Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

High frequency ultrasound to assess skin thickness in healthy adults

T.J.S. Van Mulder [a,b,*], M. de Koeijer [a], H. Theeten [b], D. Willems [a], P. Van Damme [b], M. Demolder [c], G. De Meyer [c], K.C.L. Beyers [a,d], V. Vankerckhoven [a,b]

[a] Novosanis, Bijkhoevelaan 32c, BE-2110 Wijnegem, Belgium
[b] Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, BE-2610 Wilrijk, Belgium
[c] Department of Pharmaceutical Sciences, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, BE-2610 Wilrijk, Belgium
[d] Voxdale, Bijkhoevelaan 32c, BE-2110 Wijnegem, Belgium

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Skin thickness
Intradermal
Injection device
Vaccine delivery

ABSTRACT

Background: Intradermal immunization is gaining increased attention due to multiple factors: (1) intradermal (ID) vaccination has been shown to induce improved immunogenicity compared to intramuscular (IM) vaccination; (2) ID vaccination has been shown to have a dose-sparing potential over IM leading to a reduced vaccine cost and an increased availability of vaccines worldwide. However, the currently used Mantoux technique for ID injection is difficult to standardize and requires training.
The aim of the study was (1) to assess the epidermal and dermal thickness at the proximal ventral and dorsal forearm (PVF & PDF) and deltoid in adults aged 18–65 years (2) to determine the maximum penetration depth and needle characteristics for the development of a platform of medical devices suited for intradermal injection, VAX-ID™.
Materials and methods: Mean thickness of the PVF, PDF and deltoid were measured using high-frequency ultrasound of healthy adults aged 18–65 years. Correlation with gender, age and BMI was assessed using Mann-Whitney *U* Test, Spearman correlation and Wilcoxon Signed Ranks Test, respectively.
Results: Results showed an overall mean skin thickness of 1.19 mm (0.65–1.55 mm) at the PVF, 1.44 mm (0.78–1.84 mm) at the PDF, and 2.12 mm (1,16–3.19 mm) at the deltoid. Thickness of PVF & PDF and deltoid were significantly different for men vs women ($p_{mean} < 0.001$, <0.001, <0.001, and $p_{min} < 0.001$, 0.012, <0.001, respectively). A significant association was found for age at the deltoid region ($p < 0.001$). Skin thickness for PVF, PDF & deltoid was significantly associated to BMI ($p < 0.001$).
Conclusion: Significant differences in skin thickness were seen for the PVF, PDF and deltoid region for gender, and BMI. Age only influenced the skin thickness at deltoid region. A needle length of 1.0 mm is best option for intradermal injection at the dorsal forearm (NCT02363465).

1. Introduction

The skin is one of the largest organs of the body providing the first line of defence against invading pathogens and one of the most obvious sites for achieving immune responses. The dermal skin layer is highly vascularized, has an efficient lymphatic drainage network, and contains dendritic cells [1]. All these factors stimulate the overall immune response, leading to a potential dose-sparing effect [2]. For example, ID influenza vaccination is equally or even more immunogenic compared to intramuscular (IM) vaccination [3]. At present, ID vaccines target influenza and rabies, and experimentally hepatitis B and polio [4–7] and are also used for therapeutic vaccination [8].

Most ID injections are performed using the Mantoux-technique, which implies the insertion of a needle almost parallel to the skin surface. Commonly known drawbacks include the high amount of required expertise, lack of standardization, pain sensation during injection, and decreased vaccine efficacy due to leakage [9,10]. To address these issues novel approaches for intradermal injections have been made commercially available (i.e. Soluvia™, MicronJet600™) or are in development (e.g. vaccine patch, coated, dissolving or solid microneedles), among others hollow microneedles which allow a delivery of medical substances in larger volumes [11–13]. A particular example of a hollow microneedle system is VAX-ID™, which is currently being developed by the medical device company Novosanis nv (Wijnegem, Belgium). The VAX-ID™ device contains a short and thin needle which allows

* Corresponding author at: University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, BE-2610 Wilrijk, Belgium.
E-mail address: timothi.vanmulder@uantwerpen.be (T.J.S. Van Mulder).

http://dx.doi.org/10.1016/j.vaccine.2016.07.039
0264-410X/

for a less painful injection by a perpendicular injection into the skin. Due to its unique (de)activation mechanism, by which the needle auto-retracts after injection, the use of this device is safe since it prevents needle-stick injuries and re-use [14].

The aim of the current study was to assess the epidermal and dermal thickness at the proximal ventral and dorsal forearm (PVF & PDF) and deltoid in adults aged 18–65 years. This will allow determining the maximum penetration depth and needle characteristics for the development of a platform of medical devices suited for intradermal injection, VAX-ID™ and ensure an accurate ID injection.

2. Materials and methods

2.1. Study method

Skin thickness was investigated at three body sites, i.e. the ventral and dorsal side of the proximal forearm (PVF & PDF) and the deltoid region. High-frequency ultrasound (HF-US, VEVO® 2100, VisualSonics Inc.) was used with the MS550D probe (22–55 MHz) as imaging technique.

