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

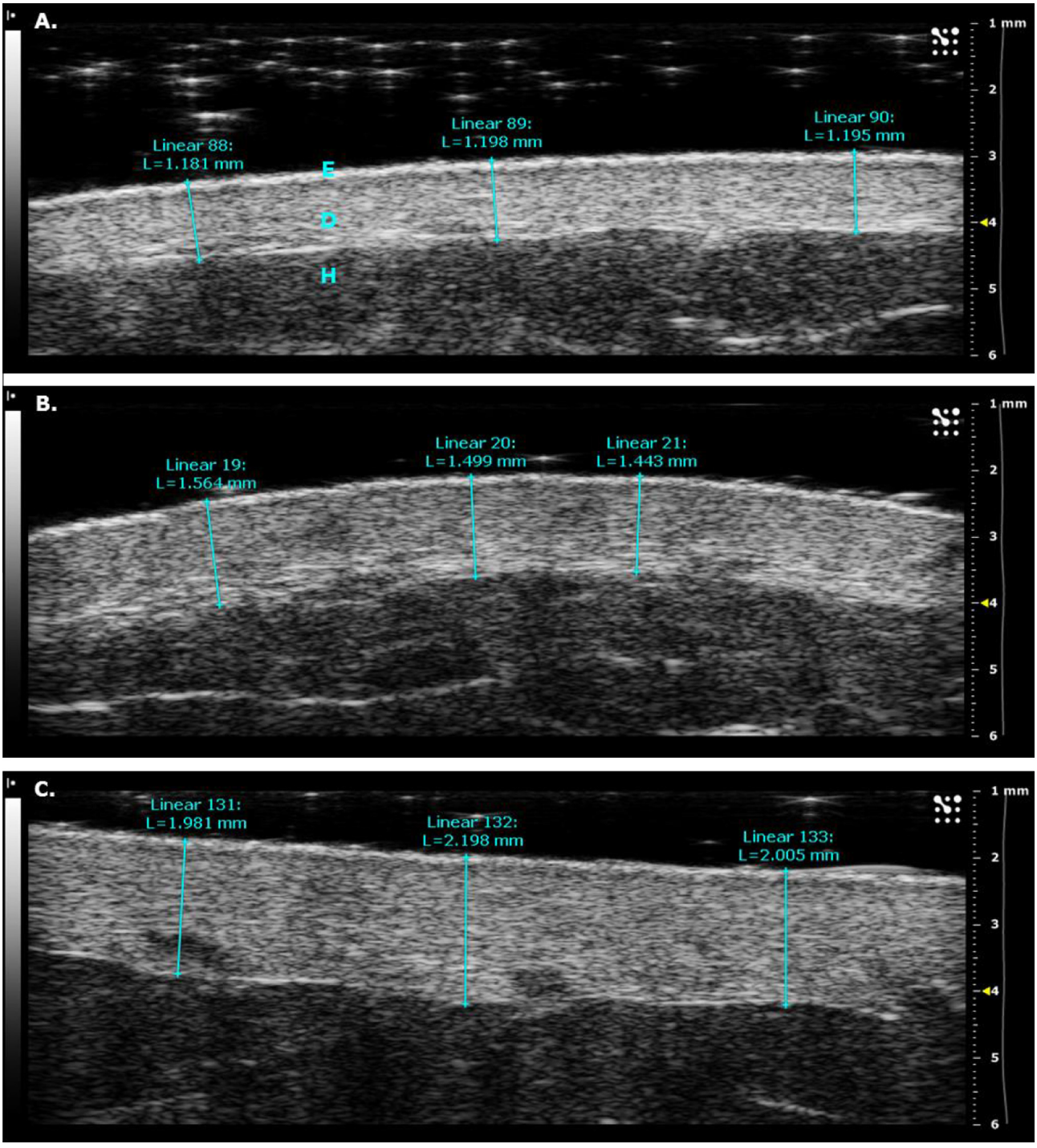

Fig. 1. Ultrasound images of mean skin thickness at three body sites. A. Ventral side of the proximal forearm. B. Dorsal side of the proximal forearm. C. Deltoid region. The ultrasound image clearly distinguished the upper three skin layers. First, the epidermis (E), characterized by a hyper-reflecting band. Second, the dermis (D), visualized as a less-reflecting band compared to the dermis. Last, the hypodermis (H), showed the lowest reflecting capacity. Skin thickness was measured perpendicular from the skin surface, including epidermis, to the junction between dermis and hypodermis (blue bars). Minor reflections appeared in the gel-layer, located on top of the epidermis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Skin thickness and gender

