Prediction of high naevus count in a healthy U.K. population to estimate melanoma risk

PREDICTION OF HIGH NAEVUS COUNT IN A HEALTHY UK POPULATION TO ESTIMATE MELANOMA RISK

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ABSTRACT

Introduction: Despite recent discoveries of germline and somatic mutations in melanoma, naevus count remains the most important risk factor for melanoma. Counting naevi on the whole body is time consuming. In order to identify patients at risk for melanoma, many studies have used naevus count on selected body sites as a proxy for total body naevus count.

Methods: The most predictive body site for total naevus count was assessed in a cohort of female healthy twins. This finding was replicated on a control group from UK a case-control study and a prediction model was after performed. The area under the receiver operating characteristics curve was used to evaluate the best cut off for the prediction of having more than 50 or 100 total body naevus counts.

Results: 3694 female twins were included. The total body naevus count showed a steady decline after the age of 30 ($p<0.001$). The most predictive sites for total body naevus count were the arms and legs: the adjusted correlation coefficients were 0.50 and 0.51 ($p<0.001$) for right and left arm respectively and 0.49 and 0.48 for right and left arm respectively ($p<0.001$). The arm remained the most predictive site for total body naevus counts when replicated in a control population including both sexes. In the twin study, women with more than 11 naevi on the right arm were approximately 9 times more likely to have more than 100 naevi (OR =9.38, 95% CI: 6.71-13.11).

Conclusion: The ability to estimate total body naevus count quickly by counting naevi on one arm could be a very useful tool in assessing melanoma risk in primary care.

INTRODUCTION

The incidence of melanoma is increasing worldwide[1]. Total body naevus count (TBNC) is one of the most important risk factor for melanoma development with much higher relative risks than environmental exposures[2,3]. Sunlight may be involved in naevogenesis, but naevi are also under genetic control, as shown by family and twin studies [4,5]. Melanoma risk increases by 2-4% per additional naevus counted on the body[6] and despite this only between 20% to 40% of melanoma arises from a pre-existing naevus [7]. Naevi are therefore mainly a marker of risk and not necessarily a precursor lesion for melanoma.

Naevi typically involute after the fourth decade of life in Caucasian populations and are rare in the elderly[4], but individuals with susceptibility for melanoma often have large numbers of common and atypical naevi, which persist until middle age or later[8]. In clinical practice, naevus count is a very important clinical marker of increased melanoma risk[9]. However total body skin examinations with mole check are rarely performed in general practice [10].

Many studies have therefore used naevus count on a few selected body sites in order to identify patients at-risk of melanoma, as TBNC is time consuming[11,12]. Most of these previous studies have small sample size. In addition, some studies are based on patients attending dermatology clinics for melanoma screening, so may not reflect population-based data. Whole arm naevus count has previously been reported to be the most predictive for TBNC in children and adults [11,12,13.]

The aim of this study was to assess the predictive value of naevus count on 17 different body sites in estimating TBNC in a large cohort of healthy UK Caucasian female subjects. Findings were replicated in a control group form a control study of Caucasian subjects. Once the site with the best predictive value for TBNC was determined, a second aim was to estimate the cut off value of naevus count at this anatomical site which best predicts the presence of 50 or 100 naevi respectively.

METHODS

Skin data

Skin examination and data collection was undertaken on 3694 twins between January 1995 and December 2003 as part of the TwinsUK study protocol and has been published previously[4]. Twins underwent a skin examination including recording skin type, hair and eye colour and freckles as well as naevus count on 17 body sites performed by trained nurses at St Thomas' Hospital in London. This protocol for naevus counting has previously been published elsewhere and has been validated in other case-control studies on melanoma[14]. A naevus was defined as a melanocytic lesion >=2 mm in diameter to avoid any confusion with ephelides. Skin type was assessed according to the Fitzpatrick classification.

