years</td>
<td>27 (26.2%)</td>
<td>50</td>
</tr>
<tr>
<td>40-49 years</td>
<td>42 (40.8%)</td>
<td>62</td>
</tr>
<tr>
<td>50-59 years</td>
<td>22 (21.4%)</td>
<td>70</td>
</tr>
<tr>
<td>60-69 years</td>
<td>5 (4.5%)</td>
<td>80</td>
</tr>
<tr>
<td>70-79 years</td>
<td>3 (2.9%)</td>
<td>90</td>
</tr>
<tr>
<td>Year of Graduation*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1940-1949</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>1950-1959</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>1969-1969</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>1970-1979</td>
<td>41</td>
<td>48</td>
</tr>
<tr>
<td>1980-1989</td>
<td>37</td>
<td>50</td>
</tr>
<tr>
<td>1990-1999</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Country of initial medical qualification**</td>
<td>Australia</td>
<td>Other country</td>
</tr>
<tr>
<td>93 (85.3%)</td>
<td>16 (14.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Number of paediatric vaccinations given or supervised weekly†

<table>
<thead>
<tr>
<th>0-2</th>
<th>3-5</th>
<th>6-8</th>
<th>9-11</th>
<th>&gt;11</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>36</td>
<td>18</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

† Nine no responses, NSW workforce age distributions 19-29 years (4.8%), 30-39 years (21.4%), 40-49 years (34.7%), 50-59 years (20.9%), 60-69 years (11.6%), 70-79 years (6.5%)  
* Two 'no' responses  
** Three 'no' responses, NSW workforce qualification distribution: Australia (72.7%), overseas (27.8%)  
† Seven 'no' responses

Table 2. Site of injection

<table>
<thead>
<tr>
<th>Site</th>
<th>2-18 months old*</th>
<th>18 months or older#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buttock</td>
<td>6 (4.3%)</td>
<td>6 (4.1%)</td>
</tr>
<tr>
<td>Anterolateral thigh</td>
<td>107 (77.5%)</td>
<td>53 (36.1%)</td>
</tr>
<tr>
<td>Medial thigh</td>
<td>2 (1.4%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>Deltoide</td>
<td>23 (16.7%)</td>
<td>87 (59.2%)</td>
</tr>
</tbody>
</table>

* All respondents gave at least one response with 26 giving more than one response  
# All respondents gave at least one response with 35 giving more than one response

Table 3. Needle length and gauge used for vaccination

<table>
<thead>
<tr>
<th>Gauge and Length</th>
<th>Respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 gauge, 16 mm</td>
<td>57 (48.7%)</td>
</tr>
<tr>
<td>25 gauge, 25 mm</td>
<td>47 (40.2%)</td>
</tr>
<tr>
<td>23 gauge, 25 mm</td>
<td>4 (3.4%)</td>
</tr>
<tr>
<td>23 gauge, 32 mm</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>Not sure</td>
<td>8 (6.8%)</td>
</tr>
</tbody>
</table>

All respondents gave at least one response, seven gave more than one response

GPs in the Hunter Rural Division of General Practice.

As with the pilot study, good compliance was observed for the prescription of buttock injection and confused was evident with respect to the vaccination site for children 18 months and older with some 36.1% of respondents being for anterolateral thigh injection (Table 2). Poor compliance with the NHMRC recommended 23 gauge 25 mm long needle was also observed (Table 3). These responses were not related to gender, age grouping (19-49 years and 50-79 years), country of initial medical qualification (Australia or overseas) or number of paediatric vaccinations administered per week (0-5 or 6-11).

Ambiguity of recommendations

The site of vaccination of children at 18 months of age is not clearly defined in the NHMRC publications with the sixth edition of the Australian Immunisation Handbook stating that: 'the anterolateral thigh is the preferred site for vaccination in infants and chil-
The anterolateral thigh is the favoured site for vaccination in children up to and including 12 months of age and there was good compliance with this site in children 2-18 months of age in the study. Available data suggest that children who are walking should not be vaccinated in the anterolateral thigh and a clear statement of this fact in the NHMRC guidelines would have avoided the confusion observed with respect to site of vaccination in children 18 months or older in the study.

Intramuscular injection results in a reduced rate of local adverse reactions with aluminum adsorbed vaccine (whole cell and acellular pertussis vaccines [DTPw and DTPa], hepatitis A and hepatitis B vaccines) and increased immunogenicity of hepatitis B and rabies vaccines.

The needle size required to ensure efficient intramuscular vaccine delivery depends on the technique employed. The technique endorsed by the NHMRC in the sixth edition of The Australian Immunisation Handbook apparently requires a 23 gauge, 25 mm needle. The World Health Organisation technique of holding the skin taut between thumb and finger and directing the needle at 90° to the skin allows intramuscular injection with a 25 gauge 16 mm needle, that most favoured in the study.

Acknowledgment
Thanks are extended to the Hunter Rural Division of General Practice for circulating and collecting the study questionnaire and to each GP who took the time to complete the questionnaire.

References
18. Zanotto J. Medical labour force annual survey. NSW: Workforce Planning Unit, NSW Health Department, 1996.
Implications of this study for the GP

- Good compliance was observed with the NH&MRC guidelines for proscription of paediatric buttock vaccination.
- The lack of clarity of these guidelines on the issue of the site of vaccination of children aged 18 months and older may have produced GP confusion.
- The lack of support for the 'blue hub' needle (23 gauge, 25 mm) recommended by the NH&MRC may reflect safety concerns with this bigger needle or the use of injection techniques for which the study preferred 'orange hub' needle (25 gauge, 16 mm).

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In a study\textsuperscript{243} of general practitioners (n = 150) in a Division of General Practice, 48.7\% recommended a 16mm long needle and 43.6\% a 25mm long needle for vaccination of infants aged 2-18 months of age.

In a study\textsuperscript{244} of general practice registrars (n = 123), 57\% nominated the Australian Immunisation Handbook recommended 23 gauge 25mm needle for the vaccination of an infant 2 months of age.

Finally, although guidelines from a number of countries recommend a 25mm long needle for the intramuscular vaccination of infants (Australia, New Zealand, Spain, Sri Lanka, UK), other needle lengths are recommended:

- Canada and USA – 22-25mm long needle
- France\textsuperscript{i}

2. Ventrogluteal Area

A single study\textsuperscript{245} (Cook & Murtagh, page 76) has been made of the tissue composition of the ventrogluteal site in infants and toddlers.

\textsuperscript{i} Helen Tixier, Group Product Manager – Pediatric vaccines, International Medical and Marketing Department, Pasteur Merieux Connaught recommends the use of 5/8\" (16mm), 25 gauge needles for proper intramuscular delivery.
Cook IF, Murtagh J. Ventrogluteal area – a suitable site for intramuscular vaccination of infants and toddlers. Vaccine 2006; 24: 2403-8.
Ventralgulteal area—a suitable site for intramuscular vaccination of infants and toddlers

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Abstract

Buttock vaccination has lower reactogenicity and similar immunogenicity to the two other recommended paediatric vaccination sites (deltoid and anterolateral thigh). Safety concerns about buttock injection derived from injections with neurotoxic agents, like penicillin but not vaccines, have become entrenched. However, the ventrolateral area is considered safe for intramuscular injection.

This study outlines the development of the ventrolateral area as a suitable site for intramuscular vaccination of infants and toddlers. Measurement was made in 642 children, aged 2–18 months and age-specific templates were prepared. These were used in an ultrasonographic study of 57 children aged 2–18 months to determine the tissue composition of the ventrolateral area compared with the recommended anterolateral thigh vaccination site.

The ventrolateral area was found to be clearly defined by the template and suitable for intramuscular injection. Subsequent vaccination studies with the area showed that it was:

• safe and had a high level of parental acceptability;
• associated with lower reactogenicity with pertussis vaccines and equivalent immunogenicity with hepatitis B vaccines compared with the anterolateral thigh vaccination site.

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Keywords: Ventrolateral; Children; Measurement; Vaccination

1. Introduction

The established use of adjuvanted diphtheria, tetanus and pertussis (DTP) vaccines in the 1940s resulted in one of the pioneers in this area publishing [1] a detailed outline of a vaccination practice (site, technique, needle gauge and size) which minimized severe local adverse effects. The practice involved deep injection into the lateral gluteal muscle mass with a 25 gauge 1/2 or 5/8 in. needle, terminating each dose with 0.1 cc of air.

This practice has been abandoned in favour of injection into the anterolateral thigh on the basis of reactogenicity, immunogenicity and safety considerations.

2. Reactogenicity

Subcutaneous vaccination in children is associated with significantly greater rates [2] of local adverse reactions than intramuscular injection and as it is contended [3] that “infants gluteal area consists mostly of fat”, buttock vaccination would be expected to be associated with higher levels of local adverse effects than other sites.
However, a review of injection site comparative studies involving pertussis vaccines, the most reactogenic of all paediatric vaccines [4] does not support this thesis. All five [5–9] published prospective studies comparing buttock with thigh injection, including one [9] using the ventrogluteal area defined by Von Hochstetter, reported significantly less local pain/swelling with buttock compared with thigh injection. A similar trend was seen in the four [6,8,10,11] published prospective studies comparing buttock with arm injection, in that local adverse reactions were significantly less common with buttock compared with arm injection in three studies [8,10,11] and not different in the other study [6].

3. Immunogenicity

Predicted poorer immune response with buttock vaccination [12] compared with other sites has been drawn from adult studies with hepatitis B vaccine [13]. This position is challenged by data from a site comparative study and non-site comparative studies with vaccines with accepted serological correlates of protection, in which the same vaccine and vaccination regimen were used.

In an open randomized study [14] comparing the immunogenicity of the hepatitis B vaccine (Engerix B 10 μg) given at 0, 1 and 6 months into the ventrogluteal area or the anterolateral thigh, comparable rates of ‘good’ antibody response (anti-HBs ≥ 100 mIU/ml) were observed, ventrogluteal (96.0%) and anterolateral thigh (93.2%). Studies with the Engerix B 10 μg in infants, given as a three dose regimen over 6 months gave comparable levels of seroprotection (anti-HBs ≥ 10 mIU/ml) for different injection sites; buttock [15] (100%), anterolateral thigh [16] (99.4%) and deltoid [17] (100%).

Similarly, with the Haemophilus influenzae type b conjugate vaccine, where the polysaccharide protein conjugate (PRP) was conjugated with tetanus toxoid (PRP-T) when this vaccine was given alone or in combination with acellular pertussis vaccine (DPTa). When given alone as a three dose regimen (2, 4 and 6 months), seroprotection (anti-PRP > 0.15 μg/ml) rates of 99, 100 and 100% were observed in three studies [18–20] with thigh injection and 100% in a study with buttock injection [21]. Combination with acellular pertussis vaccine in a three dose regimen (3, 4 and 5 months) gave seroprotection rates of 95.4–96.9% for thigh injection [22], 94.4% for buttock injection [23] and 98.6% for deltoid injection [23].

4. Safety

Neurovascular complications have been reported with intramuscular injection into all the accepted paediatric vaccination sites, buttock [24], deltoid [25] and anterolateral thigh [26] with neurotoxic agents like penicillin.

The observations by Gilles and French [24] have been pivotal in generating the shift from buttock to thigh vaccination. Buttock vaccination, despite having a better reactogenicity and comparable immunogenicity profile with the other paediatric vaccination sites, cannot be regarded as best practice if its use has the potential to cause sciatic nerve injury. However, the ventrogluteal area defined by the anterior superior iliac spine (anteriorly), greater trochanter (inferiorly) and iliac crest (superiorly) has been recognized by Von Hochstetter [27] and others [28] as being a ‘safe’ area for gluteal injection.

The aims of this study are to:

1. Measure the dimensions of the ventrogluteal area in children aged 2, 4, 6 and 18 months and prepare age-specific templates.
2. Measure the tissue composition (subcutaneous layer and muscle thickness) of the ventrogluteal area using these templates.
3. Compare the tissue composition of the ventrogluteal area with the anterolateral thigh vaccination site to determine the needle length required for intramuscular injection at both sites.

5. Methods

Measurement of the ventrogluteal area was made during routine examination of children 2, 4, 6 and 18 months, prior to vaccination, where morphometric data, clinical assessment and progress notes were recorded in their personal records. The ventrogluteal area measurements were made with the child’s pelvis at 90 degrees to the examination couch. The measurement from the greater trochanter to the anterior superior iliac spine was designated as measurement “a”, while the measurement from the greater trochanter to the iliac crest was designated measurement “b”.

Using aggregated data for each age group, the mean length of “a” and “b” was calculated and templates were constructed of single core Bell wire (0.35 mm diameter), Click Industries Australia, attachable at the three points of the ventrogluteal site with squares of self sticking “Double Mounts” Permatick 12 mm.

The ultrasound study was part of a project approved by the Monash University, Australia Standing Committee on Ethics in Research using Humans (Project No. 96/509).

A convenience sample of infants and toddlers 2, 4, 6 and 18 months of age due for primary vaccination and 18 month booster vaccination were recruited for the ultrasound study if they:

1. were in apparent good health;
2. had no history of neuromuscular disease or pelvic/back or thigh injury;
3. had written, informed consent from a parent/guardian for participation in the study.

Ventralgluteal area measurements were made with the child’s pelvis at 90° to the examination couch, with the age
appropriate template and with the transducer lightly applied at 90° to the skin's surface along the line bisecting the apex angle of the template. Anterolateral thigh measurements were made at the junction of the upper and middle third of the muscle mass with the ultrasound probe applied at 45° to the vertical, at right angles to the skin’s plane and parallel to the long axis of the leg with the child gently restrained with his or her pelvis flat on the examination couch. Subcutaneous layer and muscle layer thickness of both areas were measured using a high resolution, real time Ultrasonograph (ACUSON XP4) with a 4 cm foot print, 7 MHz linear transducer.

6. Results

Six hundred and forty-two patients aged 2–18 months had the dimensions of their ventrogluteal area measured (2 months 74M, 87F; 4 months 75M, 84F; 6 months 79M, 92F and 18 months 80M, 68F). The demographics of these patients are presented in Table 1 with ventrogluteal measurements for right side only being presented in Table 2 as there was no difference between left and right sides.

The "a" measurement (greater trochanter to anterior superior iliac spine) and "b" measurement (greater trochanter to iliac crest) increased by 5 mm across the age range 2, 4 and 6 months for both males and females without any gender difference. The "b" measurement was consistently 5 mm greater than the "a" measurement. At 18 months of age the "a" and "b" measurements still differed by 5 mm but the male measurements were greater than the female measurements by 2.5 mm.

Fifty-seven patients were recruited for the ultrasound study (2 months 10M, 4F; 4 months 6M, 7F; 6 months 7M, 11F and 18 months 8M, 4F). The demographics of these patients are shown in Table 3. The subcutaneous layer thickness (SCL) and muscle layer thickness (ML) for the right ventrogluteal area and right anterolateral thigh vaccination site are presented in Table 4, as there was no difference between the left and right side measurements.

The mean subcutaneous tissue layer thicknesses were similar for the ventrogluteal area and anterolateral thigh vaccination site at all ages. Ventrogluteal area; 2 months 9.3 mm, 4 months 9.2 mm, 6 months 9.3 mm and 18 months 8.4 mm. Anterolateral thigh vaccination site; 2 months 8.6 mm, 4 months 9.4 mm, 6 months 10.2 mm and 18 months 8.1 mm. Similarly, the mean muscle layer thicknesses were comparable for the ventrogluteal area and anterolateral thigh vaccination site at all ages. Ventrogluteal area; 2 months 11.9 mm, 4 months 12.8 mm, 6 months 13.3 mm and 18 months 17.0 mm. Anterolateral thigh vaccination site; 2 months 10.5 mm, 4 months 12.2 mm, 6 months 14.8 mm and 18 months 16.5 mm.

7. Discussion

Hughes [29] has recommended "the ventrogluteal muscles as the preferred location for all intramuscular injections" in children. The lateral muscles of the thigh were second choice as they "had observed some local post injection discomfort,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographics of patients in ventrogluteal area measurement study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>74</td>
</tr>
<tr>
<td>Weight (kg) ± S.D.</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>Length (cm) ± S.D.</td>
<td>57.7 ± 2.7</td>
</tr>
<tr>
<td>Head circumference (cm) ± S.D.</td>
<td>39.9 ± 1.2</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>87</td>
</tr>
<tr>
<td>Weight (kg) ± S.D.</td>
<td>5.30 ± 0.7</td>
</tr>
<tr>
<td>Length (cm) ± S.D.</td>
<td>55.9 ± 4.6</td>
</tr>
<tr>
<td>Head circumference (cm) ± S.D.</td>
<td>38.9 ± 3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mean measurements of ventrogluteal area* by age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Lead &quot;a&quot; (greater trochanter to anterior superior iliac spine) (cm) ± S.D.</td>
<td>4.03 ± 0.18</td>
</tr>
<tr>
<td>Lead &quot;b&quot; (greater trochanter to iliac crest) (cm) ± S.D.</td>
<td>4.52 ± 0.22</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Lead &quot;a&quot; (greater trochanter to anterior superior iliac spine) (cm) ± S.D.</td>
<td>3.97 ± 0.14</td>
</tr>
<tr>
<td>Lead &quot;b&quot; (greater trochanter to iliac crest) (cm) ± S.D.</td>
<td>4.47 ± 0.14</td>
</tr>
</tbody>
</table>

* Right and left side ventrogluteal measurements were similar.
Table 3
Demographics of patients in ultrasonographic study of ventrogluteal area

<table>
<thead>
<tr>
<th></th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males/Females</strong></td>
<td>10 M/4 F</td>
<td>6 M/7 F</td>
<td>7 M/11 F</td>
<td>8 M/4 F</td>
</tr>
<tr>
<td><strong>Mean weight</strong></td>
<td>5.3 ± 8.7</td>
<td>7.1 ± 0.9</td>
<td>8.3 ± 1.2</td>
<td>11.0 ± 1.8</td>
</tr>
<tr>
<td><strong>(kg) ± S.D.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Range (kg)</strong></td>
<td>4.4-6.0</td>
<td>5.7-8.8</td>
<td>6.6-10.5</td>
<td>9.3-15.3</td>
</tr>
</tbody>
</table>

Fig. 1. Ventrogluteal area showing vaccination template.

Fig. 2. Ventrogluteal area vaccination.

anxiety on the part of the patient from observing the procedure and the lack of stable landmarks to outline the site”.