To determine the effect of gender on skin thickness, data is graphically presented in a boxplot and Mann-Whitney tests were performed (Fig. 2). Females showed a lower skin thickness at all three body sites than males. The mean skin thickness for the PVF was 1.26 mm for males and 1.12 mm for females ($p < 0.001$). The PDF was on average 1.49 mm and 1.38 mm for males and females, respectively ($p < 0.001$). Last, for the deltoid region, mean skin thickness was 2.29 mm and 1.94 mm for males and females, respectively ($p < 0.001$).

3.4. Skin thickness and age

Spearman correlation analyses were performed generating scatterplots on age and skin thickness. A significant but weak

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

Table 1
Demographics healthy volunteers ($n = 100$).

Characteristics		Males ($n = 50$)	Females ($n = 50$)
Age (years): mean (range)		40.8 (18–64)	40.8 (18–64)
Count per age group			
	18–30	14	14
	31–40	10	10
	41–50	11	11
	51–65	15	15
BMI (kg/m^2): mean (range)[a]		25.7 (17.9–42.3)	23.1 (18.0–37.8)
Count per BMI group			
	<18.50	1	2
	18.50–24.99	24	35
	⩾25	15	12
	⩾30	10	1

[a] Mann-Whitney U test, $p = 0.002$.

correlation was found at the deltoid region ($R^2 = 0.135$; $p < 0.001$) and correlation was not significant for the proximal forearm (PVF $p = 0.116$; PDF $p = 0.146$).

Since above-mentioned analyses showed a significant influence of gender on skin thickness, the gender-specific relationship between age and skin thickness was also examined at the deltoid region. Mean deltoid skin thickness correlated stronger with age for females ($p < 0.001$; $R^2 = 0.230$) compared to males ($p = 0.013$; $R^2 = 0.140$).

3.5. Skin thickness and BMI

A positive correlation between BMI and mean skin thickness was present for all body sites and highest at the deltoid (PFV: $R^2 = 0.173$, $p < 0.001$; PDF: $R^2 = 0.184$, $p < 0.001$; deltoid: $R^2 = 0.420$, $p < 0.001$). Increasing BMI was associated with an increased skin thickness.

3.6. Multivariate analysis

Table 2 shows the final linear model as the equation of the best subset, gender was included in all models. The model with the highest predictive value ($p < 0.001$; R^2: 0.534) was seen for deltoid region. At this site, skin thickness was positively associated with gender, age and BMI. Models for skin thickness at the PVF and PDF contained gender and BMI.

Gender had the highest contribution in all three models, most at the deltoid region ($b_1 = 0.525$, $p = 0.007$), followed by the PVF ($b_1 = 0.124$, $p < 0.001$) and last the PDF ($b_1 = 0.084$, $p = 0.007$). BMI contributed most at the deltoid side ($b_1 = 0.042$, $p < 0.001$), the forearm both dorsal and ventral were less influenced (respectively, $b_1 = 0.013$, $p < 0.001$; $b_1 = 0.009$, $p = 0.002$).

Additionally, ANOVA models (Table 3) were established using only categorical data with female; 51–65 years; and BMI ⩾ 30, as reference category. These models confirmed that males had significantly higher skin thickness in all studied body sites. Only age group 2 (31–40 years) showed significantly lower skin thickness at PVF and deltoid site than the oldest age group, but not at dorsal site. BMI was only significant in the model for the deltoid site, where BMI group 2 (18.50–24.99) had significantly lower thickness than the highest BMI category.

4. Discussion

To allow determining the maximum penetration depth and needle characteristics in adults, the current study assessed and