Replication data

Our findings in the Twins UK population were then replicated in a population of 415 healthy controls population from a case-control study conducted in the UK[15]. The data collection was described previously. The control group from the case-control study was composed of 161 males and 254 female, all Caucasians. The mean age was 45+/- 15 years old. The protocol for naevus count was exactly the same as for the TwinsUK cohort with 17 body sites.. The aim of the replication was to demonstrate that our findings in the female TwinsUK cohort can be applied to other healthy Caucasian populations in the UK which is also including males.

Statistical analyses

In a preliminary analysis, we used X^2 Pearson's test and Kruskall-Wallis test to compare categorical and continuous variables respectively. Spearman's partial correlation coefficient of naevus count at each specific body site in relation to TBNC was used because naevus count was not normally distributed (including their potential transformations). The coefficients were adjusted for age, height and skin type of the volunteers at the time the naevus count was taken. The specific body sites most correlated with TBNC were identified by selecting those maximising the Spearman's partial correlation coefficients. Internal comparisons for overlapping correlations were performed [15] Thereafter, the adjusted Area Under the Receiver Operating Characteristics curve (AUC) and the true positive rates (TPR) at fixed false positive rates of interest (0.10,0.20,0.50) associated with having more than 50 or 100 TBNC respectively for well-defined cut-off points for the number of naevi on the specific body sites were calculated. Specifically adjusted-AUC can be interpreted as the probability of correctly ordering a randomly chosen case and control observation with the same covariates values and the TPR as the percent of cases detected when the covariate-specific false positive rate are held at fixed levels[16]. In order to preserve the study power, unconditional logistic regression models were carried out to examine how many naevi can predict those individuals at greater risk of developing melanoma (i.e. those with > 50 and >100 naevi). Adjustments were made for age, height and skin type[17,18]. Robust variance was used to allow for intra-group (twin) correlation. Sensitivity analyses were carried out: i) Spearman's partial correlation coefficients were estimated on a subset of women with only one twin from each family randomly selected and hence to eliminate potential sources of bias, ii) with the aim of furtherly examining if twin shared factors could affect the association between total body and specific site naevus count, a logistic regression

model differentiating the within-pair and between-pair effects with robust standard error was performed by using generalized estimating equations[19].

The replication analyses were adjusted also for sex in both non-parametric and parametric analyses. Comparison between the partial correlation coefficients of population and replication studies was performed by standard Fisher's z-transformation for independent samples[20]

Statistics were performed using statistical software STATA 12.1 (StatCorp LP, College Station, TX, USA).

RESULTS

GENERAL POPULATION STUDY

A total of 3694 twins, all females, were evaluated. The median age of the volunteers was 47 years (interquartile (iq) 37-55) (table 1) Fitzpatrick skin type was available for 77% of volunteers. Distribution of skin type was as follows: 12.9% (I), 33.4 (II), 36.3% (III), 14.2% (IV) and 3.2% (V). The median height was 162 cm (interquartile range (IQR) 158-66). Mean body naevus count was 32 (range 0-514, median 18, IQR 6-42).

TBNC changed with each decade of life (Kruskal Wallis test: $p<0.001$), showing a steady decline after 30 years old. This corresponded to an approximate decrease of 4 naevi for each decade of life from 30 to 60 years old. TBNC differed according to skin types (Kruskal Wallis test: $p = 0.01$), as well as to height (Kruskal Wallis test: $p = 0.003$), with higher naevus count in fairer skin and in women taller than 166 cm.