Our studies support this preference. The ventrogluteal area was clearly defined for injection by using the disposable template (Fig. 1) which are quickly and easily applied and caused no local reaction. Four age-specific templates were prepared for use with the most reactogenic vaccines, pertussis vaccines, at 2, 4, 6 and 18 months. Their dimensions increased by 5 mm across the ages 2, 4 and 6 months without gender difference which was apparent at 18 months with males having a 2.5 mm longer armed template. As booster pertussis vaccination at 18 months is no longer advocated [30], only three age-specific templates are now required.

Anxiety caused by the child observing the vaccination procedure is minimized with ventrogluteal area injection as the child’s attention can be easily diverted during vaccination (Fig. 2). The potential for misplaced injection in this area as a result of sudden or unexpected movement is minimized compared with the anterolateral thigh vaccination site, as the child is comfortably restrained during the procedure.

The ventrogluteal area was found, by ultrasonographic study, to be adequately muscled and did not ‘const mainly of fat’ as has been previously asserted [3]. The subcutaneous layer of the ventrogluteal area and the anterolateral thigh vaccination site (the junction of the upper and middle third of the vastus lateralis muscle) were found to be comparable, with the mean thickness ranging from 8.1 to 10.2 mm for the anterolateral thigh vaccination site and 8.4 to 9.3 mm for the ventrogluteal area. Maximal thickness observed was 12.6 mm for ventrogluteal area and 15.1 mm for anterolateral thigh vaccination site.

It was concluded that a 16 mm long needle would penetrate muscle at both sites in all children using the WHO technique [31] where the needle is entered at 90° to the skin's plane, as the procedure produces considerable compression of the subcutaneous layer, as the skin is stretched taut between the index finger and thumb.

Table 4
Mean subcutaneous and muscle layer thickness of ventrogluteal area and anterolateral thigh vaccination site* by age

<table>
<thead>
<tr>
<th>Age</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ventral gluteal area subcutaneous tissue thickness (SCT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>9.3 ± 1.9</td>
<td>9.2 ± 1.8</td>
<td>9.3 ± 1.9</td>
<td>8.4 ± 2.1</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>4.6-12.6</td>
<td>6.7-12.6</td>
<td>5.0-11.8</td>
<td>5.3-11.9</td>
</tr>
<tr>
<td><strong>Muscle layer thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>11.9 ± 3.1</td>
<td>12.8 ± 1.8</td>
<td>13.3 ± 2.7</td>
<td>17.0 ± 3.8</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.5-17.8</td>
<td>9.7-16.6</td>
<td>8.4-18.6</td>
<td>12.0-26.1</td>
</tr>
<tr>
<td><strong>Anterolateral thigh vaccination site subcutaneous tissue thickness (SCT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>8.6 ± 3.0</td>
<td>9.4 ± 2.0</td>
<td>10.2 ± 2.1</td>
<td>8.1 ± 1.7</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.0-15.1</td>
<td>6.5-13.5</td>
<td>6.7-13.5</td>
<td>5.2-14.4</td>
</tr>
<tr>
<td><strong>Muscle layer thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>10.5 ± 2.4</td>
<td>12.2 ± 2.0</td>
<td>14.8 ± 2.0</td>
<td>16.5 ± 4.6</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.2-14.2</td>
<td>9.6-15.3</td>
<td>10.1-17.1</td>
<td>9.5-22.5</td>
</tr>
</tbody>
</table>

* Right and left ventrogluteal area and anterolateral thigh vaccination site measurements were similar.
In this study, it was shown that the ventrogluteal area is clearly defined by disposable templates and is a suitable area for intramuscular injection.

Subsequent vaccination studies using the ventrogluteal area have shown that it is:

1. safe and had a high level of parental acceptability [9];
2. associated with lower reactogenicity with pertussis vaccines [9] and equivalent immunogenicity with hepatitis B vaccine compared with the anterolateral thigh vaccination site [14].

Buttck vaccination is officially recommended in Japan and Colombia and widely used in Belgium, Denmark, Germany, Italy and Slovenia.

Defining the ventrogluteal area with a template will make this a safer practice. It should be noted, however, that the description of buttck vaccination in the USA, Australia, Canada, Finland, France, Ghana, Israel, New Zealand, Netherlands, South Africa and the UK is derived from buttck injections with known neurotoxic agents, like penicillin [24] especially in viscous formulation [32], not vaccines. The validity of this description for vaccination has been canvassed by Marcuse and MacDonald [33] who concluded that the presumed hazard associated with administering vaccines in the upper outer quadrant of the buttck is poorly established and may be based on well-documented sciatic nerve injury from the administration antibiotics and antiserum at this site.

References


This study was conducted concomitantly with the study of anterolateral thigh injection site reported by Cook IF, Murtagh J.\textsuperscript{169} (page 61). Tissue composition of this area was comparable with the anterolateral thigh (Table 8).

**Table 8: Mean Subcutaneous and Muscle Layer Thickness of Ventrogluteal Area and Anterolateral Thigh Vaccination Site by Age.**

<table>
<thead>
<tr>
<th>Age</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ventralgluteal area subcutaneous tissue thickness (SCT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>9.3 ± 1.9</td>
<td>9.2 ± 1.8</td>
<td>9.3 ± 1.9</td>
<td>8.4 ± 2.1</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>4.6 – 12.6</td>
<td>6.7 – 12.6</td>
<td>5.0 – 11.8</td>
<td>5.3 – 11.9</td>
</tr>
<tr>
<td><strong>Muscle layer thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>11.9 ± 3.1</td>
<td>12.8 ± 1.8</td>
<td>13.3 ± 2.7</td>
<td>17.0 ± 3.8</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.5 - 17.8</td>
<td>9.7 – 16.6</td>
<td>8.4 – 18.6</td>
<td>12.0 – 26.1</td>
</tr>
<tr>
<td><strong>Anterolateral thigh vaccination site subcutaneous tissue thickness (SCT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>8.6 ± 3.0</td>
<td>9.4 ± 2.0</td>
<td>10.2 ± 2.1</td>
<td>8.1 ± 1.7</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.0 – 15.1</td>
<td>6.5 – 13.5</td>
<td>6.7 – 13.5</td>
<td>5.2 – 14.4</td>
</tr>
<tr>
<td><strong>Muscle layer thickness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm) ± S.D.</td>
<td>10.5 ± 2.4</td>
<td>12.2 ± 2.0</td>
<td>14.8 ± 2.0</td>
<td>16.5 ± 4.6</td>
</tr>
<tr>
<td>Range (mm)</td>
<td>6.2 – 14.3</td>
<td>9.6 – 15.3</td>
<td>10.1 – 17.1</td>
<td>9.5 – 22.5</td>
</tr>
</tbody>
</table>

**Evidence Based Summary and Answer**

Using the modified GRADE recommendations classifying the quality of evidence (page 10), there is moderate (Grade B) evidence for the use of 16mm and 25mm long needles for the intramuscular vaccination of infants.

Ultrasound studies show that a 16mm long needle is suitable for intramuscular vaccination of infants in developing countries and undernourished infants in
developed countries. Contradictory data, from small studies support the use of both 25mm and 16mm long needles in well nourished children from developed countries.

Comparative studies with needles of these lengths which might have demonstrated which was the preferred length of needles for intramuscular injection had the same methodological weakness, parental reporting of adverse reaction data.

Consequently, using the GRADE classification of recommendations (page 10) only a weak (Grade 2) recommendation only can be made for the needle length to be used for intramuscular vaccination of infants.

Adults

1. Deltoid area.

Two ultrasonographic studies have been made on the tissue composition of the deltoid muscle injection site in adults, (Table 9).

<table>
<thead>
<tr>
<th>Author</th>
<th>Adults – Age/Country/ Number</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poland et al165</td>
<td>18-59 years/ USA/ 126F, 94M</td>
<td>Intramuscular IM injection would be achieved by:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 16mm needle in females &lt; 60kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 25mm needles in all males and females 60-90kg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 38mm needle in women &gt; 90kg</td>
</tr>
<tr>
<td>Cook et al246</td>
<td>Australia/ 122 Female</td>
<td>25mm long needles would routinely penetrate muscle in males all BMI and</td>
</tr>
<tr>
<td></td>
<td>Age range 65.4 to 89.4 years, Mean age 71.3 years. 134 Males Age range 65.2 to 93.1 years Mean age 72.1 years.</td>
<td>females BMI &lt; 35, with a 32mm long needle being required in females with BMI ≥ 35</td>
</tr>
</tbody>
</table>
Definition of needle length required for intramuscular deltoid injection in elderly adults: an ultrasonographic study

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a Discipline of General Practice, School of Medical Practice and Population Health, University of Newcastle, Callaghan, NSW 2308, Australia
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Abstract

An ultrasound study in elderly patients (≥65 years) showed that body mass index (BMI) was strongly correlated with deltoid subcutaneous layer thickness in males (r = 0.69 dominant arm, 0.71 non-dominant arm) and females (r = 0.79 both arms). Females with the same BMI as males had significantly thicker subcutaneous layers (p = 0.0001) and thinner muscle layers (p = 0.0003).

Minimal needle length required for deltoid intramuscular injection where the needle was entered at 90° to the long axis of the humerus was defined by BMI group. In all BMI males and females, BMI <35, intramuscular injection could be achieved with a 25 mm long needle, whilst in females BMI ≥35, a 32 mm long needle is required.

These data will be used in studies to resolve the clinical equipoise regarding the optimal route of administration (intramuscular versus subcutaneous) of vaccines (e.g. influenza and pneumococcal vaccines), which are provided through public health programs for the elderly.

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Keywords: Elderly; Ultrasound; BMI; Vaccination

1. Introduction

Recommended routes of administration are given for vaccines used in a number of countries [1–3]. This is despite there being a total lack of route comparative studies in adults and only two such studies in children, in which injection techniques were clearly defined. Carlsson et al. [4] studied tetanus toxoid conjugated Haemophilus influenzae type b vaccine in infants aged 3, 5 and 12 months and Mark et al. [5] studied booster diphtheria/tetanus toxoid in 10 year old children. Both observed greater reactogenicity but no difference in immunogenicity when vaccines were administered subcutaneously compared with intramuscularly.

Validation of the optimal route for administration of vaccines in terms of immunogenicity and reactogenicity requires the clear definition of practices, which ensure injection into the intradermal, subcutaneous and intramuscular layers and comparative studies using these practices. For intramuscular injection, the length of needle required for injection will depend on the thickness of the subcutaneous tissue layer and this would be expected to be directly related to the adiposity of the patient. Poland et al. [6] observed a correlation between body weight and needle length required for intramuscular injection in health care workers aged 18–59 years.

In adults, the most commonly used adiposity index is the body mass index (BMI), which is the ratio of body weight (kg) to height (m²). BMI is simple to obtain, provides a direct measure of underweight and overweight and is a surrogate marker for fat mass with a better overall performance than any other weight-stature index [7].

We conducted an ultrasound study to assess the correlation between BMI in elderly patients (≥65 years) and

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doi:10.1016/j.vaccine.2005.06.098]
subcutaneous layer thickness at the deltoid injection site, the mid-point between the acromion and the deltoid tubercle. The aim of our study was to use these data to relate the BMI index groups (<20, 20–24.9, 25–29.9, 30–34.9, ≥35) to the minimal needle length required for intramuscular injection in this population.

The establishment of these relationships is pivotal to studies resolving the clinical equipoise regarding the route of administration (intramuscular versus subcutaneous) of vaccines (e.g., influenza and pneumococcal vaccines) provided in many countries through publicly funded programs for the elderly.

2. Methods

2.1. Study design and population

The study was approved by the University of Newcastle, Australia, Human Research, Ethics Committee (Approval no. H 386–0702).

A convenience sample of ambulant patients 65 years and older was recruited to the study. The sample ensured good cell size for both males and females of the five body mass index groups; low normal and underweight (BMI <20), normal weight (BMI 20–24.9), overweight (BMI 25–29.9), obese (BMI 30–34.9) and gross obesity (BMI ≥35).

Inclusion criteria were:
- No history of upper limb injury or neurological or muscular disease of the upper limb.
- Given written informed consent.

Prior to ultrasound measurements, the patients identified their dominant arm and were weighed, measured and had their body mass index recalculated.

Ultrasound measurements were made by a single sonographer, expert in high resolution, real-time ultrasonography using a linear-array, high frequency probe of 6–12 MHz (Toshiba Powervision 6000). Ultrasound measurements were made at the site of deltoid injection (midpoint between acromion and deltoid tubercle), which was marked in ink on both arms. The patient’s arms were scanned in the coronal plane with the probe held at 90° to the skin’s surface with the arm held in a relaxed position against the chest, replicating the arm position during vaccination. Compression of the deltoid tissue was avoided by light probe pressure. A “stand off pad” was used to ensure good near field resolution of the skin line to improve the accuracy of measurements. Subcutaneous layer (SCL) and muscle layer (ML) thickness were measured at each deltoid injection site.

2.2. Analysis and statistics

Body mass index was calculated as weight (kg) divided by height (m²), and grouped into 5 groups graded 1–5 representing BMI <20, 20–24.9, 25–29.9, 30–34.9, ≥35. There were four outcome variables (dominant and non-dominant arms, SCL and ML thickness).

For each variable, a two-sample t test or Wilcoxon rank sum test, depending on the normality of the data, was performed to test the overall difference between males and females. Correlation analysis was performed to assess the strength of the relationship between weight and BMI as continuous variables and BMI groups and the weight groups defined for females (<60, 60–90, and >90kg) by Poland et al. [5] as categorical variables with each of the four outcome variables.

Spearman’s non-parametric correlation analysis methods were used because both weight and BMI and outcome variables were not normally distributed.

3. Results

Two hundred and fifty-six patients were recruited for the study; 122 females (mean age 71.3 years, range 65.4–90.4 years), 134 males (mean age 72.1 years, range 65.2–93.1 years). BMI group distributions for these patients are shown in Table 1.

There was no statistically significant difference between males and females in BMI (p = 0.9202, Wilcoxon rank sum test) although there was a statistically significant difference

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subcutaneous (SCL) and muscle (ML) layer thickness (mm) by Gender and BMI Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>BMI group</td>
</tr>
<tr>
<td>Female</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>20–24.9</td>
</tr>
<tr>
<td></td>
<td>25–29.9</td>
</tr>
<tr>
<td></td>
<td>30–34.9</td>
</tr>
<tr>
<td></td>
<td>≥35</td>
</tr>
<tr>
<td>Male</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>20–24.9</td>
</tr>
<tr>
<td></td>
<td>25–29.9</td>
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<tr>
<td></td>
<td>30–34.9</td>
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<tr>
<td></td>
<td>≥35</td>
</tr>
</tbody>
</table>

a Subcutaneous layer thickness (SCL), p < 0.0001. Wilcoxon rank sum test, female compared with male.

b Muscle thickness (ML), p = 0.0000. Two sample t test, female compared with male.
between males and females in weight overall with males being heavier than females (p < 0.0001, Wilcoxon rank sum test).

There was no apparent difference between dominant and non-dominant arm so for brevity, data on the dominant arm SCL and ML thickness only are presented.

Females had significantly greater SCL thickness than males (p < 0.0001, Wilcoxon rank sum test) and significantly less ML thickness than males (p = 0.0003, two sample t test), Table 1.

Spearman's correlations were similar for weight and BMI as continuous variables with SCL thickness (males weight r = 0.66 both arms, males BMI r = 0.69 dominant arm and r = 0.71 non-dominant arm. Females weight r = 0.80 both arms, females BMI r = 0.79 both arms).

The correlation between BMI as a continuous variable and SCL thickness was not dissimilar to the correlation between BMI as a categorical variable and SCL thickness (male BMI r = 0.69 dominant arm, r = 0.71 non-dominant arm, male BMI groups r = 0.68 dominant arm, r = 0.69 non-dominant arm; female BMI r = 0.79 both arms, female BMI groups r = 0.77 dominant arm, r = 0.78 non-dominant arm).

The correlation for females of BMI groups with SCL thickness (r = 0.77 dominant arm, r = 0.78 non-dominant arm) was stronger than the correlation between the weight groups defined by Poland et al. and SCL thickness (r = 0.69 both arms).

4. Discussion

Body mass index (BMI) has been shown to be an accurate predictor of percentage body fat in elderly adults [8], fat mass in an elderly [9] and metabolic abnormalities in elderly men [10].

In our study, BMI and SCL groups were well correlated with SCL thickness at the deltoid injection site in the elderly (male BMI r, dominant arm, r = 0.71 non-dominant arm, male BMI groups r = 0.68 dominant arm, r = 0.69 non-dominant arm; female BMI r = 0.79 both arms, female BMI groups 0.77 dominant arm, r = 0.78 non-dominant arm).

Deltoid intramuscular injection has been defined [6] as penetration of the muscle by 5 mm or more. On this basis we concluded that a 25 mm long needle would routinely penetrate muscle in all BMI males and in females BMI <35, with a 32 mm long needle being required in females with BMI ≥35, using the World Health Organization injection technique [11] where the needle is injection at 90° to the long axis of the humerus.

These results are similar to those reported [6] in health care workers aged 18–59 years where it was suggested that “true deltoid intramuscular injection [11] would be achieved by the use of 25 mm long needles in all body weight men, with needle length being related to body weight groups in females, 60 kg (16 mm), 60–90 kg (25 mm) and >90 kg (38 mm).

In our study the correlation, for females, of BMI groups with SCL thickness (r = 0.77 dominant arm, r = 0.78 non-dominant arm) was stronger than the correlation between these weight groups and SCL thickness (r = 0.69 both arms).

Use of a 16 mm long needle would not have achieved intramuscular injection in significantly more elderly patients than the younger health care workers previously studied [6] 76.2% (82/112) female, 48.4% (61/126) males, compared with 41.8% (56/134) females, 17% (16/94) males. This difference reflects the documented [9] greater percentage body fat in the elderly compared with younger adults, as both studies had comparable percentage of patients >60 kg (our study 69.9%, Polish study 76.5%).

Review of studies with vaccines used in publicly funded vaccinations campaigns, split, trivalent influenza vaccines and 23 valent pneumococcal vaccines retrieved 83 and 137 studies, respectively.

Four studies [12–15] with influenza vaccine and two studies [16,17] with the pneumococcal vaccine indicated the length of needle used to achieve intramuscular injection. This varied from 16 to 25.4 mm, with the two studies by Jackson et al. [12–16] using 16 mm long needles.

Our study shows that intramuscular injection would not have been achieved in a significant proportion of the vaccine recipients in the studies, by Jackson et al.

If route of administration is a determinant of the reactogenicity and immunogenicity of influenza and pneumococcal vaccines in elderly adults, then the BMI group related needle lengths defined in our study will be pivotal in ensuring intramuscular injection.