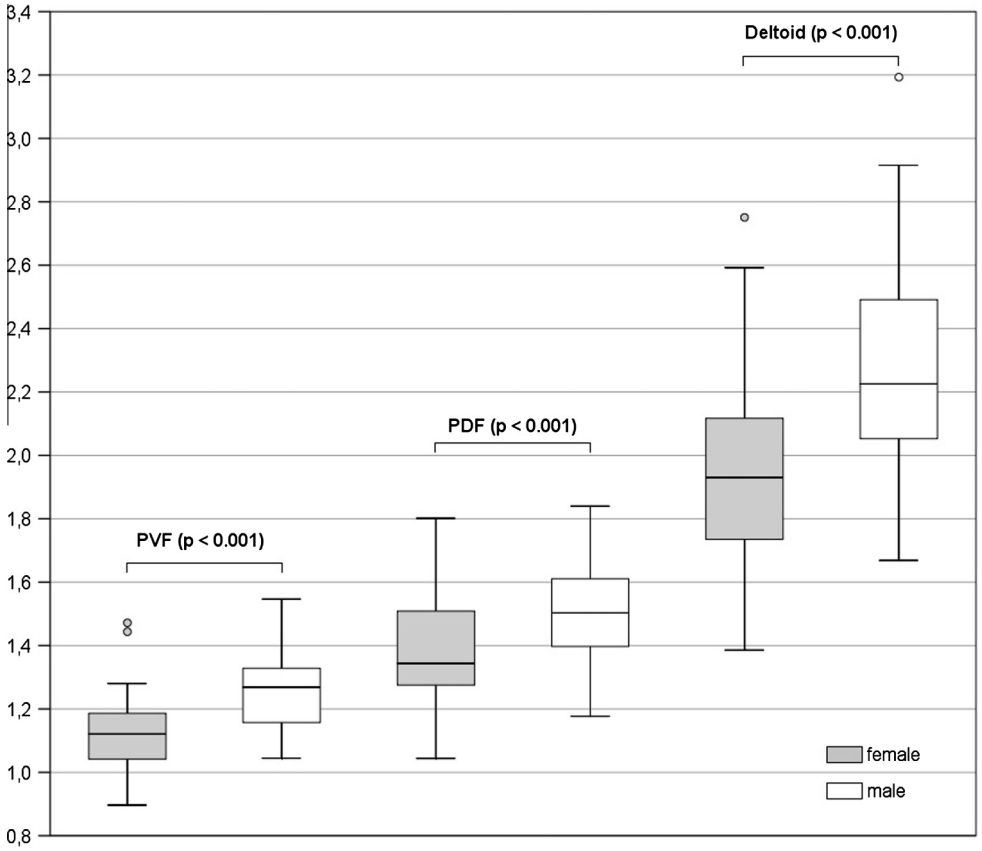

Fig. 2. Comparison of mean skin thickness between males and females at 3 body sites (Mann-Whitney U test).

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

Table 2
Linear models to predict mean skin thickness at investigated body sites.

Model	Equation of best subset	Constants	*p*-value	R^2
Mean Ventral	Skin Thickness = $b_0 + b_1 * \text{Gender} + b_2 * \text{BMI}$	$b_0 = 0.912$	<0.001	0.345
		$b_1 = 0.124$	<0.001	
		$b_2 = 0.009$	0.002	
Mean Dorsal	Skin Thickness = $b_0 + b_1 * \text{Gender} + b_2 * \text{BMI}$	$b_0 = 1.077$	<0.001	0.232
		$b_1 = 0.084$	0.005	
		$b_2 = 0.013$	<0.001	
Mean Deltoid	Skin Thickness = $b_0 + b_1 * \text{Gender} + b_2 * \text{Age} + b_3 * \text{BMI} + b_4 * \text{Gender} * \text{Age}$	$b_0 = 0.691$	<0.001	0.534
		$b_1 = 0.525$	0.001	
		$b_2 = 0.007$	0.007	
		$b_3 = 0.042$	<0.001	
		$b_4 = -0.007$	0.052	

For categorical variable gender, females (gender = 2) were selected as the reference category.

Table 3
ANOVA models to predict mean and minimal skin thickness at investigated body sites.

Model	Parameter	Constants	*p*-value	R^2
Mean Ventral	Intercept	1.159	<0.001	0.323
	[Gender = 1]	0.147	<0.001	
	[Age group = 1]	−0.055	0.065	
	[Age group = 2]	−0.096	0.004	
	[Age group = 3]	−0.038	0.233	
Mean Dorsal	Intercept	1.516	<0.001	0.187
	[Gender = 1]	0.088	0.030	
	[BMI group = 1]	−0.114	0.096	
	[BMI group = 2]	−0.156	0.050	
	[BMI group = 3]	−0.116	0.053	
Mean Deltoid	Intercept	2.322	<0.001	0.476
	[Gender = 1]	0.271	<0.001	
	[Age group = 1]	−0.185	0.014	
	[Age group = 2]	−0.240	0.002	
	[Age group = 3]	−0.043	0.554	
	[BMI group = 1]	−0.318	0.072	
	[BMI group = 2]	−0.327	0.001	
	[BMI group = 3]	−0.115	0.220	

Last categories were selected as reference categories, for gender (gender = 2, females); for age (age group = 4, 51–65 y); and for BMI group (BMI group = 4, $\geqslant 30$ kg/m^2).

compared the skin thickness at the proximal forearm (PVF & PDF) and deltoid in an age and gender stratified sample using highly accurate imaging.