Out of 3694 women, 840 were excluded because of missing data on skin type. The Spearman's partial correlation coefficients for naevus count at different body sites in relation to TBNC adjusted for age, height and skin type are reported in Table 1 (n=2854). The sites most associated with TBNC were arms and legs: the adjusted correlation coefficients were 0.50 ($p<0.001$) and 0.51 ($p<0.001$) for right and left arms respectively and 0.49 ($p<0.001$) and 0.48 ($p<0.001$) for right and left legs respectively. Laterality did not affect the correlation. There were no differences between correlation coefficients corresponding to right arm and leg ($p=0.65$), while there were differences when comparing arm or leg to other body specific sites ($p<0.001$). Correlations were as follows: 0.31 for chest, 0.43 for back and 0.16 for buttocks ($p<0.001$ for each correlation). ~~When the correlation coefficients were also adjusted for the Fitzpatrick skin type, the number of women included into the analyses decreased to 2323. However the results previously obtained on the larger sample of women were confirmed and strengthened: partial correlation coefficients increased to 0.50 for right whole arm, to 0.51 for left whole arm, 0.49 and 0.48 for right and left whole leg, respectively ($p<0.001$).~~ Overall Bonferroni's correction for multiple comparisons as well as the sensitivity analysis performed on the subset of women including only one twin for family did not affect the associations.

REPLICATION STUDY

We replicated these analyses in a cohort of controls from the UK which were part of a UK melanoma case control study published previously[14]. This study used exactly the same protocol with TBNC divided in 17 body sites. This cohort was composed of 415 UK Caucasian controls, 162 men (39%) and 253 women (61%), with a mean age of 45 years (range: 0-78). The average TBNC was 33 (range: 0-323): 30 (0-167) in males and 35 (0-323) in females, which is comparable to the TwinsUK sample.

Spearman's partial correlation coefficients were adjusted for age, height, skin type and sex. The most predictive site was the right arm in both males and females (adjusted r=0.86, p<0.001), and in particular the right arm above elbow (adjusted r=0.83, p<0.001)(Online Table 1). These results were confirmed for both males and females (males: adjusted r=0.85, p<0.001; females: adjusted r=0.88, p<0.001). In addition the back was strongly associated with TNBC but in males only (adjusted r=0.84, p<0.001) (Online Table 2). The correlation coefficients corresponding to the arm differed significantly from the coefficients corresponding to other body sites (p<0.001). The correlation coefficients were also different when comparing the TwinsUK population with the replication study (p<0.001).

Out of 410 women with data on age, sex, height and skin type, 86 (21.0%) had more than 50 TBNC and 23 (5.6%) had more than 100 TBNC. The thresholds optimizing the Adjusted Area Under the Receiver Operating Characteristics Curve (AUC) in the prediction of more than 50 and 100 naevi for right arm were 7 (adjusted AUC=0. 89, 95% CI: 0.85-0.94) and 11 respectively (adjusted AUC=0.93, 95% CI: 0.91-0.96) with the true positive rates (TPR) of 0.91 (95% CI: 0.84-0.97) and of 1.00 (95% CI: 1.00-1.00) at the fixed false positive rates (FPR) of 0.20 respectively (Online Table 3). The thresholds optimizing the adjusted AUC for the right arm above elbow were 5 (adjusted AUC=0.86, 95% CI: 0.81-0.91) with TPR of 0.85 (95% CI: 0.77-0.93) at fixed FPR of 0.20 and 8 (adjusted AUC=0.93, 95% CI: 0.86-0.99) with TPR of 0.96 (95% CI: 0.87-1.00) at fixed FPR of 0.20 for above 50 or 100 TBNC respectively. There was no difference in the predictive ability between whole arm and arm above elbow on having more than 50 (p=0.17) or more than 100 TBNC (p=0.23).

MODEL FOR PREDICTION ON THE MOST SIGNIFICATIVE SITE

Naevus count on the arm was the site that best predicted TBNC in both the TwinsUK cohort and the replication study. The arm is also easily accessible in a clinical setting; we therefore focused on the arm and its components. Out of 2854 TwinsUK female twins, 582 (20.4%) had more than 50 TBNC and 187 (6.5%) had more than 100 TBNC. According to AUC, the most accurate thresholds in terms of naevi on right arm were 7 (adjusted AUC=0.74, 95% CI: 0.71-0.76) with TPR of 0.67 (95% CI: 0.63-0.71) at fixed FPR of 0.20 and 11 (adjusted AUC=0.73, 95% CI: 0.69-0.78) with TPR of 0.62 (95% CI: 0.55-0.70) at fixed FPR of 0.20 in predicting women having more than 50 naevi and more than 100 naevi, respectively (Table 2).