Acknowledgements

We wish to thank all the patients who so willingly participated in this study and the Mayne Health Group who facilitated the ultrasonography of these patients. We also wish to thank the Hunter Rural Division of General Practice for providing part funding for the statistical analysis conducted by Datapharm Australia.

References


There are limited data on the influence of needle length on injection site reaction in adults using this site of injection. In the study by Beeching et al\textsuperscript{247} involving two different hepatitis A/typhoid vaccines, Viatim\textsuperscript{®} and Hepatyrix\textsuperscript{®}, injected intramuscularly into the deltoid muscle with 16mm and 25mm long needles respectively, injection site reactions were significantly greater with the shorter needle.

2. Gluteal Area

Determination of needle length required for intramuscular injection has been studied by ultrasonographic and CT scanning techniques and in an autopsy study, (Table 10).

### Table 10: An Ultrasonographic Study, Computerised Tomographic Studies and An Autopsy Study of the Gluteal Area in Adults

<table>
<thead>
<tr>
<th>Author</th>
<th>Imaging</th>
<th>Adult/Age</th>
<th>Results</th>
<th>Mean SCT</th>
</tr>
</thead>
</table>
| Zaybak et al\textsuperscript{248} | Turkey  | 59 female 60 males mean age 38.6yrs range 21-69yrs Recruited from a university hospital. No inclusion or exclusion criteria given. | Dorsogluteal site
Overweight n=20 female 50.2±0.92 male 27.6±1.12
Obese n=66 female 51.7±1.25 male 30.7±1.03
Extremely obese n = 33 female 53.2±1.38 male 31.7±1.19
Ventreogluteal site
Overweight n= 20 female 50.4±1.37 male 31.2±0.88
Obese n=66 female 54.3±1.09 male 37.0±1.05
Extremely obese n=33 female 59.2±1.76 male 41.3±1.04 Concluded that 38mm needle gave IM injection in 2% females, 63% males at dorsogluteal site, 3% females, 43% males at ventrogluteal site in healthy adults with BMI > 24.9. |
<p>| Cockshott et al\textsuperscript{249} | CT Scan | Convenience sample having CT Scan (no inclusion or exclusion criteria). Study involved both inpatients and | Dorsogluteal site concluded if needle hub projects 5mm from the skin, intramuscular injection with 38mm long needle would occur in less than 5% of females and 15% of |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Sample Description</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burbridge²⁵⁰</td>
<td>CT Scan</td>
<td>298 adults booked for CT Scan included those having both emergency and routine scans and inpatients and outpatient, females and males. Only exclusion criteria &lt; 16 years of age. Age 19 – 89 years, mean age females 58.4 years, mean age males 55.9 years. 150 males 148 females.</td>
<td>Dorsogluetal site: Females SCT 8.3 to 81.5mm (mean 33.2 ± 17.54) Males SCT 1.1 to 80.4 (mean 23.1 ± 9.13). Using a 37mm long needle, allowing for 6mm penetration of gluteal muscle, intramuscular injection would not have occurred in: Females 81/148 (54.76%) Males 21/150 (14%)</td>
</tr>
<tr>
<td>Nisbet²⁵¹</td>
<td>CT Scan</td>
<td>100 consecutive patients, 39 male, 61 female age 22 – 65 years mean 47.8 ± 11.3 No inclusion or exclusion criteria.</td>
<td>Ventrogluteal site Females 10/61 (16%) adipose tissue &gt;35mm Males 2/39 (5%) adipose tissue &gt;35mm Dorsogluetal site: Females 35/61 (51%) &gt; 35mm Males 8/39 (21%) &gt; 35mm</td>
</tr>
<tr>
<td>Chan et al²⁵²</td>
<td>CT Scan</td>
<td>50 adults, inpatients (25 males , 25 females) aged 21 to 87 years, mean age 53.3 years. Inclusion criteria: Patients requiring a CT Scan abdomen/pelvis and who were also receiving IM medication. Exclusion criteria: Patients who were neutropenic, thrombocytopenic and/or coagulopathic. Injection was made with a 23 gauge 30mm long needle using the WHO technique into the dorsogluetal area. 1ml of air was given with the prescribed medication.</td>
<td>Dorsogluetal site: All males fat thickness &lt;35mm and most women &gt;30mm. Injection with a 30mm long needle gave intramuscular injection in 56% males and 8% females due to the fact that the needle was not always completely inserted to the hub during injection.</td>
</tr>
</tbody>
</table>
### Evidence Based Summary and Answer

Using the modified GRADE recommendations classifying the quality of evidence and recommendations (page 10), there is high (Grade A) evidence and a strong (Grade 1) recommendation can be made that a 25mm long needle will give intramuscular injection in the deltoid muscle in all adult males and females less than 90 kg weight or BMI less than 35.
There is also high (Grade A) evidence that the standard 38mm long needle will not routinely give intramuscular injection in the dorso-gluteal site in women, especially if obese, even if fully inserted to the hub. A strong (Grade 1) recommendation can be made against the use of a 38mm long needle for routine dorso-gluteal intramuscular injection in this group.
CHAPTER 4:
WHAT IS THE BEST TECHNIQUE FOR INTRAMUSCULAR INJECTION OF VACCINES?

Background Evidence

Following comprehensive review of the literature, Warren\textsuperscript{256} concluded that the best practice for intramuscular injection is a 90° angle of needle insertion to the skin’s surface. Certainly, vaccination guidelines from Canada\textsuperscript{40}, Mexico\textsuperscript{46,47}, Saskatchewan\textsuperscript{42}, Spain\textsuperscript{51}, UK\textsuperscript{53,57} and USA\textsuperscript{62,65}, Colorado\textsuperscript{67}, Los Angeles County\textsuperscript{74}, recommend this angle of needle insertion with an angle of 80° - 90° being recommended in guidelines from Ireland\textsuperscript{44} and Minnesota\textsuperscript{70}. However in Australia\textsuperscript{30-32}, New Zealand\textsuperscript{48-50} and Sri Lanka\textsuperscript{52}, angled needle insertion is recommended (45° – 60°).

The practice of angled needle insertion for vaccination resulted from a recommendation\textsuperscript{257} made following the development of femoral artery thrombosis necessitating toes amputation in a 3 month old child injected with procaine penicillin using a 22mm long needle introduced into the lateral thigh. Subsequently, a technique for angled intramuscular injection in the thigh of infants was formulated by Bergeson et al\textsuperscript{258} and promulgated in the USA by the Advisory Committee on Immunization Practice\textsuperscript{64} (ACIP) and the American Academy of Pediatrics\textsuperscript{59} (1994) for intramuscular vaccination of infants and toddlers.
A technique henceforth called the USA technique involved the clinician bunching the muscle of the lateral thigh with the free hand and inserting the needle inferiorly to the long axis of the leg and at an angle to reach the muscle whilst avoiding nearby neurovascular structures and bone. In its original formulation, the needle was inserted at an angle of 45° to the long axis of the leg and posteriorly at a 45° angle to the table top with the patient supine.

In the technique developed in Australia and promoted in New Zealand and Sri Lanka, the needle is inserted at the junction of the upper and middle thirds of the lateral thigh at an angle of 45° - 60° to the long axis of the thigh and directed downwards towards the patella. Expert opinion from Australia, in support of this technique, asserts that “inserting the needle at a 60° angle results in less tissue resistance as the needle penetrates the muscle”.

A technique promoted by the World Health Organization in 1984 involved stretching the skin flat between the index finger and thumb with the needle inserted at 90° to the long axis of the thigh.

The question of whether angled needle entry offered clinical advantage over entry at 90° to the long axis of the thigh was addressed in a study by Cook and Murtagh (page 95).
Optimal technique for intramuscular injection of infants and toddlers: a randomised trial

Ian F Cook and John Murtagh

ABSTRACT

Objective: To compare the rates of adverse reactions and parental approval ratings for three different techniques for anterolateral thigh vaccination in children aged 2, 4, 6 and 18 months.

Design: Randomised, observer-blind trial.

Participants: 375 children who received pertussis-containing vaccines in a regional New South Wales town between 29 May 2001 and 30 June 2002.

Interventions: Children were randomised to receive intramuscular injection with acellular pertussis-containing and Haemophilus influenzae type b vaccines with one of three recognised injection techniques (Australian, World Health Organization or United States).

Main outcome measures: Local adverse reactions (bruising and redness/swelling), systemic adverse reactions (irritability, perceived fever, persistent crying/screaming, drooling, vomiting/poor feeding) and parental acceptance were assessed 24 hours after injection.

Results: 361 children (96%) were evaluated 24 hours after vaccination. The WHO technique resulted in significantly fewer children, than with the other two techniques, with the systemic adverse reaction variable "irritability" (P = 0.0039). There was a significant difference between the technique groups overall for the local adverse reaction "bruising" with acellular pertussis containing vaccines (P = 0.0119), due to a lower reaction rate in the WHO group compared with the US group (P = 0.0356).

Conclusion: The WHO technique appears to be the optimal technique for anterolateral thigh injection in children — it ensures that the injection is intramuscular, results in fewer adverse reactions, and is the easiest technique to perform as it does not require angling of the needle to the long axis of the femur.

MJA 2005; 183: 60–63

METHODS

Vaccination

Vaccinations were given according to the Australian Childhood Immunisation Schedule, with diphtheria-tetanus-acellular pertussis-hepatitis B vaccine (Infanrix®) and (Ciba-Geigy®) (children aged 2, 4 and 6 months) and diphtheria-tetanus-acellular pertussis vaccine (Infanrix) (GlaxoSmithKline) (children aged 18 months). Haemophilus influenzae type b conjugate vaccine (Pedvax (Merck Sharp & Dohme) was given concurrently with the same technique as the acellular pertussis vaccine into the contralateral thigh of children aged 2 and 4 months. Oral polio (Sabin) vaccine (two drops) was given to children aged 2, 4 and 6 months.
Vaccination technique
The three intramuscular injection techniques used were:

**Australian** — the needle was inserted at the junction of the upper and middle thirds of the vastus lateralis with the needle angled at 45° to the skin and pointing down towards the knee.11

**World Health Organization** — the needle was inserted into the anterolateral thigh at an angle of 90° to the long axis of the femur with the skin compressed between the index finger and the thumb.11

**United States** — the needle was inserted into the upper lateral quadrant of the thigh at an angle of 45° to the long axis of the femur and posteriorly at an angle of 45° to the table top, with the baby supine. The thigh muscle was braced at the injection site to increase muscle mass and to minimize the chance of striking bone.11

**Needle gauge and length**
The injections using the Australian and US techniques were made with 23 gauge, 25 mm long needles, and the WHO technique injections were made with a 25 gauge, 16 mm long needle.
The shorter needle was used with the WHO technique, as previous studies have shown that a 25 mm long needle would make bony contact if fully inserted.8,9

**Participants**
Children aged 2, 4, 6 and 18 months attending a solo practice in Taree, New South Wales, from 29 May 2001 to 30 June 2002, were included in the study if:
- they were in apparent good health at the time of vaccination; and
- written informed consent was obtained from the child’s parent(s)/guardian.

**Study design**
This was a single centre, randomised, observer-blind trial. Randomisation to the three techniques was on a 1:1:1 basis using computer-generated random numbers. At recruitment, the practice nurse recorded the child’s details and arranged a follow-up vaccination review appointment at the practice the next day. She gave the vaccinator (H.C.) a sealed envelope containing the random number. The vaccinator prepared the vaccines accordingly, and placed them in a covered kidney dish. The vaccines were injected with the vaccinator’s body obscuring as much as possible the parents’ view of the procedure and without being seen by the practice nurse. The child’s details (age, technique) were recorded in a manifest available only to the vaccinator.

**Postinjection assessment**
The study outcome measures — local adverse reactions (bruising and redness/swelling), systemic adverse reactions (irritability, perceived fever, persistent crying/screaming, drowsiness, vomiting/poor feeding) and parental acceptance — were assessed 24 hours after injection by the practice nurse, as in other pertussis vaccine reactogenicity studies.11,12,13

A previously validated research instrument14 was used to objectively assess local reactions — bruising and redness/swelling — on a visual analogue scale (VAS), where 0 = no reaction and 5 = whole leg involved; and subjectively (parent) reported irritability, perceived fever, persistent crying/screaming, drowsiness, vomiting/poor feeding on a VAS, where 0 = no reaction and 5 = very severe reaction. Likewise, parental rating of vaccination outcome was scored 0 = very happy and 5 = very unhappy.

**Ethical approval**
Our study was approved by the Monash University Standing Committee on Ethics in Research using Humans.

---

**1 Profile for clinical trial of three vaccination techniques (Australian, World Health Organization and United States)**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Enrolled n = 375</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australian</strong></td>
<td>n = 125</td>
</tr>
<tr>
<td><strong>WHO</strong></td>
<td>n = 125</td>
</tr>
<tr>
<td><strong>US</strong></td>
<td>n = 125</td>
</tr>
</tbody>
</table>

*Allocated at random to:

Not evaluable
n = 4

Evaluable n = 121
Age group* No. (%) 2 months 24 (19.0%) 4 months 36 (28.8%) 6 months 26 (21.5%) 16 months 36 (29.0%)

Evaluable n = 120
Age group* No. (%) 2 months 25 (20.8%) 4 months 27 (22.5%) 6 months 32 (26.8%) 16 months 33 (27.5%)

Evaluable n = 120
Age group* No. (%) 2 months 23 (18.3%) 4 months 32 (26.7%) 6 months 35 (29.2%) 16 months 31 (25.8%)

* Age ranges were as follows: 2 months, range 8–10 weeks; 4 months, range 15–18 weeks; 6 months, range 22–28 weeks; 18 months, range 68–78 weeks.

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**Statistical analysis**
The sample size was based on the anticipated proportion of patients with redness/swelling after vaccination. It was expected that 37.6% of patients would experience redness/swelling with the WHO technique,11 compared with 20% with each of the US and Australian techniques. With an α level of 5%, adjusted for multiple comparisons and power of 80%, it was calculated that 360 participants would be required, 120 in each of the three groups. Thus, 125 participants per group were recruited as, from our previous study,13 a "drop out" rate of less than 4% was expected.

Statistical analysis was performed using SAS version 8.2 (SAS Institute, Cary, NC, USA), based on a modified intention-to-treat population (i.e., excluding children who did not return for follow-up). Multivariate logistic regression analysis was performed for each reaction parameter, with the outcome classified into no reaction (VAS score 0) and any reaction (VAS scores 1–5). Age and technique groups were included as factors in the model, together with their interaction. When there were small sample sizes in any one cell (e.g., bruising), a Fisher's Exact Test was also used. A non-parametric Kruskal-Wallis test was used to compare parental acceptability, treated as a continuous vari-
be a scale of 0–5 (very happy to very unhappy) across the three technique groups.

RESULTS

A total of 375 consecutive children were enrolled in the study, all satisfying the inclusion criteria at presentation. The reason for unavailability of all 14 children who could not be evaluated 24 hours after vaccination was parental non-compliance rather than adverse effects. This was ascertained in follow-up contact by the practice nurse. The study groups were similar in terms of numbers per age group for the three techniques (Box 1).

No statistically significant interaction was found between technique and age in any of the logistic regression models, so this interaction factor was removed from the analysis model. Where the age factor was not significant, it was also removed from the analysis model. Age was significant in the analysis of redness/swelling, bruising, irritability and persistent crying, and was kept in these models. In Box 2, the P values for these parameters reflect the significance of the test after adjusting for age.

The WHO technique resulted in significantly fewer patients with the systemic adverse reaction variable “irritability” (30.0%) compared with the Australian technique (49.2%) (P = 0.0030). There was a significant difference between the groups overall for bruising with acellular pertussis vaccine (P = 0.0418) after controlling for age. The difference was due to a 6.7% bruising for the US technique compared with 0.8% for the WHO technique (P = 0.0356), but this was not statistically significant at the α = 0.025 level after adjusting for multiple comparisons (Box 2).

Most parents recorded parental acceptability scores of zero (“very happy”), the highest score being “3” recorded by one parent in the Australian technique group, and there were scores of “2” in the other two groups. The mean (95% CI) parental acceptability scores were 0.34 (0.23–0.45) for the Australian technique, 0.30 (0.20–0.38) for the WHO technique and 0.41 (0.30–0.52) for the US technique. There were no statistically significant differences in parental acceptability between the three techniques (P = 0.2927).

DISCUSSION

Ascertaining the best technique for paediatric vaccination is mandated by increasing concern about vaccine-induced adverse

THE CONSULTATION — RESEARCH

2 Local and systemic adverse reactions by technique (Australian, World Health Organization and United States)

<table>
<thead>
<tr>
<th>Reaction/vaccination/technique</th>
<th>Any reaction (score 1–5)</th>
<th>Overall P</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Redness/swelling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infantrix/Infanrix/HepB</td>
<td>0.0752*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>31/121 (25.6%)</td>
<td>0.655 (0.370–1.158)</td>
<td>0.1458</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>40/120 (33.3%)</td>
<td>1.253 (0.730–2.150)</td>
<td>0.4133</td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>46/120 (38.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedvax</td>
<td>0.1365*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>10/120 (8.3%)</td>
<td>0.800 (0.322–1.989)</td>
<td>0.6306</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>11/120 (9.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>18/120 (15.0%)</td>
<td>1.773 (0.784–4.015)</td>
<td>0.1667</td>
<td></td>
</tr>
<tr>
<td><strong>Bruiising</strong></td>
<td>0.0418↑</td>
<td>0.6219↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infantrix/Infanrix/HepB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>3/121 (2.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>1/120 (0.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>8/120 (6.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedvax</td>
<td>0.3296↑</td>
<td>0.922↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>1/120 (0.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>2/120 (1.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Systemic reactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infantrix/Infanrix/HepB/Pedvax</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>55/121 (45.5%)</td>
<td>1.969 (1.147–3.379)</td>
<td>0.0139</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>36/120 (30.0%)</td>
<td>2.437 (1.417–4.192)</td>
<td>0.0013</td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>59/120 (49.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived fever</td>
<td>0.3103</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>4/121 (3.3%)</td>
<td>0.422 (0.126–1.409)</td>
<td>0.1605</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>9/120 (7.5%)</td>
<td>1.000 (0.363–2.613)</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>9/120 (7.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent crying/screaming</td>
<td>0.5162↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>10/120 (8.3%)</td>
<td>0.638 (0.270–1.505)</td>
<td>0.3042</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>15/120 (12.5%)</td>
<td>1.000 (0.452–2.212)</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>14/120 (11.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0.9428</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>6/121 (5.0%)</td>
<td>1.200 (0.356–4.043)</td>
<td>0.7866</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>5/120 (4.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>6/120 (5.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting/poor feeding</td>
<td>0.8118</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>13/121 (10.7%)</td>
<td>1.083 (0.473–2.481)</td>
<td>0.8498</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>12/120 (10.0%)</td>
<td>1.000 (0.452–2.212)</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>10/120 (8.3%)</td>
<td>0.818 (0.339–1.973)</td>
<td>0.6551</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted P value, after controlling for age in the model.
† Calculated using Fisher’s Exact test; all others derived from logistic regression analysis.
‡ Not statistically significant at α = 0.025 (adjusting for multiple comparisons).
THE CONSULTATION — RESEARCH

reactions in the context of a decreasing incidence of vaccine-preventable diseases. This concern is highlighted in a recent Australian study of children with incomplete vaccination, in which it was found that 70% of those who disagreed with or were concerned about immunization had concerns about adverse reactions.