The probe was set at 40 MHz and image depth 6.00 mm, resulting in a 40 μm axial resolution and a 90 μm lateral resolution. One focal zone was set at the junction between dermis and hypodermis.

For the deltoid region, scans were taken manually from the base of the deltoid muscle until the level of the acromion.

Ultrasound images clearly distinguished upper three skin layers (Fig. 1). Distances were measured by drawing straight lines perpendicular from the skin surface to the dermal – hypodermal junction using VisualSonics Vevo® LAB 1.7.0 software. From these measurements mean skin thickness per body site per subject was calculated.

2.2. Study population

This study was conducted at the University of Antwerp. Eligible subjects included Dutch speaking healthy, Caucasian adults aged 18–65 years. Pregnant or lactating women were excluded, as well as people using an corticoid containing ointment, crème, or gel and persons suffering from skin diseases. Recruitment took place from January to April 2015 via the University of Antwerp, the Antwerp University Hospital, the Centre for the Evaluation of Vaccination (CEV, University of Antwerp) and social media.

Demographics and regular use of medication were surveyed through a questionnaire. Upon entering the study subjects were weighed (kilograms) and measured (meters) to calculate the Body Mass Index (BMI). Measurements were done without shoes and coats, weight of remaining clothes was estimated 1 kg and body weight was corrected as such. Age was divided into different categories, adjusted from Laurent et al. [17]. BMI categories were based on WHO criteria [WHO Expert Committee, 1995].

2.3. Statistical methods

Prior to the study, a sample size calculation for multiple regression analysis (Danielsoper.com) pointed out that at least 86 persons were needed. This number was calculated based on an effect size of 0.15; a statistical power of 85%; the measurement of 3 predictors; and a p-value of 0.05. The effect size of 0.15 was chosen because it was considered clinically relevant. If the average thickness is 1.5 mm, a deviation towards 1.275 or 1.725 (15%) would implicate that another needle type is needed for an accurate intradermal injection.

SPSS 22.0 was used for statistical processing of the data. Mean skin thickness was calculated per body site. The influence of gender on skin thickness was examined using boxplots and Mann-Whitney tests. Subsequently, the association of age and BMI with skin thickness was investigated using scatterplots and Spearman correlation analyses. To analyze whether the different sections within one body site differed significantly in skin thickness, pairwise comparisons of means were performed using the Wilcoxon test. Correlation with gender, age and BMI was assessed using Mann-Whitney *U* Test, Spearman correlation and Wilcoxon Signed Ranks Test, respectively. The three locations were compared using Wilcoxon tests. A p-value of <0.05 was considered statistically significant.

To investigate the coinciding influence of multiple demographic characteristics on skin thickness, both linear models and ANOVA models were generated. First, linear models in which all continuous variables were added and only gender was inserted as a categorical variable. For these models, the adjusted R^2 provided the predictive value of the model. In addition, AIC values were compared to evaluate model strength. In the step-wise model building selection criterion for excluding a variable was set at $p > 0.10$. Second, ANOVA models in which all variables were inserted in categories, to be able to compare outcomes to prior knowledge.

2.4. Ethical considerations

The study was approved by the Ethics Committee of the University Hospital Antwerp, Belgium (Belgian Registration Number B300201523257) and registered at clinicaltrials.gov (NCT02363465). All subjects gave their informed consent prior to participation in the study. All collected data was coded.

3. Results

3.1. Subjects

A total of 100 subjects were enrolled aged 18–64 years (Table 1). 50 males and 50 females were evenly distributed over the four age groups. Therefore mean age and range between males and females are similar (mean: 40.8, range: 18–64). In total, 3% of the study population was underweight (BMI < 18.5), 59% had a BMI in the normal range (18.50–24.99), 27% was overweight (BMI ⩾ 25), and 11% suffered from obesity (BMI ⩾ 30). Mean BMI significantly differed for gender ($p = 0.002$), the effect was provoked by age groups 41–50 and 51–65 years ($p = 0.002$, data not shown). In these groups, BMI was significantly higher in males compared to females.

3.2. Skin thickness at three body sites

Skin thickness gradually increased from ventral to dorsal side of the proximal forearm and further to the deltoid region. The mean skin thickness was 1.19 mm at the PVF (95%CI: 1.16–1.22), 1.43 mm at the PDF (95%CI: 1.40–1.47) and increasing to 2.12 mm at the deltoid (95%CI: 2.05–2.19). The studied body sites significantly differed in mean skin thickness ($p < 0.001$). Representative ultrasound images of mean skin thickness for all three body sites are shown (Fig. 1).

Pairwise comparisons of skin thickness on left and right side were performed using the Wilcoxon test. The difference between mean skin thickness of left and right PVF was 0.030 mm ($p < 0.001$), for the dorsal side, the mean difference was 0.019 mm ($p = 0.003$) and no difference was found for the deltoid ($p = 0.747$). Although differences are statistically significant, these small differences don't affect needle characteristics. Consequently, left and right regions were joined in further analyses.

Please cite this article in press as: Van Mulder TJS et al. High frequency ultrasound to assess skin thickness in healthy adults. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.07.039