The results clearly demonstrated that overall mean thickness increased from the PVF to the PDF and deltoid region. An important gender effect was found as well. Skin thickness was higher at the PVF as well as the PDF and the deltoid in males compared to females, respectively by 12.5%, 8.0% and 18.0%. These findings are in accordance with those of Escoffier et al. [15] who investigated skin thickness at the PVF using ultrasound in 54 men and 69 women, aged 5–90 years. They concluded that the skin of men (range 0.7–0.9 mm) was 16% thicker than that of women (range 0.6–0.7 mm). Similarly, de Rigal et al. [16] concluded that the PDF (1.0–1.2 mm) was approximately 17% thicker than PVF (0.9–1.0 mm) in 142 women and relatively constant over age, diminishing after 70 years old.

However both lower and upper ranges in the current study are considerably higher compared to the findings of Escoffier et al. [15] and de Rigal et al. [16] potentially due to technologic progression of the ultrasound equipment allowing more detailed measurements. Moreover, de Rigal et al. [16], included only women which might decrease overall skin thickness, due to the significant influence of gender. Studies by Laurent et al. [17], Gibney et al. [18] and Derraik et al. [19] also found significant differences in skin thickness related to gender. Except for Laurent et al. [17] these studies investigated other body sites than we did: (1) suprascapular, (2) waist, (3) thigh, (4) upper arm and (5) abdomen. Comparing to results of the current study, the deltoid skin thickness was equal to the findings of Laurent et al. [17].

In contrast to the current study where a significant increase was seen for the deltoid skin thickness in relation to age, Laurent et al. [17] did not find an impact of age on the deltoid skin thickness. Also Gibney et al. [18] reported only a minor increase in skin thickness with age (study population 18–65 years), whereas Derraik et al. [19] concluded that a slight decrease in dermal skin thickness was associated with age (study population 20–81 years). However in these two latter studies, body sites again differed from the current body sites. Nevertheless the absence of subjects older than 65 years in the current study might explain why there was no negative association between age and skin thickness as seen in the study of de Rigal et al. [16].

An increase in BMI was associated with an increase in skin thickness in our study, which is comparable to the findings of other studies [17–19]. With respect to BMI study subjects were randomly chosen and the BMI distribution was similar to that reported for Flanders in 2008. Approximately 50% of the Flemish population had a normal BMI, compared to 59% of the subjects in the study [20]. In 2008, 47% of the population was suffering from overweight of which approximately 14% suffered from obesity. In our study, 38% of the subjects suffered from overweight and significantly more males were obese compared to females ($p < 0.001$), similarly as in the reference population. The impact of the BMI on the intradermal penetration depth and therefore needle length is in fact only important for people suffering from underweight. In the current study as well as in the Flemish population, 3% suffered from underweight, most frequently occurring in young females. A predefined needle length based on the overall population mean skin thickness could cause too deep injection in people suffering from underweight.

Among the three investigated body sites, the deltoid region clearly had the thickest skin, followed by the PDF and the PVF. Additionally, abovementioned research confirms the effect of gender on skin thickness at any of the studied body sites. Males have a significantly thicker skin compared to females. Most findings agreed to a positive relation between skin thickness and BMI, but the impact of BMI is lower at the forearm sites. Age appeared to have a weak relation to the skin thickness especially for the deltoid region, although we did not investigate adults aged +65 years and we only tested for linear relationships. Because also infants would benefit from such new intradermal immunization possibilities, ongoing research explores the skin thickness in children aged 0–18 years.

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

Based upon these data and according to the ease of use of the forearm (as done with the Mantoux technique), we propose the proximal dorsal forearm as the designated injection site for an intradermal injection device in adults. The maximal penetration depth is mostly determined by the skin thickness in females, which should according to the current study data not exceed 1.0 mm.