In our study, the WHO technique was associated with fewer children having the adverse reaction "intensity" than with the other techniques and, for the acellular pertussis vaccine, less bruising compared with the US technique.

This outcome does not support the hypothesis (underpinning the US and Australian techniques) that angling of the needle to the long axis of the femur with intramuscular injection in children gives less adverse reactions.

The conclusion on the needle length aspect of vaccination practice by Diggie and Deeks was weakened by the use of needles with different gauges (23 gauge/25 mm vs 25 gauge/16 mm). Similarly, the inability to control needle length and gauge as potential variables in our study may have weakened the conclusions drawn regarding differences between the different techniques of injection. Our choice of needle was dictated by the recommendation of 23 gauge/25 mm long needles with the US technique and previous ultrasound studies showing that 25 mm long needles would routinely make bony contact if used with the WHO technique. Elimination of needle gauge as a possible confounding variable was not possible, as 23 gauge/16 mm long needles are not commercially available.

The WHO technique best fulfills the requirements of an optimal injection technique in children — it ensures that the injection is intramuscular, results in fewer adverse reactions, and is the easiest technique to perform, as it does not require angling of the needle to the long axis of the femur.

ACKNOWLEDGEMENTS

We wish to thank all the parents/guardians who so willingly allowed their children to participate in this study. We would also like to thank the Hunter Division of General Practice for providing funding for the statistical analysis conducted by Distapharm, Australia.

COMPETING INTERESTS

None identified.

REFERENCES


(Received 30 Mar 2005, accepted 9 Jun 2005)
In this study, 375 consecutive healthy children aged 2, 4, 6 and 18 months were randomised to receive pertussis containing vaccines with either the USA, Australian or WHO injection technique into the anterolateral thigh.

The WHO technique, angle insertion at 90° to the long axis of the thigh is the optimal technique as it:

1. results in fewer adverse reactions –

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>Comparison</th>
<th>NNH (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>USA vs WHO technique</td>
<td>18 (9 to 78)</td>
</tr>
<tr>
<td>Irritability</td>
<td>Aust vs WHO technique</td>
<td>7 (4 to 34)</td>
</tr>
<tr>
<td></td>
<td>USA vs WHO technique</td>
<td>5 (3 to 13)</td>
</tr>
</tbody>
</table>

2. is easier to perform as it does not require angling of the needle to the long axis of the femur.

**Evidence Based Answer**

Using the modified GRADE recommendations to classify the quality of evidence (page 10) there is high (Grade A) evidence that vaccines should be administered by needle insertion at 90° to the long axis of the thigh in infants and toddlers. A strong (Grade 1) recommendation can be made regarding this technique of needle insertion in this age group in the anterolateral thigh.
CHAPTER 5:
WHAT SITES ARE SUITABLE FOR INTRAMUSCULAR INJECTION OF VACCINES?

Background Evidence

There is a general consensus that vaccines administered by the intramuscular route should be given into the deltoid muscle in adults and older children – guidelines Australia, Canada, Ireland, Mexico, New Zealand, Spain, UK and USA. However, for infants, Schechter et al260 have recently concluded that “additional research comparing the various sites with respect to pain, local reaction, antigenicity and parental acceptability is necessary.” “Only then can recommendations that are supported by adequate evidence be generated”.

Dorsogluteal Site

The first detailed outline of a vaccination practice (site, technique, needle gauge and size) in infants which minimised severe local adverse reactions was made by Sauer261, a pioneer of pertussis vaccination programs in the 1940s. The practice involved deep injection into the lateral gluteal muscle mass with a 25 gauge ½ or 5/8 inch needle, terminating each dose with 0.1cc of air. The injection site used with this practice was the dorsogluteal site (Figure 1.) which is defined anatomically as the upper, outer quadrant of the buttock.
Figure 1. The dorsogluteal site

Its use for vaccination has been abandoned in favour of injection into the anterolateral thigh on the basis of reactogenicity (side effects), immunogenicity (immune response) and safety considerations in guidelines from Australia, Brazil, Canada, British Columbia, Saskatchewan, India, Malaysia, Mexico, New Zealand, Spain, Sri Lanka, UK, USA, Colorado, Georgia, New Mexico, Allegheny, and WHO.

However, other sites for intramuscular injection of vaccines have been used or are recommended in infants < 12 months of age.

1. Clinical Trial Data

A search was made of Medline, Embase, Scopus, Biological Abstracts, Science Citation Index, Cochrane Database of Systematic Reviews (CDSR), NHS Database of Abstracts of Review of Effects (DARE); using the following search terms and their word variants “vaccines”, “vaccination”, immunization”, and “infants”, “children”,...
“babies”, “less 12 months of age”. Bibliographies of all relevant articles were searched for additional studies. Studies with live virus vaccines like; measles, mumps, rubella or combination vaccine of these antigens, varicella and yellow fever were excluded as these vaccines are traditionally given by subcutaneous injection. 834 studies were found, from early studies with pertussis vaccines in the 1930s to December 2007 with monovalent and multi-antigen vaccines. In 408 studies (49%) anatomic site of vaccine injection was reported, a similar rate (59%) of reporting of injection site was observed by Poierer262 in a limited study of vaccine trials. Sites other than the thigh were reported in 111 studies85,87-89,98,102,104,107-111,180,183,189,190,196,223,239,263-355 from 32 countries; Australia, Belgium, Brazil, Canada, China, Chile, Czechoslovakia, Finland, France, Gambia, Germany, Holland, Hong Kong, Iceland, India, Iran, Italy, Japan, Mexico, New Zealand, Niger, Pakistan, Papua New Guinea, Philippines, Taiwan, Thailand, Tonga, Turkey, UK, USA, Vietnam and Yugoslavia.

Intramuscular injection was made in the upper arm/deltoid site in 76 studies89,104,107-111,183,200,239,263-308,311,312,315-330,341,351, gluteal site in 42 studies85,87-89,98,102,104,108,180,183,189,190,196,223,283,305,306,309,310,313,314,331-350,352,354,355 and subscapular area in 1 study353. Sites other than the anterolateral thigh were used predominantly in Belgium (5/6 studies deltoid), China (3/3 studies deltoid), Chile (5/7 studies deltoid), Finland (8/18 gluteal and 2/18 deltoid), Hong Kong (4/4 gluteal), Japan (2/2 upper arm-triceps/deltoid).
2. Expert Opinion

Sites other than the thigh are recommended for intramuscular injection of vaccines by public health officials and paediatricians in other countries. Gluteal area is recommended in

- Belgium\(^a\)
- Croatia\(^b\)
- France\(^c\)
- Italy\(^d\)
- In Bulgaria\(^e\), the subscapular area is recommended.

\(\text{ii}\)

**Basis of Recommendation Against Use of the Dorsogluteal Site**

The recommendations against buttock vaccination in infants were discussed in the Introduction (page 8).

To review, these are:

**Injection Site Reactions (Reactogenicity)**

It was contended\(^{258}\) that the “infants gluteal area consists mostly of fat” and as a consequence buttock vaccination would be expected to be associated with greater rates of injection site reactions than other paediatric vaccination sites (anterolateral thigh and deltoid area). The converse was found in site comparative studies, with

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\(^{a}\) Personal communication from Dr A Bochner, Campus Kinderziekenhuis, Antwerpen advised by facsimile on 16/6/97 that “practically most of the nurses and doctors are vaccinating in the buttock, without any neurological complications to my knowledge”.

\(^{b}\) Personal communication from Dr Renata Mazuran, Institute of Immunology, Zagreb advised by facsimile on 21/8/97 that “DTP vaccine is given as intra-gluteal injection by the nurses and physicians in primary health care”.

\(^{c}\) Personal communication from Dr Emmanuel Grimprel, Paris, advised by facsimile 19/8/97 that “buttock for IM injection of vaccines”.

\(^{d}\) Personal communication from Professor Alberto E Tozzi, Roma advised by facsimile on 28/6/97 that “most immunizations are given in the buttock”.

\(^{e}\) Personal communication from Associate Professor Nina Gatcheva and Professor Bogdan Petrunov advised by facsimile on 9/10/97 that “DTP – at 2,3 and 4 months of age for primary immunization, DTP – deep subcutaneous at the lower angle of the shoulder-blade”.

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buttock vaccination having lower rates of injection site reaction than these other sites. 

**Immune Response (Immunogenicity)**

On the basis of the well documented impaired response of hepatitis B vaccine given into the buttock compared with the deltoid area in adults, it has been suggested that the immune response of vaccines given into this site in infants will be impaired compared to other sites. This has not been supported by a route comparative study (buttock vs anterolateral thigh) with a hepatitis B vaccine and studies with *Haemophilus influenzae* type b conjugate vaccine in non-comparative studies using different sites of injection where similar rates of seroprotection were observed.

**Safety**

Buttock injection of vaccines has been proscribed on the basis of the well documented neurovascular complications observed with opaque, viscous penicillin preparations (benzathine and procaine penicillin) and other drugs, chloroquine, paraldehyde, chlorpromazine, diazepam, sulphadoxine-pyrimethene with children at apparently greater risk than adults.

Weir and Fearnnow have suggested that neurovascular injury following benzathine penicillin injection is due to microvascular occlusion by penicillin crystals which can result in sciatic nerve injury, peripheral arterial damage and transverse myelitis. This mechanism has also been suggested as the cause of Nicolau syndrome (embolic cutis medicamentosa) condition characterized by severe injection site pain and variable degrees of skin necrosis which has been observed with intramuscular
injection of penicillin\textsuperscript{371-373}. Animal studies \textsuperscript{374-376} have shown that nerve injury can also result from direct nerve trauma which is dependent on the dose and agent used with penicillin, chlorpromazine and diazepam being the most toxic agents.

Vascular injury has also been observed following intramuscular injection into the thigh. Limb ischaemia has been observed\textsuperscript{377-380} with injection of long acting penicillin preparations into the thigh probably due to vascular occlusion. Femoral nerve injury with subsequent vastus lateralis atrophy and neuralgia paresthetica have been observed with intramuscular injection of meperidine\textsuperscript{381} and anti-emetic preparations\textsuperscript{382} respectively.

Neurovascular injury has also been reported after intramuscular injection into the deltoid muscle. Radial and axillary nerve lesions have been reported following injections of penicillin\textsuperscript{383}, an anti-emetic\textsuperscript{384}, premedication\textsuperscript{385}, quinine\textsuperscript{386}, tetanus antisera\textsuperscript{387} and vaccines\textsuperscript{388,389} into the deltoid muscle. Meirelles and Filho reporting\textsuperscript{388} a case of axillary nerve injury following deltoid injection with influenza, diphtheria and tetanus vaccines (the vaccine given into the affected arm not stated), consider that the deltoid muscle should not be considered the site of choice for the injection of vaccines due to the anatomical variation in site of the anterior branch of the axillary nerve. Ling and Loong reported\textsuperscript{389} a case of radial nerve palsy following intramuscular injection of tetanus toxoid into the left deltoid of a 47 year old Chinese male.

Vascular complication following intramuscular injection into the deltoid is rare with only a single case being reported\textsuperscript{390}, with a large area (15cm x 8cm) of skin necrosis
developing over the right deltoid in a 7 year old female following injection of
Novalgin® 4 days previously. It was considered that necrosis was due to prolonged
spasm of the perforator vessel due to perivascular injection or thrombosis secondary
to intravascular injection.

No neurovascular injury has been reported following intramuscular injection of
vaccines in the gluteal area. Marcuse and MacDonald, after canvassing reports of
significant adverse reactions with gluteal vaccination, concluded82 “that the hazard of
administering vaccines in the upper outer quadrant of the buttock is not well
established and may have been presumed based on analogy to well documented
sciatic nerve injury related to the administration of antibiotics and antisera at this
site”. Not withstanding this conclusion, dorsogluteal vaccination cannot be regarded
as best practice if its use has the potential to cause sciatic nerve injury.

Ventrogluteal Site

The ventrogluteal site (Figure 2.), whose boundaries are the anterior superior iliac
spine (anteriorly), the iliac crest (superiorly) and the greater trochanter was identified
by Von Hochstetter391 as being an injection area in the buttock which is free of major
nerves and blood vessels. This site, has also been called the ‘hip’ site392. Support for
the use of this site for intramuscular injection is prominent in nursing literature392-396.

Figure 2. The ventrogluteal site

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It had been endorsed\(^7\) by the Oregon State Public Health Division, DHS immunization program for intramuscular injection of children 3 years through to adults and has been mentioned as a site for intramuscular injection in the recent American Academy of Pediatrics “Red Book”\(^6\) and Center for Diseases Control “Pink Book”\(^6\).

The dimensions of the ventrogluteal site were measured\(^2\) (Cook & Murtagh, page 75) in 642 infants aged 2-18 months of age, and using aggregated data for each age and sex group templates attachable at the three parts of the ventrogluteal area were prepared.

Tissue composition of the ventrogluteal site defined with these templates was measured by ultrasonography in 57 subjects aged 2-18 months. Similar measurement of the tissue composition of the anterolateral thigh vaccination area (the junction of the upper and middle third at the vastus lateralis muscle) was made in these subjects (page 61). The ventrogluteal site was adequately muscled and did not ‘consist mainly
of fat’ as has been previously asserted\textsuperscript{258}. The subcutaneous layer of the ventrogluteal site and the anterolateral thigh vaccination site were comparable with mean thickness varying from 8.4 to 9.3mm for the former and 8.1 to 10.2mm for the latter across the age range 2-18 months.

It was concluded that a 16mm long needle would routinely penetrate muscle at both sites in infants and toddlers using the WHO technique where the needle is entered at 90\degree to the skin’s surface as the procedure produces considerable compression of the subcutaneous layer, as the skin is stretched taut between the index finger and the thumb.

Comparison of the immune response to administration of vaccine at these sites was made using hepatitis B vaccine, a vaccine for which there is a serological correlate of protection\textsuperscript{397}. In an open, randomised study\textsuperscript{189} (Cook & Murtagh, page 110) of 200 healthy infants, Engerix B 10\(\mu\)g was given as a 0, 1, 6 month regimen with hepatitis B surface antibody (anti-HBs) measured 4 – 6 weeks after the last dose.
Cook IF, Murtagh J. Comparative immunogenicity of hepatitis B vaccine J administered into the ventrogluteal area and anterolateral thigh in infants. Paediatr Child Health 2002; 38: 393-6.

doi: 10.1046/j.1440-1754.2002.00013.x
Good antibody response (anti-HBs titre ≥ 100iu/ml) was not significantly different for the two sites (ventrogluteal 96.6%, anterolateral thigh 93.2%) and geometric mean titre (GMT) for anti-HBs were also comparable (ventrogluteal 2071.2 ± 5.8mIU/ml and anterolateral thigh 2073.2 ± 5.2mIU/ml). Seroconversion (antiHBs ≥ 10iu/ml was 98.9% ventrogluteal site and 97.7% anterolateral thigh.

These seroconversion rates were comparable with other studies where Engerix B 10µgm was administered to infants as a three dose region over 6 months, buttock355 (100%), anterolateral thigh398 (99.0%) and deltoid288 (100%).

The seroconversion rates were similar to those in the study by Alves et al98 where Engerix B 10µgm was given as a 3 dose regimen at 2, 4 and 9 months into the dorsogluteal site or anterolateral thigh site (99.2% and 99.3% respectively). However good antibody response was significantly lower with the dorsogluteal site (87.8% compared with the anterolateral thigh 94.8%) possibly reflecting a thicker subcutaneous layer at the dorsogluteal site compared with the ventrogluteal site in infants, as seen with adults (Table 10, page 89).

As with other studies with hepatitis B123 (Cook , page 49), a sex-difference in antibody response was seen, with females having a greater geometric mean titre in both groups (ventrogluteal area, females 2220mIU/ml, males 1947mIU/ml; anterolateral thigh, females 2176mIU/ml, males 1955mIU/ml).
Comparison of the reactogenicity (adverse reactions) and parental approval of vaccines administered at these sites was made with whole cell and acellular pertussis vaccines. In randomised, trained, observer ‘blind’ studies (Cook & Murtagh, page 117) 283 and 566 children aged 2-18 months of age received whole cell pertussis and acellular pertussis vaccine respectively.
Cook IF, Murtagh J. Comparative reactogenicity and parental acceptability of pertussis vaccines administered into the ventrogluteal area and anterolateral thigh in children aged 2, 4, 6 and 18 months. Vaccine 2003; 21:3330-4.
Comparative reactogenicity and parental acceptability of pertussis vaccines administered into the ventrogluteal area and anterolateral thigh in children aged 2, 4, 6 and 18 months

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Abstract

The importance of site of injection of combined pertussis/diphtheria/tetanus vaccines was investigated in two single blind studies. In the pilot study, in which the research instrument was trailed, 283 children aged 2–18 months received whole cell pertussis vaccine (DTPw) by the intramuscular route either into the anterolateral thigh or the ventrogluteal site. In the larger randomised study, 566 children aged 2–18 months were similarly injected with acellular pertussis vaccine (DTPa). Adverse reactions monitored for 24 h showed the same lower rates for both vaccines with ventrogluteal injection compared with anterolateral thigh injection for systemic reactions (irritability ($P < 0.0001$), decreased feeding ($P < 0.0001$), persistent crying/screaming ($P < 0.0001$) and local reactions (bruising ($P < 0.0001$)) and redness/swelling ($P < 0.0001$)). The Haemophilus influenzae type b vaccine (HiB TITER) given concurrently in the contralateral site to the pertussis vaccine showed the same lower rates in both studies for ventrogluteal injection compared with anterolateral thigh injection for local reactions (redness/swelling both studies ($P < 0.0001$) and bruising DTP study ($P < 0.0001$) and DTPa study ($P < 0.0004$)). Parental acceptability was greater ($P < 0.0001$) in both studies for ventrogluteal injection compared with anterolateral thigh injection.

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Keywords: Pertussis vaccines; Ventrogluteal area; Anterolateral thigh

1. Introduction

Definition of best paediatric vaccination practice (site and technique of injection) is mandated by increasing concerns about vaccine induced adverse reactions in the context of a decreasing incidence of diseases vaccines prevent [1].

The first clear statement of this practice was made by Sauer [2] and outlined buttock injection. This site has subsequently been proscribed for childhood vaccination on the basis of tangential safety and immunological data. Gilles and Matson [3] and Gilles and French [4] have observed that gluteal injection has been associated with neurovascular injury in infants, especially sciatic nerve palsy. No such neurovascular complications have been reported after administration of any vaccine into this area.

Neurovascular complications have been reported in [5,6] following injection into the two now preferred paediatric vaccination sites (deltoid and anterolateral thigh sites).

Concerns about the immunogenicity of paediatric vaccines administered to the gluteal area have arisen from adult studies with hepatitis B [7] and rabies [8] vaccines in which lower seroconversion and antibody titres were observed when these vaccines were given into the gluteal site compared with the deltoid region.

The ventrogluteal site, whose boundaries are the anterior superior iliac spine (anteriorly), the iliac crest (superiorly) and the greater trochanter (inferiorly), was identified by Von Hochstetter [9] as being an injection site in the buttock which is free of major nerves and blood vessels, and which is now accepted [10] as an appropriate site for intramuscular injection.

Using a rigid, disposable template to define this area tightly in 57 children aged 2–18 months, an ultrasonographic study showed that the ventrogluteal site was similar to the anterolateral thigh vaccination site in these children, with each site having 10 mm or less subcutaneous fat and 10 mm or more muscle. Injections using the World Health Organization injection technique (skin stretched taut and needle entered at 90° to the skin) and a 25 gauge, 16 mm long needle would routinely ensure intramuscular injection at both sites.

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Ventrogluetral and anterolateral thigh injection of hepatitis B vaccine with this technique in an open design study of 200 babies aged up to 10 months at onset of vaccination showed [11] that the two sites were immunologically comparable for this vaccine.

The aim of the present studies were to compare the reactogenicity and parental acceptability of whole cell and acellular pertussis/diphtheria/tetanus vaccines and Haemophilus influenzae type b vaccine (HibTITER) given by intramuscular injection into the ventrogluetral and anterolateral thigh injection sites.

2. Methods

2.1. Study design and population

The study was approved by the Monash University, Australia, standing committee on ethics in research using humans (Project No. 96/509).

Infants aged 2, 4, 6 and 18 months were involved in the study if they:

- were in apparent good health at the time of vaccination;
- had written informed consent from a parent/guardian.

Infants were excluded from the study if they:

- had active or progressive neurological disease;
- had a history of severe immediate allergic or anaphylactic reaction to any vaccine antigen used in the study.

The ventrogluetral site was defined with the previously described template and injection was made, with the skin taut between the thumb and index finger, with a 25 gauge, 16 mm needle, fully entered at 90° to the skin's surface with the needle directed anteriorly.

Anterolateral thigh injection was made at the junction of the upper third and lower two-thirds of the anterolateral thigh muscle mass with a 25 gauge, 16 mm needle fully entered at 45° to the vertical, at 90° to the skin's surface, with the skin taut between the thumb and index finger.

The whole cell pertussis/diphtheria/tetanus vaccine comparative study was used as a pilot study to validate the research instrument which contained adverse reaction parameters identified by Feery [12] for whole cell pertussis/diphtheria/tetanus vaccine. Assessment was made 24 h after vaccination, as in other pertussis vaccine studies [13,14], by the practice nurse who was 'blind' with respect to vaccination site and vaccine used at each site. Subjective (parent) responses to the systemic reaction variables (irritability, perceived fever, persistent crying/screaming, drowsiness and vomiting/poor feeding) were recorded before making objective assessment of local reactions (redness/swelling and bruising). This sequence of assessment was made to minimize potential observer bias with respect to vaccination site.

Reaction parameters and parental acceptability were scored on visual analogue scales; 0: no reaction or very happy to 5: highest level of reaction or very unhappy.

In the whole cell pertussis vaccine study, infants attending the medical practice were given a sequential vaccination number. Children given an odd number were given vaccines into the anterolateral thigh, while those given an even number were given vaccines into the ventrogluetral site. H. influenzae type b conjugate vaccine was given concurrently into the contralateral ventrogluetral or anterolateral site as the pertussis vaccine, with oral polio vaccine (two drops in 0.2 ml of vaccine syrup), given to children aged two, four and six months.

In the subsequent acellular pertussis vaccine study infants were randomised on a 1:1 basis for ventrogluetral and anterolateral thigh site vaccinations with H. influenzae type b vaccine and polio vaccines given as in the earlier study.

2.2. Vaccines

Four vaccines were used in the study:

(i) Whole cell pertussis vaccine (DTPw), produced by Commonwealth Serum Laboratories, Australia with each 0.5 ml dose containing diphtheria toxoid 30 LF, tetanus toxoid 6 LF, Bordetella pertussis not more than $20 \times 10^6$ organisms, 50 mcg thiomersal as preservative, 1 mg aluminium phosphate adjuvant.

(ii) Acellular pertussis vaccine (DTPa), produced by Smith Kline Beecham with each 0.5 ml dose containing diphtheria toxoid 25 LF, tetanus toxoid 10 LF, PT 25 mg, FHA 25 mg and 69 kDa OMP 8 mg. The components were adsorbed on aluminium 0.5 mg in the form of aluminium hydroxide and suspended in isotonic sodium chloride.

(iii) H. influenzae type b conjugate vaccine produced by Lederle Laboratories, with each 0.5 ml dose containing purified Haemophilus b saccharide 10 μg and approximately CRM197 protein 25 μg in isotonic sodium chloride.

(iv) Oral poliomyelitis vaccine (OPV), produced by Smith Kline Beecham, containing live attenuated polio virus (types 1–3) and neomycin B sulphate 5 μg.

2.3. Statistics

The sample size calculation of 280 infants in the pilot study was based on the assumption of a 40.6% rate of any redness after thigh vaccination with a 16 mm long needle [15] and the hypothesis that a reduction to 25% could be demonstrated with an alpha of 5% and study power of 80%.

Fisher's exact test or Chi-square ($\chi^2$), where appropriate, were used to compare visual analogue scores of none, moderate (scores 1–2) and severe (scores 3–5) observed with the vaccines.
Table 1
Local and systemic visual analog scores by vaccination site for whole cell pertussis, diphtheria and tetanus vaccine (DTPw)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Site</th>
<th>Visual analogue score (n)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>None (scores 0–2)</td>
<td>Moderate (scores 1–3)</td>
</tr>
<tr>
<td>Local reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPw</td>
<td>Bruising</td>
<td>Ventrolateral 115 (85.8)</td>
<td>19 (14.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 42 (30.7)</td>
<td>92 (67.2)</td>
</tr>
<tr>
<td></td>
<td>Redness/swelling</td>
<td>Ventrolateral 86 (64.2)</td>
<td>47 (35.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 11 (8.0)</td>
<td>85 (62.0)</td>
</tr>
<tr>
<td>Hib/TTITER</td>
<td>Bruising</td>
<td>Ventrolateral 134 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 122 (89.3)</td>
<td>15 (10.9)</td>
</tr>
<tr>
<td></td>
<td>Redness/swelling</td>
<td>Ventrolateral 124 (92.5)</td>
<td>9 (6.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 101 (73.7)</td>
<td>35 (25.5)</td>
</tr>
<tr>
<td>Systemic reaction</td>
<td>Irritability</td>
<td>Ventrolateral 37 (27.6)</td>
<td>87 (64.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 12 (8.8)</td>
<td>67 (48.9)</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
<td>Ventrolateral 89 (66.4)</td>
<td>43 (32.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 46 (33.6)</td>
<td>75 (54.7)</td>
</tr>
<tr>
<td></td>
<td>Persistent crying</td>
<td>Ventrolateral 91 (67.9)</td>
<td>35 (26.1)</td>
</tr>
<tr>
<td></td>
<td>screaming</td>
<td>Anterolateral thigh 33 (25.5)</td>
<td>72 (52.6)</td>
</tr>
<tr>
<td></td>
<td>Drowsiness</td>
<td>Ventrolateral 96 (71.6)</td>
<td>35 (26.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 88 (64.2)</td>
<td>45 (32.8)</td>
</tr>
<tr>
<td></td>
<td>Vomiting/poor feeding</td>
<td>Ventrolateral 121 (90.3)</td>
<td>13 (9.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anterolateral thigh 114 (83.2)</td>
<td>23 (16.8)</td>
</tr>
</tbody>
</table>

P-value is from either χ² or Fisher’s exact test where appropriate with significance level of 0.05. Values in parentheses are percentages.

3. Results

In the pilot study, whole cell pertussis vaccine was given to 283 children. From two demographically similar populations (139 ventrolateral and 144 anterolateral thigh).

There were 12 'protocol violators' (8 females, 4 males, 5 ventrolateral sites and 7 anterolateral thigh sites) but these were not due to adverse reactions. Local and systemic visual analog scores are shown in Table 1.

For both DTPw and Hib/TTITER injections into the anterolateral thigh were associated with significantly greater rates of local reaction (bruising and redness/swelling) than injections into the ventrolateral site.

Systemic reactions (irritability, perceived fever and persistent crying) were also observed at a significantly greater rate with anterolateral thigh than ventrolateral site injection. There was no significant difference between the two sites for rates of drowsiness and vomiting/poor feeding.

Parent acceptability was greater for ventrolateral injection than anterolateral thigh injection for each age group and overall. Good parental acceptability score (score 0), 2 months, ventrolateral 89.2%, anterolateral thigh 41.7%; 4 months, ventrolateral 84.4%, anterolateral thigh 26.1%; 6 months, ventrolateral 92.1%, anterolateral thigh 37.8% and 18 months, ventrolateral 96.3%, anterolateral thigh 4.0%.

Ventrolateral site compared with anterolateral thigh P < 0.0001. Parental acceptability dramatically decreased for the 18-month anterolateral thigh injection but not with ventrolateral injection at this age.

In the acellular pertussis study, 283 vaccinees were recruited into each injection group. "Protocol violation", not due to vaccine adverse reactions, resulted in the loss of 11 vaccine recipients (5 males, 6 females) from each group for evaluation.

As with the DTPw study (Table 2), both DTPs and Hib/TTITER injections into the anterolateral thigh were associated with significantly greater rates of local reaction (bruising and redness/swelling) than injection into the ventrolateral site.

Similarly, systemic reactions (irritability, perceived fever and persistent crying) were also observed at a significantly greater rate with anterolateral thigh injection than ventrolateral site injection. Again, there was no significant difference between the two sites for rates of drowsiness and vomiting/poor feeding. Also, the parental acceptability level was greater for ventrolateral injection than anterolateral thigh injection for each age group and overall. Good parental acceptability score (score 0) 2 months, ventrolateral 94.7%, anterolateral thigh 86.6%; 4 months, ventrolateral 94.5%, anterolateral thigh 74.3%; 6 months, ventrolateral
Table 2
Local and systemic visual analog scores by vaccination site for acellular pertussis, diphtheria and tetanus vaccine (DTPa)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Site</th>
<th>Visual analogue score (x)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Moderate (scores 1-2)</td>
<td>Severe (scores 3-5)</td>
</tr>
<tr>
<td><strong>Local reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPa</td>
<td>Bruising</td>
<td>271 (99.6)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Ventrogluteal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>252 (92.6)</td>
<td>20 (7.4)</td>
</tr>
<tr>
<td>Redness Swelling</td>
<td>Ventrogluteal</td>
<td>255 (93.8)</td>
<td>16 (5.9)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>179 (62.6)</td>
<td>97 (33.3)</td>
</tr>
<tr>
<td>HIBTITER</td>
<td>Bruising</td>
<td>272 (100.0)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ventrogluteal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>260 (95.6)</td>
<td>12 (4.4)</td>
</tr>
<tr>
<td>Redness Swelling</td>
<td>Ventrogluteal</td>
<td>263 (96.7)</td>
<td>9 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>234 (86.9)</td>
<td>76 (27.2)</td>
</tr>
<tr>
<td><strong>Systemic reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>Ventrogluteal</td>
<td>243 (89.3)</td>
<td>28 (10.3)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>196 (72.1)</td>
<td>75 (27.6)</td>
</tr>
<tr>
<td>Fever</td>
<td>Ventrogluteal</td>
<td>265 (97.4)</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>264 (99.8)</td>
<td>74 (26.8)</td>
</tr>
<tr>
<td>Persistent crying/screaming</td>
<td>Ventrogluteal</td>
<td>269 (98.9)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>254 (93.4)</td>
<td>17 (6.3)</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>Ventrogluteal</td>
<td>269 (98.9)</td>
<td>3 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>262 (96.3)</td>
<td>10 (3.7)</td>
</tr>
<tr>
<td>Vomiting/poor feeding</td>
<td>Ventrogluteal</td>
<td>261 (96.6)</td>
<td>10 (3.7)</td>
</tr>
<tr>
<td></td>
<td>Anterolateral thigh</td>
<td>247 (90.8)</td>
<td>25 (9.2)</td>
</tr>
</tbody>
</table>

Total number of subjects: 544.272 in each group. P-value is from either $\chi^2$ or Fisher’s exact test where appropriate with significance level of 0.05. Values in parentheses are percentages.

96.2%, anterolateral thigh 75.0%; and 18 months, ventrog- luteal 81.3%, anterolateral thigh 71.8%. Ventrogluteal site compared with anterolateral thigh $P < 0.0001$.

For both sites, high parent acceptability (score 0) was lower with the 18-month booster injection. The 18-month anterolateral thigh booster accounted for a disproportionate number and percentage of the higher levels of parent unacceptability scores (grades 2 and 3, 10; group reaction rate 12.8%) compared with the pooled 2-, 4- and 6-month age groups (grades 2 and 3, 12; group reaction rate 6.1%). DTPa was significantly more reactivogenic than DTP for both local and systemic adverse reactions at both ventrog- luteal and anterolateral thigh sites.

4. Discussion

In 1983, Weir and Fearnow [16], reviewing the causation of transverse myelitis in children following intramuscular penicillin injection into the gluteal musculature concluded that, “with non-viscous, lucent injectables, cautious gluteal injections are probably safe but viscous, opaque suspensions, particularly bexaamidine penicillin should be avoided in the gluteal region of infants, probably in other sites as well”. Bergeson [17], the enunciator of the American technique for anterolateral thigh injection in children, in response to this publication, posed the question [18] of, “How safe is the injection of non-viscous, lucent materials in this area?”

This issue with respect to childhood vaccination has been canvassed by MacDonald and Marcuse [19,20], who concluded [21] that “the presumed hazard associated with administering vaccinations in the upper outer quadrant of the buttock is poorly established and may be based on well-documented septic injury from the administration of antibiotics and anisec at this site”.

Previously reported [22,23] paediatric site comparative studies with pertussis vaccines have shown the superiority of the buttock over thigh injection in terms of local and systemic adverse effects.

Baraff et al. [22], using whole cell pertussis, diphtheria, tetanus vaccines, observed that local reactions (pain and swelling) and systemic reaction (fever) were significantly less when the vaccine was given into the buttock rather than into the thigh.

While Tozzi et al. [23], using two acellular and one whole cell pertussis/diphtheria/tetanus vaccine observed that buttock injection was associated with a significantly lower rate of local adverse reaction (swelling and tenderness but not
redness) and systemic adverse reaction (rectal temperature >38°C) and irritability than thigh injections.

An injection technique which has the potential for serious adverse reaction cannot be considered as best practice. However, this concern was covered for the ventrogluteal site, in our study, by the use of rigid, disposable templates, which tightly defined this area. The ventrogluteal site was superior to the anterolateral thigh (in our studies), with respect to local adverse reaction (bruising and redness/swelling) for DTPw, DTPa and the H. influenzae type b vaccine and systemic reactions (irritability, perceived fever and persistent crying and screaming but not vomiting/poor feeding and drowsiness).

The other significant return associated with using the ventrogluteal site was the high level of parental acceptability and this is important in the context of maintaining good compliance with Government funded vaccination programs.

Acknowledgements

We wish to thank all the parents/guardians who so willingly participated in these studies. We also wish to thank the Hunter Rural Division of General Practice for providing funding for the statistical analysis conducted by Datapharm Australia.

References

In both studies anterolateral thigh injection was associated with significantly greater rates of local and systemic adverse reactions than ventrogluteal injection. Numbers needed to harm (NNH) data show the clear superiority of the ventrogluteal(VG) site over the anterolateral thigh(AL) site with pertussis containing vaccines, particularly the whole cell vaccine, (Table 11).

Table 11: Adverse Reactions with DTPw and DTPa given by Intramuscular Injection into the Anterolateral Thigh(ALT) or Ventrogluteal Site(VG).

<table>
<thead>
<tr>
<th>Reaction at 24 hrs</th>
<th>Comparison (ALT vs VG)</th>
<th>DTPw (NNH)</th>
<th>DTPa (NNH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td>(ALT vs VG)</td>
<td>1.81</td>
<td>14.3</td>
</tr>
<tr>
<td>Redness/swelling</td>
<td>(ALT vs VG)</td>
<td>1.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Irritability</td>
<td>(ALT vs VG)</td>
<td>5.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Fever</td>
<td>(ALT vs VG)</td>
<td>3.0</td>
<td>15.2</td>
</tr>
<tr>
<td>Persistent crying/screaming</td>
<td>(ALT vs VG)</td>
<td>2.4</td>
<td>17.9</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>(ALT vs VG)</td>
<td>13.5</td>
<td>38.5</td>
</tr>
<tr>
<td>Vomiting/poor feeding</td>
<td>(ALT vs VG)</td>
<td>14.1</td>
<td>19.6</td>
</tr>
</tbody>
</table>

**Evidence Based Answer**

Using the modified GRADE recommendations classifying the quality of evidence and recommendations (page 10), there is high (Grade A) evidence and a strong (Grade 1) recommendation can be made for intramuscular administration of vaccines into the ventrogluteal site of infants and toddlers.
CONCLUSION

Application of the principles of evidence based medicine to vaccines has resulted in the development of evidence based vaccinology (EBV). This has been defined as “the identification and use of the best evidence in making and implementing decisions during all of the stages of the life of a vaccine, including pre-licensure vaccine development and post licensure manufacture and research, and utilization of the vaccine for disease control”.

Vaccination practice (route and site of administration of the vaccine) is an integral component of EBV.