Disclosure

This research was partly funded by the Flemish Government Agency for Innovation by Science and Technology (IWT KMO 140465) and INTERREG.

Acknowledgements

We thank all healthy volunteers who participated in this study.

References

[1] Young F, Marra F. A systematic review of intradermal influenza vaccines. Vaccine 2011;29:8788–801.
[2] Zehrung D, Jarrahian C, Wales A. Intradermal delivery for vaccine dose sparing: overview of current issues. Vaccine Skin Vaccin. Summit 2011 2013;31:3392–5.
[3] Vankerckhoven V, Van Damme P. Clinical studies assessing immunogenicity and safety of intradermally administered influenza vaccines. Expert Opin Drug Deliv 2010;7:1109–25.
[4] Filippelli M, Lionetti E, Gennaro A, Lanzafame A, Arrigo T, Salpietro C, et al. Hepatitis B vaccine by intradermal route in non responder patients: an update. World J Gastroenterol 2014;20:10383–94.
[5] Kouiavskaia D, Mirochnitchenko O, Dragunsky E, Kochba E, Levin Y, Troy S, et al. Intradermal inactivated poliovirus vaccine: a preclinical dose-finding study. J Infect Dis 2015;211:1447–50.
[6] Kulkarni PS, Sapru A, DGÇÖcosta PM, Pandit A, Madhusudana SN, Yajaman AB, et al. Safety and immunogenicity of a new purified vero cell rabies vaccine (PVRV) administered by intramuscular and intradermal routes in healthy volunteers. Vaccine 2013;31:2719–22.
[7] Laurent PE, Bonnet S, Alchas P, Regolini P, Mikszta JA, Pettis R, et al. Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. Vaccine 2007;25:8833–42.
[8] Chen D, Bowersock T, Weeratna R, Yeoh T. Current opportunities and challenges in intradermal vaccination. Therap Deliv: Future Sci 2015;6:1101–8.
[9] Combadiere B, Liard C. Transcutaneous and intradermal vaccination. Hum Vaccin 2011;7:811–27.
[10] Flynn PM, Shenep JL, Mao L, Crawford R, Williams BF, Williams BG. Influence of needle gauge in mantoux skin testing. Chest 1994;106:1463–5.
[11] Levin Y, Kochba E, Hung I, Kenney R. Intradermal vaccination using the novel microneedle device MicronJet600: Past, present, and future. Hum Vaccin Immun 2015;11:991–7.
[12] Tuan-Mahmood TM, McCrudden MOTC, Torrisi BM, McAlister E, Garland MJ, Singh TRR, et al. Microneedles for intradermal and transdermal drug delivery. Europ J Pharm Sci 2013;50:623–37 (Trans)dermal drug delivery: Emerging trends to study and overcome the skin barrier.
[13] Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, Almagor Y, Sharon O, Levin Y. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. Vaccine 2009;27:454–9.
[14] Van Mulder TJS, Verwulgen S, Beyers KCL, Scheelen L, Elseviers MM, Van Damme P, et al. Assessment of acceptability and usability of new delivery prototype device for intradermal vaccination in healthy subjects. Hum Vaccin Immun 2014;10:3746–53.
[15] Escoffier C, de Rigal J, Rochefort A, Vasselet R, Lévêque JL, Agache P. Age-related mechanical properties of human skin: An in vivo study. J Invest Dermatol 1989;93:353–7.
[16] de Rigal J, Escoffier C, Querleux B, Faivre B, Agache P, Lévêque JL. Assessment of aging of the human skin by in vivo ultrasonic imaging. J Invest Dermatol 1989;93:621–5.
[17] Laurent A, Mistretta F, Bottigioli D, Dahel K, Goujon C, Nicolas JF, et al. Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. Vaccine 2007;25:6423–30.
[18] Gibney MA, Arce CH, Byron KJ, Hirsch LJ. Skin and subcutaneous adipose layer thickness in adults with diabetes at sites used for insulin injections: implications for needle length recommendations. Curr Med Res Opin 2010;26:1519–30.
[19] Derraik JGB, Rademaker M, Cutfield WS, Pinto TE, Tregurtha S, Faherty A, et al. Effects of age, gender, BMI, and anatomical site on skin thickness in children and adults with diabetes. PLoS ONE 2014;9:e86637.
[20] Drieskens S. Voedingsstatus: BMI verdeling België. Wetenschappelijk Instituut Volksgezondheid; 2008. p. 711–69.