In this thesis, studies are presented which allow evidence based guidelines for vaccination practice to be formulated:

**Route of Administration**

- For route of administration of vaccines a strong (Grade 1) recommendation can be made for the intramuscular in preference to the subcutaneous route. This recommendation is based on high (Grade A) quality data derived from trials with a split, trivalent influenza vaccine\(^1\) (Cook et al, page 18) and a 23 valent pneumococcal vaccine\(^2\) (Cook et al, page 29) and supported by a review of route comparative studies in the literature\(^3\) (Cook, page 39). This preference is also seen for live virus vaccines, such as measles, measles-mumps-rubella, varicella and yellow fever, which have traditionally been given by subcutaneous injection. Further investigation of this preference could be made with Zostavax\(^4\), a live
varicella vaccine shown to be effective\textsuperscript{401} in the prevention of herpes zoster and post herpetic neuralgia. A difficulty in conducting this study in the general practice context will be cost, as cellular immune response needs to be measured to determine vaccine protection\textsuperscript{402,403}.

The determinants of intramuscular injection are needle length and technique of injection:

- For needle length in infants, only a weak recommendation (Grade 2) can be made for a particular length needle (16mm or 25mm) for intramuscular injection of the anterolateral thigh and ventrogluteal site. Moderate (Grade B) quality data from ultrasound studies, needle length comparative studies and clinical trials support the use of 16mm and 25mm long needles for intramuscular vaccination of infants and toddlers in the anterolateral thigh. The ultrasonographic study by Cook & Murtagh\textsuperscript{169} (page 61) showed that a 16mm long needle would routinely penetrate muscle of the anterolateral thigh in children aged 2, 4, 6 and 18 months when entered at 90 degrees to the skin’s surface as with the World Health Organization technique\textsuperscript{75}. The 16mm long needle was also preferred by Australian general practitioners for the intramuscular injection of vaccines in children aged 2-18 months\textsuperscript{243} (Cook & Murtagh, page 69). This controversy could be resolved by conducting an ultrasound study in an appropriately numbered study involving infants of all ages and/or a needle length comparative study with outcomes assessed by a ‘blind’, trained observer, not parents as parental reporting has been implicated in the genesis of the highly variable results with meningococcal serotype C conjugate vaccine\textsuperscript{79,175-178} and whole cell pertussis containing vaccines\textsuperscript{171,172}. 

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• For needle length in adults a strong (Grade 1) recommendation can be made for the use of a 25mm long needle for intramuscular injection into the deltoid area. This recommendation is based on high (Grade A) quality data from two ultrasound studies\textsuperscript{165,246} and one route comparative study\textsuperscript{247} with a 16mm long needle. These data argue that vaccine manufacturers in Australia should be presenting influenza vaccines for adult use with a 25mm long needle rather than the 16mm long needle currently provided.

• A strong (Grade 1) recommendation can be made not to use the ‘standard’ 38mm long needle for intramuscular injection of the dorso and ventrogluteal sites, especially in obese adult females. High (Grade A) evidence from an ultrasound and a CT scan study showed that subcutaneous layer (SCL) thicknesses were greater than 50mm in this group. Intramuscular injection defined\textsuperscript{165} as penetration of the muscle layer by 5mm or more would not be achieved with a 38mm long needle unless the SCL was compressed by almost 20mm.

• For injection technique, a strong (Grade 1) recommendation can be made for introducing the needle at a $90^\circ$ angle to the skin’s surface. This recommendation is based on high (Grade A) quality data derived from a randomised, trained observer, blind study with an acellular pertussis containing vaccine\textsuperscript{259} (Cook & Murtagh, page 96). This recommendation brings the technique of administration of vaccines into line with the intramuscular injection of other agents\textsuperscript{58}. This recommendation is now endorsed in “The Australian Immunisation Handbook, 9\textsuperscript{th} edition 2008.”\textsuperscript{404}
Site of Injection

(a) Deltoid muscle:

- The deltoid muscle is the recommended site for intramuscular injection in adults and children with adequate muscle mass (usually > 12 months of age). However, there is considerable variation in the recommended site for intramuscular injection of this muscle, with injection recommended to be given:
  - 2.5cm below the acromion\(^{395}\)
  - Two or three finger breadths below the acromion\(^{405}\) (2.5cm – 5.0cm) and South Australian Guidelines 1989\(^{33}\) (2 finger breadths), Los Angeles County Vaccination Guidelines\(^{74}\) (3 finger breadths).
  - 3-5cm below the acromion\(^{406}\).
  - Halfway between the acromion and a line drawn horizontally across the lower aspect of the deltoid muscle from the apex of the angle made by the medial border of the biceps and the lateral border of the pectoralis muscles. (Vaccination Guidelines. Saskatchewan\(^{42}\), Ireland\(^{44}\), New Zealand\(^{49,50}\)).
  - Halfway between the acromion and the deltoid insertion into the mid humerus (deltoid tuberosity)\(^{258,407,408}\).

A call to establish reliable protocols for intramuscular injection of the deltoid muscle was made following a questionnaire study of 50 general practitioners and 50 practice nurses by McGarvey and Hooper\(^{409}\). In this study in Ireland, only two general practitioners and one practice nurse used the acromion to identify the injection site,
64% of the general practitioners and 70% of the practice nurses recommended administration of the injection into the middle one third of the muscle, 26% of the general practitioners and 25% of the practice nurses recommended the upper half whilst the remainder of the two groups (10% and 5% respectively) recommended use of the lower half of the muscle.

These protocols are necessary to prevent axillary and radial nerve injury with intramuscular injection of vaccines into the deltoid muscle\textsuperscript{388,389}. As Australian nurses now give many routine injections, including vaccines into the deltoid muscle, a study like that conducted in Ireland by McGarvey and Hooper could be made to assess current practice and to develop injection protocols for training purposes.

(b) Ventrogluteal Site:

In infants and toddlers a strong (Grade 1) recommendation can be made for the ventrogluteal site making it an alternative to the anterolateral thigh site. High (Grade A) quality data from an ultrasound study showed that the ventrogluteal site had similar subcutaneous layer and muscle layer thickness to the anterolateral thigh site in infants aged 2, 4, 6 and 18 months and did not consist “mainly of fat” as asserted by Bergeson et al\textsuperscript{258}.

High (Grade A) quality data about immune response with ventrogluteal site injection were obtained with hepatitis B vaccine with comparable immune response to the anterolateral thigh site being observed in infants in an open, randomised study\textsuperscript{189} (Cook & Murtagh, page 110) “Good” antibody response\textsuperscript{397} defined as anti-HBs $\geq$ 100iu/ml, was 96.6% for ventrogluteal site and 93.2% for anterolateral thigh and
geometric mean titre (GMT) for anti-HBs ventrogluteal site 2071 ± 6mIU/ml and anterolateral thigh site 2073 ± mIU/ml.

High (Grade A) quality data for randomised, “trained” observer blind studies\(^\text{190}\) (Cook & Murtagh, page 117) with pertussis containing vaccines showed that ventrogluteal site injection gave lower rates of adverse reactions and was preferred by parents to the anterolateral thigh site.

Support still exists for dorsogluteal intramuscular injection – In response to the question posed to the Primary Care Question Answering Service 27/7/07, “Why is the dorsogluteal site preferred over the ventrogluteal site when administering an intramuscular injection?” It was answered\(^\text{410}\) “I think the NLHQ&A service would struggle to explain ‘why’ this situation has arisen. Frequently clinical practice has been driven by precedent and/or ‘expert opinion’. To answer why would probably require an historical analysis”.

Somewhat paradoxically the agents, procaine penicillin\(^\text{411}\) and benzathine penicillin\(^\text{412}\), which helped generate the transformation from buttock to anterolateral thigh vaccination in infants are still recommended to be given by injection into this site.

The ventrogluteal site is widely promoted by the nursing profession as a safe site for intramuscular injection. However, despite the formal promotion\(^\text{413}\) of the ventrogluteal site to nurses in New Zealand, there was resistance to its usage with
some workshop attendees continuing to use the dorsogluteal site as they stated “I’ve never had a problem with my practice, why should I change”.

The ventrogluteal site has also been recommended for intramuscular injection in Ireland where it was noted\textsuperscript{414} that “there is a dearth of research in this area in Ireland as to the extent to which the ventrogluteal site is used”.

In Australia, there are no data on the ventrogluteal site use by nursing and medical practitioners. A questionnaire study of its use in this group could be used to develop protocols for teaching intramuscular injection at this site which has now been recommended in The Australian Immunisation Handbook, 9\textsuperscript{th} edition, 2008\textsuperscript{404}.

It is also noteworthy that the pharmaceutical industry has also endorsed the ventrogluteal site for the administration of parenteral iron (Ferrosig\textsuperscript{®} Sigma Pharmaceutical\textsuperscript{415} and Ferrum\textsuperscript{®} Aspen Pharmacare\textsuperscript{416}).

In this thesis, sex-difference was observed in antibody response with a split, trivalent influenza vaccine\textsuperscript{116} (Cook et al, page 18) and a hepatitis B vaccine\textsuperscript{189} (Cook & Murtagh, page 110) and in adverse reactions with both this influenza vaccine\textsuperscript{116} and a 23 valent pneumococcal polysaccharide\textsuperscript{118} (Cook et al, page 29) vaccine. Despite suggestions\textsuperscript{121,122} that limited data exist on sex-difference with vaccines, 97 studies were found with antibody differences\textsuperscript{123} (Cook, page 49) and 29 studies with differences in adverse reactions (page 55).
Consistent with these sex-differences in antibody response were sex-differences in disease attack rates, females less disease than males (influenza, hepatitis A and hepatitis B infections) and males less disease than females (pneumococcal and diphtheria infection) in vaccinated populations \(^{123}\) (Cook, page 49). Sex-differences in clinically significant adverse reactions were seen with measles, rubella and yellow fever vaccines in vaccinated populations.

Sex-differences in disease attack rate have also been seen with other vaccines in vaccinated populations, Herpes simplex vaccine \(^{417}\) (females less disease than males) and BCG vaccine, tuberculosis \(^{418,419}\) and leprosy \(^{420-423}\) (females less disease than males) and leprosy \(^{424,425}\) (males less disease than females).

Sex is clearly an important determinant of the immune response and clinical outcome with some vaccines. Since the submission of the thesis, a review “Cook IF. Sex differences in injection site reactions with human vaccines. Human Vaccines 2009; 5: 1-9.” has been accepted for publication and is included to complete the topic of sex-differences with vaccines.
Cook IF. Sex differences in injection site reactions with human vaccines.

Sex differences in injection site reactions with human vaccines

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Key words: sex-difference, human vaccines, immunisation, injection site, adverse reactions

Adverse events following immunization (AEFI) are not uncommon, with injection site reactions (ISRs) being the most common.

Predictors of injection site reactions are vaccine factors (antigen characteristics, antigen dose, dose number of antigen, antigen adjuvanted and type of diluent), vaccine administration factors (site and route of administration) and vaccinee factors (age and sex, the latter the subject of this review).

1,074 studies which reported ISRs were retrieved by searching of online journals and databases. Analysis of these data for sex-differences was only reported in 57 studies, with 54 of these studies reporting a sex-difference (42 in subjects >17 years and 12 in subjects <17 years).

In accord with the well documented greater pain sensitivity in females compared with males, in all studies with vaccines which reported pain during and post vaccination (hepatitis A, B, diphtheria/tetanus toxoid, diphtheria/tetanus/perussis/DTaP and Tdap, anthrax and inactivated influenza), females reported a greater rate of pain than males.

The pathophysiology of the sex-difference in local reactions (induration, tenderness, erythema, pruritus) following vaccination is clearly multifactorial with hypersensitivity reaction (type III, Arthus reaction-antigen/antibody immune complex formation), route of administration and hormonal factors being suggested.

The data presented in this review demonstrate that studies of AEFI should recruit similar numbers of females and males and that these data should be analysed for sex-difference. Additionally, unlike as at present, reporting of analysis of AEFI data by sex should become standard practice.

Sexual dimorphism is well documented for immunity and pain perception in humans.14,15 However, the impact of these sex-differences on the immunogenicity and reactogenicity of vaccines has received little attention. Furthermore, the quality of the available data range from 'high grade' (from prospective, randomized trials with well defined endpoints) to 'low grade' (from observational studies).

Sex-difference in childhood mortality has been reported in observational studies with a high dose measles vaccine9,10 and with difference sequences of childhood vaccination.11 However, the results of these studies are not universally accepted12,13 due to perceived methodological deficiencies.

Antibody (humoral) response to vaccines has been chosen14 to assess the sex-difference in immune response to vaccines at serological levels of protection are available for many vaccines and it offers a convenient, objective means of assessing these differences.

In a comprehensive review15 of the literature, sex-difference in immune response was reported in 97 studies with 14 different vaccines. Sex-difference was antigen dependent with females having greater antibody response than males for the following vaccines: influenza (elderly adults, young adults with acute stress and eccentric exercise), hepatitis A, hepatitis B, rubella, measles (adults), diphtheria (undenatured infants, primary vaccination), tetanus, brucella and rabies and males having a greater antibody response than females for the following vaccines: influenza (young adults, life events and social support effects), pneumococcal polysaccharide (healthy and alcoholic adults), diphtheria (booster dosing), measles (pre-pulmonary), yellow fever, meningococcal A (adults, acute stress), meningococcal C (most age groups), Venezuelan equine encephalitis and rabies.

Injection site reactions (ISRs) have been chosen to assess the sex-difference in reactogenicity (adverse events following immunization—AEFI) as they are the most common16 and clearly causally related18 AEFI.

Data for this study were obtained by:
(A) Hand searching journals for prospective studies, assessing immunogenicity, efficacy and/or safety/reactogenicity of vaccines administered to humans, independently of study design. Excluded were all studies analysing data subsequent to prior studies (i.e., followup on immunogenicity and/or antibody persistence) and population based studies (i.e., effectiveness or efficacy of immunisation programs) and studies in which a particular vaccine per se was not the primary target of investigation (i.e., non-safety subanalysis of larger trials, effect on underlying disease or outbreak control).

The following journals were searched from the date in parenthesis to Feb 2009:

Sex differences in injection site reactions with human vaccines


(B) The following databases: Medline, Embase, Scopus, Biological abstracts, Science Citation Index, Cochrane Database of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL) and NHS Database of Abstracts of Reviews of Effects (DARE) were also searched for additional studies using the word searches "prospective vaccine studies/trials" and "immunogenicity/reaction/safety" with the exclusions listed above.

Bibliographies of all relevant publications obtained by these searches were then searched for additional studies.

These searches retrieved 1,074 studies which reported injection site reaction data. Journals with more than 10 such publications were: Vaccine (559), Pediatrics Infectious Disease Journal (91), Pediatrics (63), Journal of Infectious Diseases (43), Journal of Pediatrics (36), Human Vaccine (26), Lancet (23), American Journal of Tropical Medicine and Hygiene (23), JAMA (23), Infection and Immunity (22), American Journal of Diseases of Childhood (18), New England Journal of Medicine (16), Developmental Biological Standards (14), Canadian Medical Association Journal (11), Bulletin of the World Health Organization (11), Clinical and Vaccine Immunology (11).

In 57 studies, injection site reaction data were analysed with sex as an independent variable. In 3 studies, no sex-difference was found and in the remainder sex-difference was as reported in Table 1.

Sex-difference in vaccination and post vaccination pain was seen with hepatitis A, hepatitis B, diphtheria/tetanus toxoid, diphtheria/tetanus/percuttis (DTaP, Tdap), anthrax and inactivated influenza vaccine with females reporting more pain than males. This accords with the previously reported large body of data, observing that females have a lower pain threshold and are less tolerant to painful stimuli than males. The difference would appear to be underpinned by biological, psychological and social factors. Pira et al. observed that during vaccination of 4 month old children, parents made significantly more coping-promoting statements and generally talked more to female infants than male infants. These data offer explanation for the genesis of the well documented sex-difference in gender role expectations in pain perception, with masculinity conferring stoicism and femininity more sensitivity.

Miller and Whitney gave a physiological explanation for the sex-difference in vaccination pain with hepatitis B vaccine. They suggested that because women were likely to have smaller deltoid muscles than men, injection into this muscle would cause more muscle distortion and hence give an increased rate of pain reporting in the former compared with the latter.

Predictors of Local Injection Site Reactions (swelling, induration, erythema and tenderness) include:

1. Vaccine factors
   (a) Antigen characteristics—e.g., acellular compared with whole cell pertussis containing vaccines.9
   (b) Antigen dose—e.g., diphtheria vaccines.80
   (c) Dose number of antigen—e.g., revaccination with 23 valent pneumococcal polysaccharide vaccines.81
   (d) Antigen adjuvanted—e.g., aluminium adjuvanted vaccines more ISRs than plain vaccines.82
   (e) Vaccine diluent—e.g., M-M-R II compared with Priorite.83
   2. Vaccine administration factors
      (a) Site of Injection—e.g., buttock/ventrolateral site compared with thigh and deltoid sites.84
      (b) Route of administration—e.g., intramuscular compared with subcutaneous route.85
   3. Vaccinee factors
      (a) Age—e.g., young adults compared with older adults with pneumococcal polysaccharide and pneumococcal conjugate vaccines,80 diphtheria/tetanus/percuttis (DTaP), tetanus-diphtheria (Td).86
      (b) Sex of vaccinee, as shown in this review.

The pathophysiology of these local reactions is unclear but likely to involve a number of mechanistic pathways. For instance, it has been suggested that these reactions are of two types: immediate and delayed. Immediate reactions are thought to be mediated by IgE-mediated reactions and delayed reactions are due to delayed-type hypersensitivity (DTH).87

IgE-mediated reactions are thought to be of two types: immediate and delayed. Immediate reactions are thought to be mediated by IgE-mediated reactions and DTH reactions are due to delayed-type hypersensitivity (DTH).87

Inflammation responses following local reactions are characterized by a number of inflammatory mediators, including proinflammatory cytokines, chemokines, and other inflammatory molecules. These mediators play a critical role in the regulation of the immune response and are thought to be involved in the pathogenesis of local reactions following vaccination. In particular, the cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) have been shown to be involved in the development of local reactions following vaccination.88

In addition to the role of cytokines, the role of the innate immune system in the development of local reactions following vaccination has also been studied. It has been shown that natural killer cells, Toll-like receptors, and other innate immune cells play a role in the regulation of the immune response and in the development of local reactions following vaccination.89

The role of inflammation in the development of local reactions following vaccination is thought to be mediated by the activation of the innate immune system and the release of inflammatory mediators. These mediators then act on the adaptive immune system, leading to the development of local reactions.90

The role of inflammation in the development of local reactions following vaccination has been the subject of much research, with studies focusing on both the innate and adaptive immune responses. It is thought that the development of local reactions is due to the activation of the innate immune system and the release of inflammatory mediators. These mediators then act on the adaptive immune system, leading to the development of local reactions.91

In summary, the local reactions following vaccination are thought to be mediated by a number of different mechanisms, including IgE-mediated reactions, delayed-type hypersensitivity, and inflammation. Further research is needed to fully understand the role of these mechanisms in the development of local reactions following vaccination.
### Table 1 Vaccines and sex differences in injection site reaction (ISRs)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Study design and number of subjects in study</th>
<th>Injection site reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B (Plasma derived, alum adsorbed)</td>
<td>Prospective, randomised, placebo controlled, double blind, trial. n = 199</td>
<td>Pain F &gt; M with injection rate 10 s/ccc at 0 hr.</td>
</tr>
<tr>
<td>Hollinger et al.</td>
<td>placettes n = 75; vaccine n = 124; Overall mean age 25.7 yrs ± 5.0.</td>
<td>Trend towards significance F &gt; M, pain at 0 hr, 24 hr, with injection rate 50 s/ccc and 12 hrs, 24 hrs, with injection rate 10 s/ccc.</td>
</tr>
<tr>
<td>Hepatitis B (Recombinant aluminium adsorbed)</td>
<td>Randomised, crossover study of two injection rates (10 seconds and 30 seconds). n = 30, 41 M, 41 F. Adults, no age data.</td>
<td>Pain, local erythema, pruritus and swelling at injection site; F &gt; M.</td>
</tr>
<tr>
<td>Mitchell &amp; Whitney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabies (Pasteur vaccine VPRP)</td>
<td>Retrospective, post vaccination questionnaire study. n = 329. 204 M, 125 F. Age range 20-99 yrs.</td>
<td>Pain, local tenderness at the injection site. F &gt; M for both vaccines.</td>
</tr>
<tr>
<td>Merieux vaccine M-DACY</td>
<td></td>
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</tr>
<tr>
<td>Kagawa et al.</td>
<td></td>
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</tr>
<tr>
<td>Hepatitis A (Epaxol and Havrix)</td>
<td>Prospective, randomised trial with vaccine given in 4:1 ratio; Epaxol: Havrix as single dose, n = 560. 291 M, 249 F, Age range 9-76 yrs.</td>
<td>F &gt; M.</td>
</tr>
<tr>
<td>Clarke et al.</td>
<td></td>
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<tr>
<td>Recombinant canarypox</td>
<td>Prospective, multicentre, randomised, double blind clinical trials. ANAVAC alone, n = 416; 284 M, 132 F, mean age 35 ± 9.6 yrs.</td>
<td>Pain and swelling at injection site; F &gt; M.</td>
</tr>
<tr>
<td>HIV vaccines de Bruyn et al.</td>
<td>442 M, 267 F, mean age 34 ± 9.6 yrs.</td>
<td></td>
</tr>
<tr>
<td>HIV-1 lipopeptide vaccines</td>
<td>Prospective, open trials. Eight preventive trials: n = 200 adults, 131 M, 69 F. Median age 46.3 yrs. range 22.7 to 57.6 yrs. Two therapeutic trials, n = 48 HIV infected adults, 39 M, 9 F, median age 40.5 yrs. Age range 23.7 to 64.3 yrs.</td>
<td>Pain, local reaction; F &gt; M.</td>
</tr>
<tr>
<td>Durier et al.</td>
<td></td>
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<tr>
<td>Malaria vaccine SP66</td>
<td>Prospective, open, phase 1 study; n = 11; 7 M, 4 F. Age range 18-44 yrs.</td>
<td>Local inflammation: erythema, induration or both; F &gt; M in group aged 1-14 yrs and &gt;14 yrs.</td>
</tr>
<tr>
<td>Amador</td>
<td></td>
<td></td>
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<tr>
<td>Malaria vaccine SP66</td>
<td>Prospective, pseudo randomised study, n = 266</td>
<td>Erythema, induration, warmth, tenderness. F &gt; M.</td>
</tr>
<tr>
<td>Migasena et al.</td>
<td>AV, n = 134; FT, n = 132</td>
<td>Swelling, M &gt; F.</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>Children aged 15-16 yrs.</td>
<td>Local reaction, F &gt; M.</td>
</tr>
<tr>
<td>(adsorbed tetanus vaccine—AV; plain tetanus vaccine—FT)</td>
<td>Assessed at 2 days post vaccination.</td>
<td>Local reaction, F &gt; M.</td>
</tr>
<tr>
<td>Collier et al.</td>
<td>AV, n = 142 M, 48 F; FT, n = 120, 53 M, 65 F</td>
<td>Swelling, M &gt; F.</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td></td>
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<tr>
<td>White et al.</td>
<td></td>
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<tr>
<td>Diptheria/tetanus toxoid (Td)</td>
<td>Prospective, open study; n = 1,493 M, 379 F.</td>
<td>Local reaction, F &gt; M.</td>
</tr>
<tr>
<td>Bayot et al.</td>
<td></td>
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<tr>
<td>Tetanus toxoid</td>
<td>Age range 13-65 yrs.</td>
<td>Local reaction, F &gt; M.</td>
</tr>
<tr>
<td>Diptheria/tetanus toxoid (Td)</td>
<td>Prospective, non-randomised study; Previously vaccinated: n = 201.</td>
<td>Local reaction, F &gt; M.</td>
</tr>
<tr>
<td></td>
<td>61 M, 140 F; age range 19-30 yrs. Not previously vaccinated: n = 147, 65 M, 82 F; aged &gt;45 yrs.</td>
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<tr>
<td>Diptheria/tetanus toxoid</td>
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<tr>
<td>Mark et al.</td>
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<tr>
<td>Diptheria/tetanus toxoid</td>
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<tr>
<td>Mark et al.</td>
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<tr>
<td>Diptheria/tetanus toxoid</td>
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<tr>
<td>Diptheria/tetanus toxoid Mark et al.</td>
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</tr>
<tr>
<td>Diptheria/tetanus toxoid</td>
<td>Prospective, open study; n = 111.</td>
<td>Sore arm and arm swelling at injection site, F &gt; M.</td>
</tr>
<tr>
<td>Diptheria/tetanus toxoid Middelkoop</td>
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</tr>
<tr>
<td>Diptheria/tetanus toxoid</td>
<td>Children aged 16 yrs.</td>
<td>Sore arm and arm swelling at injection site, F &gt; M.</td>
</tr>
<tr>
<td>Diptheria/tetanus toxoid</td>
<td>Safety data n = 92, 39 M, 53 F.</td>
<td></td>
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<tr>
<td>Diptheria/tetanus toxoid</td>
<td></td>
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</tr>
<tr>
<td>Diptheria/tetanus toxoid (DTP/P and DTP/P)</td>
<td>Retrospective, cohort, questionnaire study, n = 2000</td>
<td>No sex or age data.</td>
</tr>
<tr>
<td>Chung et al.</td>
<td>467 questionnaires returned and</td>
<td></td>
</tr>
<tr>
<td>Diptheria/tetanus toxoid (DTP/P and DTP/P)</td>
<td>30% of questionnaires responded. No sex or age data.</td>
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**Human Vaccine**

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135
<table>
<thead>
<tr>
<th>Table 1 Vaccines and sex differences in injection site reaction (ISRs) (continued)</th>
</tr>
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<tbody>
<tr>
<td><strong>Diphtheria-tetanus toxoid (DTaP)</strong></td>
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<tr>
<td>CDCR75</td>
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<tr>
<td><strong>Diphtheria-tetanus toxoid (Td)ap</strong></td>
</tr>
<tr>
<td>Blatter et al.50</td>
</tr>
<tr>
<td>n = 2,286, adults 19-64 years.</td>
</tr>
<tr>
<td>Tdap+ group: n = 1,148, 531 M, 917 F</td>
</tr>
<tr>
<td>Tdap- group: n = 728, 267 M, 461 F</td>
</tr>
<tr>
<td><strong>Diphtheria-tetanus toxoid (DTaP)</strong></td>
</tr>
<tr>
<td>Jackson et al.57</td>
</tr>
<tr>
<td>Arm injection: n = 1174, 611 M, 563 F</td>
</tr>
<tr>
<td>Thigh injection: n = 141, 70 M, 71 F</td>
</tr>
<tr>
<td><strong>Diphtheria-tetanus toxoid (dTP/VP and Tdap)</strong></td>
</tr>
<tr>
<td>Schrieks et al.38</td>
</tr>
<tr>
<td>n = 288 pre-immunization serology. 145 dTP/VP and 143 Tdap</td>
</tr>
<tr>
<td><strong>Diphtheria-tetanus toxoid (DTaP and dTP/VP)</strong></td>
</tr>
<tr>
<td>Skowronski et al.50</td>
</tr>
<tr>
<td><strong>Diphtheria-tetanus toxoid (Tdap)</strong></td>
</tr>
<tr>
<td>and Tetanus-diphtheria (Td)</td>
</tr>
<tr>
<td>Pichichero et al.52</td>
</tr>
<tr>
<td>Healthy adolescents and adults, Age 11 to 64 years. Single dose Tdap at 1st 1000 in each adolescent stratum, 11-13, 14-17 years and 800 in each adult stratum, 18-39, 40-49, 50-64 years.</td>
</tr>
<tr>
<td><strong>Inactivated, split-tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Cook et al.51</td>
</tr>
<tr>
<td>mean age 72.9 ± 8.9 yrs.</td>
</tr>
<tr>
<td><strong>Inactivated, subunit, tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Nichol et al.42</td>
</tr>
<tr>
<td>mean age 37.2 ± 10.6 yrs.</td>
</tr>
<tr>
<td><strong>Inactivated, split-tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Gwaltney et al.42</td>
</tr>
<tr>
<td>1,800 responded to questionnaire.</td>
</tr>
<tr>
<td><strong>Inactivated, subunit and whole cell tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Cote et al.44</td>
</tr>
<tr>
<td><strong>Inactivated, subunit tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Honkama et al.42</td>
</tr>
<tr>
<td>Number of males and females not given.</td>
</tr>
<tr>
<td>Age 66 yrs.</td>
</tr>
<tr>
<td><strong>Inactivated, split tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Darsalio et al.48</td>
</tr>
<tr>
<td>Age range 60-80 yrs.</td>
</tr>
<tr>
<td><strong>Inactivated, subunit tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Beyrer et al.49</td>
</tr>
<tr>
<td><strong>Inactivated, subunit whole virus or adsorbed whole virus tetradent inflammation vaccine</strong></td>
</tr>
<tr>
<td>Mazur et al.46</td>
</tr>
<tr>
<td>108 age range 14-30 yrs, 49 M, 59 F.</td>
</tr>
<tr>
<td>193 age range 31-60 yrs, 90 M, 103 F.</td>
</tr>
<tr>
<td><strong>Inactivated, split and fractionated meningeal and tetradent inflammation vaccines</strong></td>
</tr>
<tr>
<td>Ruchti et al.50</td>
</tr>
<tr>
<td>Analysis of n = 605 with data on 397 subsets (271 M, 104 ≥ 35 yrs, 167 ≥ 35 yrs, 7% M, 19% ≥ 35 yrs, 19% ≥ 35 yrs.)</td>
</tr>
<tr>
<td>Vaccines and sex differences in injection site reaction (ISRs) (continued)</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Inactivated, whole and split influenza vaccine</td>
</tr>
</tbody>
</table>
| Maslow et al.  
| Inactivated, zonal centrifuged, monovalent influenza vaccine  |
| Phillips et al.  
| Inactivated, split trivalent influenza vaccine                |
| Jackson et al.  
| Inactivated, split trivalent influenza vaccine                |
| Robb et al.  
| Inactivated, whole and split monovalent and trivalent influenza vaccine |
| Cox et al.  
| Inactivated, subunit trivalent influenza vaccine             |
| Kehrl et al.  
| Inactivated, split trivalent influenza vaccine                |
| Couch et al.  
| Inactivated, whole cell, trivalent influenza vaccine         |
| Panke et al.  
| 23 valent pneumococcal polysaccharide vaccine                |
| Sicon et al.  
| 23 valent pneumococcal polysaccharide vaccine                |
| Cook et al.  
| Anthrax vaccine                                               |
| Pitman et al.  
| Anthrax vaccine Pitmann  
| Pitmann et al.  
| Anthrax vaccine Wassenman et al.  
| Anthrax vaccine McNeil et al.  
| Anthrax vaccine Hoffman et al.  
| Anthrax vaccine Marano et al.  
| Anthrax vaccine Green et al.  
| Mumps or Measles-mumps vaccine                                |
| Seager et al.  
| Pentavalent Botulinum toxoid                                  |
| Pitman et al.  |

**Table 1**

Table 1 shows the vaccines and sex differences in injection site reaction (ISRs) (continued). The table includes various vaccines such as inactivated influenza, anthrax, and mumps, along with the number of participants and the sex distribution. For example, in the inactivated, whole and split influenza vaccine by Maslow et al., 4100 participants were observed, with 2380 females and 1720 males. The table also includes prospective and randomized studies with varying sample sizes and results.
pertussis-toxin (PT) IgE than those without reaction. However, the post-booster levels of PT IgE and post-booster levels of pertactin and diphtheria IgG were also greater in those with reaction compared with those without reaction.

The significance of the association between post-vaccination IgE elevation and local reactions, other than it is associated with aluminium adjuvancing is unclear.

Cell mediated immunity is clearly implicated in the generation of ISRs, with Schefele et al. observing that rates of positive skin testing with diphtheria/tetanus and acellular pertussis antigens were significantly higher in subjects with extensive injection site reactions than in those with none. These reactions with acellular pertussis-booster vaccination are associated with Th1 helper 2 (Th2) cytokine production due to Th2 polarizing of cellular memory to DTaP. Other explanations for the sex-difference in ISRs include injection route and hormonal status.

White et al. has suggested that this difference reflects subcutaneous rather than intramuscular injection of vaccine in females compared with males, as it is well documented that subcutaneous injection is associated with a greater rate of ISRs than intramuscular injection. Certainly, ultrasound studies in adults have shown that females have a thicker subcutaneous layer than males of comparable weight and body mass index (BMI). Consequently, injection with the same needle length is more likely to give subcutaneous injection rather than intramuscular injection in females compared with males.

This explanation may also be applicable to infants and children as although ultrasound studies have shown no sex difference in subcutaneous layer thickness, in the MRI/CT study by Lippert and Wall, females had thicker subcutaneous layers than males, thigh (subjects 2 months to 6 years) and deltoid (12 months to 18 years). These authors contend that the discrepancy between ultrasound and MRI/CT scan generated data reflects greater accuracy of the latter in measuring and showing the distinction between muscle and fat layers and lack of compression of the site being screened with MRI/CT scanning compared with ultrasonography. Also of interest in this context is the observation by Schefele et al. that in children aged 4-6 years the risk of large local reaction was significantly higher in children with a body mass index above the median value, suggesting that “the vaccine may not have been reliably injected into muscle in larger children”.

Although the Indian Academy of Pediatrics recommends pertussis containing vaccines be given by intramuscular injection, 8 cases (2 male, 6 female) of DTP vaccine-induced liposclerosis have been reported in a retrospective review conducted between 2000–2005 in three hospitals in New Delhi. The reason female fatty tissue exhibits this reaction is unclear but Haas et al. have suggested “hormone-related differences in resistance of adipocytes or in the receptor status of human adipose tissue”. Clearly, this explanation does not explain the female preponderance of this condition in infants vaccinated with DTP.

Finally, Migasen et al. suggest that the sex-difference in ISRs seen with the measles vaccine MMR in adults but not with children, reflects an endocrine rather than a chromosomal effect.

Sex-difference in injection site reactions have been observed in passive surveillance programs with vaccines in Australia, Canada, and the USA with females reporting a greater rate of ISRs than males. This method of assessing adverse events following immunization (AEFI) has a number of well recognized deficiencies. These include under reporting, potential for reporting bias and inability to determine the incidence of AEFI’s and to distinguish events caused by immunization from co-occurring events. The impact of gender bias on reporting in these programs is uncertain but this bias is evident in the study by Robb et al. and in studies with vaccinia vaccine. In a study with a split virulent influenza vaccine, Robb et al. observed that females reported a significantly greater rate of vaccine adverse reactions (most commonly soreness at the injection site) than males in a questionnaire survey (passive surveillance) with this effect considerably reduced in a random telephone survey (active surveillance). A randomized, controlled trial with vaccinia vaccine observed that females had a lower rate of ISRs than males. This result contrasts with that from a passive surveillance program in Australia in which females had a 1.6 times higher rate of adverse reactions with this vaccine compared with males.

Zhou et al. reporting on the Vaccine Adverse Event Reporting System (VAERS)—USA 1991–2001, concluded “in all of the adult age groups, a predominance among the number of women reporting was observed, but the difference in sex was minimal among children”, with a study conducted in 12/54 (22%) studies with subjects <1 year to 17 years.
Sex differences in injection site reactions with human vaccines

Failure to appreciate these data may explain why Jackson et al. (1997) offered an alternative explanation ("differential reporting of pain by parents based on the child's gender") for the greater rate of post-vaccination pain in females compared with males after administration of a 5th dose of a diphtheria/tetanus/acellular pertussis vaccine. Analysis of the sex-difference in ISRs was not reported in the majority of published vaccine clinical trials (1:0.7) but its recognition in 54 studies with 16 vaccines warrant that studies of vaccine reactogenicity recruit similar numbers of females and males that sex should be included as an independent variable in the statistical analysis of such trials. Additionally reporting of analysis of AEFI data by sex should become standard practice.

References

Typically, these differences have been recognised with subgroup analysis of trial results. It should now be mandatory that vaccine trialists recruit a representative sample of males and females into their studies to be able to detect sex-differences in immune response/adverse reactions with vaccines.

A study with sex-difference in immune response as its primary objective could be conducted with adult diphtheria/tetanus (ADT) vaccine in subjects over 60 years of age. A serological study in Australia showed that immunity in this population was deficient in 47% of subjects for diphtheria and 37% for tetanus. Previous studies (Cook, page 49) have shown that females have a reduced booster immune response to diphtheria toxoid compared with males, so a study with ADT could be conducted with subjects ≥ 60 years with no immunity to diphtheria to assess sex-difference in response.

Protection against vaccine preventable disease ought to be the right of every individual. However, as public fear shifts from concerns about sequelae of vaccine preventable diseases to concerns about “side effects” of vaccines, some patients and parents of patients are electing not to exercise this right. This has led to a resurgence of vaccine preventable diseases as seen with declining rates of measles-mumps-rubella (MMR) vaccination in the UK and Japan.
To address this situation conscious efforts are being made to advocate vaccination\textsuperscript{432} by increasing knowledge about vaccines and spreading this information in a clear, understandable manner to both policy makers and health professionals and patients or parents of patients.

Vaccination practice (route and site of administration of vaccines) is a fundamental yet poorly researched area of vaccinology. In this thesis, studies are presented which allow evidence based guidelines to be formulated for vaccination practice. These should help retain public confidence in vaccination programs by minimising the adverse reactions of vaccines whilst maintaining their efficacy.
APPENDIX 1. Guidelines and Recommendations for Vaccination by County/State/Province/County

<table>
<thead>
<tr>
<th>County/State/Province/County</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australia</strong></td>
<td></td>
</tr>
<tr>
<td>Immunisation Procedures, NH&amp;MRC 1st ed²⁵ 1975</td>
<td>No needle gauge or length, or angle of injection recommended. ADT, CDT, Triple antigen given by subcutaneous or intramuscular injection into alternate limbs.</td>
</tr>
<tr>
<td>Immunisation Procedures NH&amp;MRC 2nd ed²⁶ 1982</td>
<td>No needle gauge or length, or angle of injection recommended. ADT, CDT, Triple antigen given by deep subcutaneous or intramuscular injection into alternate limbs.</td>
</tr>
<tr>
<td>Immunisation Procedures NH&amp;MRC 3rd ed²⁷ 1986</td>
<td>No needle gauge or length or angle of injection recommended. Comment on site: p3 “Currently, preferred sites for intramuscular injections are the deltoid muscle of the upper arm and the anterolateral aspect of the upper thigh. This latter site is now recommended by the World Health Organization for routine immunisation of infants. In the past, the upper, outer quadrant of the buttock was the common site of intramuscular vaccination. The buttocks should not be routinely used as a vaccination site for infants and children; and, to avoid injury to the sciatic nerves, they are generally not used in adults. The central region of the buttocks should be avoided for all injections, the upper, outer quadrant should be used only for the largest volumes of injection or when multiple doses need to be given, such as when large doses of Ig must be administered. The site selected should be well into the upper, outer mass of the gluteus maximus and away from the central region of the buttocks. There is recent evidence that the immune response to a vaccine may be impaired by administration into the gluteal region.”</td>
</tr>
<tr>
<td>Immunisation Procedures NH&amp;MRC 4th ed²⁸ 1991</td>
<td>No needle gauge or length or angle of injection recommended. Comment on site: p8. “Currently, the preferred site for intramuscular injections is the deltoid muscle of the upper arm in adults and children aged 12 months and over; and the anterolateral aspect of the upper thigh in infants less than 12 months. Gluteal intramuscular injection is not recommended for vaccines.”</td>
</tr>
</tbody>
</table>
| The Australian Immunisation Procedures Handbook 5th edition²⁹ 1994 | A 23 gauge needle, 25mm in length, should be used for deep subcutaneous or intramuscular injections. No angle of injection recommended. Comment on site: p5 “The anterolateral thigh is the preferred site for intramuscular or deep subcutaneous injection in infants and children up to 12 months of age. In older children and adults, the deltoid may be used as an

p10,11:
Needle: “The standard needle for vaccine injections is 23 gauge and 25mm in length.”
Angle: “The needle should be inserted at an angle of 45 to 60 degrees, neural and vascular damage are more likely if the needle is inserted at a 90 degree angle. Insertion at 45 to 60 degrees may result in less tissue resistance as the needle penetrates the muscle.”
Comment on site: “The anterolateral thigh is the preferred site for vaccination in infants and children under 12 months of age. The deltoid region is an alternative site for vaccination in older children (those who have commenced walking) and adults. The risk of sciatic nerve damage from gluteal injections is greatest in infants because the position of the nerve is more variable. Another reason for preferring the anterolateral thigh is that the muscle mass is larger than in the gluteal region; some attempted gluteal intramuscular injections result in unintended subcutaneous injection, with more severe local reactions. Finally, hepatitis B and rabies vaccines are less immunogenic if injected in the buttock; these vaccines should not be injected into the buttocks in subjects of any age. Despite the above recommendations to use the anterolateral thigh for injection of vaccines, some health care providers have been reluctant to abandon their traditional practice of injection into the buttock. Because of this, we are obliged to repeat the warning that if vaccines are injected into the buttock, particular care must be taken to inject into the upper, outer quadrant to avoid the sciatic nerve.”


p8
Needle: “The standard needle for administering intramuscular (IM) vaccines is 23 gauge and 25mm in length.”
Angle: “Inserting the needle at a 45 to 60 degree angle results in less tissue resistance as the needle penetrates the muscle. The 23 gauge needle allows the vaccine to be injected slowly into the muscle rather than being forced in under high pressure, which can cause injection pain.”
Comment on site: “The anterolateral thigh is the preferred site for vaccination in infants under 12 months of age. The deltoid region is the preferred site for vaccination in older children (those who have commenced walking) and adults. The reasons for preferring the vastus lateralis muscle in infants under 12 months are as follows:

- It avoids the risk of sciatic nerve damage from gluteal injections. The risk of sciatic nerve damage from gluteal injections is greatest in infants because
the position of the nerve is more variable.
- Some vaccines (e.g., hepatitis B vaccine) are less immunogenic if injected into the gluteal region.
- The anterolateral thigh has a larger muscle mass than the gluteal region and therefore has a reduced risk of severe local reactions.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Page/Site</th>
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</table>
Angle: angled entry at 60 degrees results in less tissue resistance as the needle penetrates the muscle.”  
Comment on site: p6 “In adults, vaccine injections should not be given in the buttocks because of the possibility of a suboptimal response. However, IM immunoglobulin can be administered into the upper, outer aspect of the buttocks.” |
| Immunisation Guidelines, South Australian Health Commission33 1989.      | p10 “Generally either 25 or 27 gauge needles are used for the majority of routine child immunisation (with the exception of BCG).” |
| About Immunisation – for Providers, South Australian Department of Health34, 200234. | Page 1. Comment on site: “The top, outer part of the thigh is the preferred site for injections for infants under the age of 12 months. The buttocks should never be used because of the risk of sciatic nerve damage.” |
| Immunisation – The Basics, South Australian Immunisation Co-ordination Unit35, 2004. | p33, Same as Australian Immunisation Handbook 7th edition, p8. “Using a 23 gauge 25mm needle, inserting the needle at an angle (60 degrees) results in less tissue resistance as the needle penetrates the muscle.” |
| Brazil                                                                  |                                                                           |
| Manual de Normas de Vacinacao, Brazil 3rd edition37 2001                | p25, Comment on site: “Hepatitis B vaccine in infants, not given in gluteal area as associated with reduced antibody production, at least in adults.” |
| Canada                                                                   |                                                                           |
| Canadian Immunization Guide 5th edition38 1998                          | For intramuscular injection: 22mm (7/8”) length needle for infants, 25mm (1”) for others, no angle of injection mentioned.  
Comment on site: “Because of decreased immunogenicity reported with several vaccines, the buttock is not recommended as an immunization site, except when large volumes must be given, e.g., immunoglobulin. If the buttock is used, care must be exercised to avoid injury to the sciatic nerve by selecting a site in the upper, outer quadrant of the gluteus maximus and avoiding the central area.” |
<table>
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<tr>
<th>Source</th>
<th>Needle and Angle Recommendations</th>
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</table>
Angle: “IM injections are administered at a 90 degree angle into the vastus lateralis muscle (anterolateral thigh) in infants < 1 year of age.” 
Comment on site: “The buttock should not be used for active immunization. Immunogenicity is lower for hepatitis B and rabies vaccine if given in the buttock, probably because of injection into adipose tissue where the vaccine is not well mobilized.” |
| British Columbia Communicable Disease Control Immunization Program Section IV – Vaccine Administration 2003 | Needle: 
- “For infants and toddlers a 7/8” – 1” needle is usually used, depending on the muscle size and the amount of subcutaneous tissue. 
- Use a 21 to 25 gauge needle depending on the viscosity of the biological product.” 
Comment on site: Ventrogluteal site: 
- Do not use this site for vaccine administration. 
- The ventrogluteal site is the preferred site for the IM injection of large volumes of immune globulin preparations. 
- This site can be used in those over 7 months of age.” |
| Saskatchewan Immunization Manual 2006                                 | Needle: 25g, 1” or longer needle for vaccines given intramuscularly. 
Angle: 90° angle into vastus lateralis. 
Comment on site: “Use the dorsogluteal site, upper, outer quadrant of the buttock only for large volume administration (e.g. gamma globulin), as decreased immunogenicity from the site has been reported. An alternative location is the ventrogluteal injection site. Research shows it is safer for injection than the dorsogluteal site.” |
| India                                                                 | No needle gauge or length or angle of injection recommended. 
Comment on site: “The DTP must be injected intramuscularly and the preferred site is the anterolateral aspect of the thigh. The gluteal region is better avoided for 2 reasons: 
Occasionally, the needle (and the vaccine itself) hits the sciatic nerve, causing injury which may result in foot drop or even more extensive paralysis. 
Secondly, the vaccine may be deposited in the fat pad adjacent to the muscle tissue, in that case, the immune response is likely to be less than what would occur after true IM injection. This phenomenon has been shown to be important in the case of HB and rabies vaccine;” |
although not shown directly with DPT, it is better to adhere to the principle of no IM injection gluteally.”

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<tr>
<th>Ireand</th>
<th>Immunisation Guidelines for Ireland. 2002 edition&lt;sup&gt;44&lt;/sup&gt;</th>
<th>Needle: birth to 12 months of age, 22-25 gauge, 25mm needle. Angle: Insert needle at an 80° to 90° angle to the skin. Comment on site: Vastus lateralis in infants (birth to 12 months of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaysia</td>
<td>Malaysian Immunisation Manual, Academy of Medicine of Malaysia, College of Paediatrics&lt;sup&gt;45&lt;/sup&gt;. 2001</td>
<td>Comment on site: Upper outer quadrant of buttock – is associated with reduced antibody level production.</td>
</tr>
<tr>
<td>Mexico</td>
<td>Manual de Vacunacion, Mexico&lt;sup&gt;47&lt;/sup&gt; 2005.</td>
<td>p185-9 Needle/angle: Intramuscular injection, needle at 90° to skin’s surface in anterolateral thigh in infants. Comment on site: Tendency not to use gluteal region for intramuscular injection to avoid injury to the sciatic nerve.</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Immunisation Handbook New Zealand&lt;sup&gt;48&lt;/sup&gt; 1996</td>
<td>p 30 and 34 Needle size: 25g 25mm for vastus lateralis. Angle: “An angle of 45° to 60° to the skin enables the needle to penetrate muscle fibres smoothly.” Comment on site: “Gluteal injections are not recommended use of the buttock for vaccines administration is to be avoided because of: • Poor vaccine uptake in fat which has been documented for hepatitis B vaccine and may apply to other vaccines (the gluteal region is mostly fat until the child has been walking for some time). • Possible increased risk of abscess formation. • Possible risk of sciatic nerve involvement.</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Immunisation Handbook New Zealand&lt;sup&gt;49&lt;/sup&gt; 2002.</td>
<td>p37-8 Needle: 23-25 x 16mm or 25mm into vastus lateralis. Angle: “needle should be inserted at a 60-70 degree angle to the long axis of the leg.” Comment on site: “The buttock should not be used for the administration of vaccines in infants or young children, as the buttock region is mostly subcutaneous fat until the child has been walking for at least 9-12 months.”</td>
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<td>Source</td>
<td>Notes</td>
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<tr>
<td><strong>Immunisation Handbook New Zealand</strong> §50 2006</td>
<td>Needle: as for Handbook 2002. Angle: “The needle should be inserted at a 60-70 degree angle (or 90 degree World Health Organization technique) towards the long axis of the leg. Comment on site: as for the 2002 edition plus “use of the buttock is not recommended for adult vaccination either, as the buttock subcutaneous layer can vary from 1-9cm and IM deposition may not occur.”</td>
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<td><strong>Spain</strong></td>
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<tr>
<td>Manual de Vacunas en Pediatric, Spain §51 2005</td>
<td>p216-21. Needle: 22-23 gauge, 25mm vastus lateralis in infants. Angle of injection: 90° Comment on site: “Gluteal injection is advised against in infants for two reasons – possible injury to the sciatic nerve and thick fat over area makes possibility of vaccine being deposited into muscle smaller with reduced immune response.”</td>
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<tr>
<td><strong>Sri Lanka</strong></td>
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<tr>
<td>Immunization Handbook, National Expanded Programme on Immunization. Sri Lanka §52 2002.</td>
<td>p12 Needle: 23g, 25mm Angle: “the needle should be inserted at an angle of 45 to 60 degrees into the vastus lateralis.” “Comment on site and technique: Neural and vascular damage are more likely if the needle is inserted at a 90 degree angle. Insertion at 45-60 degrees may result in less tissue resistance as the needle penetrates muscle. The hepatitis B and rabies vaccines are less immunogenic if injected into the buttock. Therefore these vaccines should not be injected into the buttock in subjects of any age.”</td>
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<td><strong>United Kingdom</strong></td>
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<tr>
<td>Immunisation against Infectious Disease. Edward Jenner Bicentenary edition §53 1996</td>
<td>p 15 and 103. Needle: 25 gauge Angle: 90° Comment on site: anterolateral thigh in infants. The buttock must not be used because vaccine efficacy may be reduced.</td>
<td></td>
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<tr>
<td>Immunizing Children. Mayon-White and Moreton §54 1998</td>
<td>p 43 Needle: 23 gauge, “the wider bore needle allows the vaccine to dissipate over a wider space, thus reducing the risk of localized redness and swelling.”</td>
<td></td>
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<tr>
<td>UK Guidelines on Best Practice in Vaccine Administration §55 2001</td>
<td>Needle: 25mm Angle: 90° to the skin’s surface for intramuscular injection Comment on site: anterolateral thigh in infants.</td>
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<tr>
<td>Position Statement on Injection techniques, UK §56. 2002</td>
<td>Needle: insufficient evidence to recommend whether a larger (1”) needle should be used in infants and young</td>
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<tr>
<td>Source</td>
<td>Needle, Angle and Site Details</td>
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<tr>
<td>Immunization against Infectious Disease. “The Green Book”*57</td>
<td>Needle: 23gauge or 25 gauge, 25mm. Angle: 90° to the skin for IM injections. Comment on Site: “Anterolateral thigh in infants. Immunisation should not be given into the buttock due to the risk of sciatic nerve damage and possibility of injecting the vaccine into fat rather than muscle. Injection into fatty tissue of the buttock has been shown to reduce the immunogenicity of hepatitis B and rabies vaccines.”</td>
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<tr>
<td>United States of America</td>
<td>p 19, 23rd edition; p9, 24th edition; p20, 27th edition. Comment on site: Ordinarily, the upper, outer aspect of the buttock should not be used for immunisation in infants because the gluteal region consists mostly of fat until the child has been walking for some time and because of the possibility of damaging the sciatic nerve.</td>
<td></td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention. Epidemiology and prevention of vaccine preventable diseases. “The Pink Book” 9th edition 62. 2006.</td>
<td>Needle, Angle and site: As for 9th edition. Comment on site: “The muscles of the buttock have not been used for administration of vaccines in infants and children because of concern about potential injury to the sciatic nerve, which is well documented after injection of antimicrobial agents into the buttocks. If the gluteal muscle must be used, care should be taken to define the anatomic landmarks (if the gluteal muscle is chosen, injection should be administered lateral and superior to a line between the posterior, superior iliac spine and the greater trochanter or in the ventrogluteal site, the center of a triangle bounded by the anterior, superior iliac spine, the tubercle of the iliac crest and the upper border of the greater trochanter).”</td>
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</tbody>
</table>
| General Recommendations on Immunization: Recommendations of the Advisory Committee on Immunization Practice (ACIP) MMWR*64 1994. | p6. Needle: 22 to 25gauge, 7/8 to 1” long. Comment on site: “Anterolateral thigh in infants < 12 months of age. The buttock should not be used routinely for active vaccination of infants, children or adults because of the potential risk of injury to the sciatic nerve. In addition, injection into the buttock has been associated with decreased immunogenicity because of inadvertent
<table>
<thead>
<tr>
<th>Reference</th>
<th>Needle, Angle, Site</th>
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<tr>
<td>General Recommendation on Immunization: Recommendations of the Advisory Committee on Immunization Practice (ACIP) and the American Academy of Family Physicians (AAFP). MMWR^65 2002</td>
<td>Needle: 22 to 25 gauge, 7/8 to 1” long in infants &lt; 12 months of age. Angle: 90°. Comment on site: “Anterolateral thigh. The buttock should not be used for administration of vaccines or toxoids because of the potential risk of injury to the sciatic nerve.” Other comment as for 1994 statements in MMWR.</td>
</tr>
<tr>
<td>General Recommendations on Immunization: Recommendations of the Advisory Committee on Immunization Practice (ACIP) MMWR^66 2006.</td>
<td>Needle, angle and site as for MMWR 2002. Comment on site: “Variation from the recommended route and site can result in inadequate protection. In adults but not in infants, the immunogenicity of hepatitis B is substantially lower when the gluteal rather than the deltoid site is used for administration.”</td>
</tr>
<tr>
<td>Georgia Immunization Program Manual^68, 2006</td>
<td>Comment on site: “Hepatitis B vaccine should be administered in the deltoid or anterolateral aspect (vastus lateralis) of the thigh. It should not be administered in the buttock.”</td>
</tr>
<tr>
<td>Oregon State Public Health Division DHS Immunization Program^72 2006</td>
<td>Needle/site: 22 – 25 gauge, 1 – 2” in vastus lateralis in infants and toddlers lacking adequate deltoid mass. Angle: 90° Comment on site: “Ventrogluteal site recommended for diphtheria, tetanus, pertussis, Haemophilus influenzae type b, hepatitis A and inactivated influenza but not hepatitis B vaccine for children 3 years to adults. The dorsal gluteal buttock site should never be used as a vaccination site for active vaccination.”</td>
</tr>
<tr>
<td>Allegheny County Health Department Immunization Policy Manual^73 1993</td>
<td>p23. Comment on site: “Hepatitis B vaccine: vaccine must be given intramuscularly, but not in the gluteal region.”</td>
</tr>
<tr>
<td>Los Angeles County, Department of Health Services^74</td>
<td>Needle: 23 – 25 gauge, 1”. Angle: 90° to the skin’s surface for IM injection.</td>
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<td>Year</td>
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<td>Immunization in Practice: A guide for health workers who give vaccines: when and how to give vaccines. WHO75 1984</td>
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<td>2001</td>
<td>Introduction of hepatitis B vaccine into childhood immunization services. WHO76 2001</td>
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<td>2001</td>
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   when and how to give vaccines. WHO, Geneva. EPI/PHW/84/3Rev.1

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