When examining the upper and lower arm separately, the threshold optimizing the AUC in predicting more than 50 naevi was 3 (AUC=0.69, 95% CI: 0.66, 0.72) with TPR of 0.59 (95% CI: 0.54-0.63) at fixed FPR of 0.20 for right forearm and 4 (AUC=0.72, 95% CI: 0.69-0.74) with TPR of 0.61 (95% CI: 0.53-0.69) at fixed FPR of 0.20 for right arm above elbow. Individuals with 3 naevi on the right forearm (AUC=0.69, 95% CI: 0.65-0.74; at FPR of 0.20: TPR=0.56, 95% CI: 0.46-0.66) or 8 above

the elbow on the right arm (AUC=0.73, 95% CI: 0.68-0.77; at FPR of 0.20: TPR=0.59, 95% CI: 0.52-0.67) were likely to have a TBNC of more than 100. There was no difference in the predictive ability of the whole arm and the arm above elbow on having more than 50 naevi (p=0.60) or more than 100 (p=0.58) naevi.

Women with more than 7 naevi on the right arm had approximately nine times the risk of having >50 TBNC compared to women with less than 7 naevi (adjusted OR for right arm: 8.81, 95% CI: 7.03-11.04). Women with more than 11 naevi on the right arm had a nine fold increased risk of having >100 TBNC compared to women with less than 11 naevi respectively (adjusted OR for right arm: 9.38, 95% CI: 6.71-13.11). The right arm above elbow was also predictive of TBNC: adjusted OR of having >50: 7.04, 95% CI: 5.67-8.75 for women with more than 4 naevi on the right upper arm and adjusted OR of having >100 TBNC with more than 8 naevi on the right upper arm: 9.83, 95% CI: 7.10-13.60). Odds ratios for having more than 50 and 100 TBNC dependent on the naevus count on the arm and arm above elbow obtained by fitting restricted cubic splines with three knots are shown in Figure 1. When sensitivity analyses were performed to evaluate if twin shared factors could affect the association between total body and specific site naevus count, the similarity between the estimated within- and between-pairs effects indicated that the effect of an unit increase of naevus count in the arm on having more than 50 and 100 TBNC was the same independently of whether the comparison was made between two twins or two unrelated women in the twin population (data not shown).

DISCUSSION

This study based on a large population of UK healthy female Caucasian twins not selected for any specific diseases shows that arm naevus count is more predictive of TBNC than any other body site. Although legs were also predictive of TBNC, the arm yielded the highest correlations and for clinical practice will be a more accessible site. This was also replicated in a UK control population including males. The predictive value of naevi as a marker of melanoma risk is consistent in all Caucasian populations despite very different levels of sun exposure across the Caucasian populations studied[3,4,6]. Naevi confer the same magnitude of risk for melanoma at all latitudes suggesting that sun exposure is not an important factor in this association[6]. Being able to estimate TBNC by quickly counting naevi on one arm could be a very useful tool in predicting melanoma risk in healthy populations.

Other smaller studies have reported that naevus count on the arm was the most predictive of TBNC[11,12]. Gallus *et al.*[13] reported an association between arm naevus count and TBNC in 3406 schoolchildren. We confirmed an association between face naevi with TBNC as recently described[21] even if this last was not the strongest association in the study reported here. In contrast to previous studies, our UK volunteers were not recruited in a dermatology department or during a skin cancer screening campaign, thus avoiding potential selection bias and ensuring applicability to a general population.

The demographic profile of the studied population reflects a Caucasian skin phenotype at significant risk of melanoma with a high prevalence of Fitzpatrick skin phototype I and II and a median age of 47 which is not far from the mean age of diagnosis of melanoma. Our analyses confirmed that arm naevus count is, in fact, the more strongly correlated to TBNC as previously described[22,23,24]. Byles *et al.*[25] found a correlation of 0.71 between the left arm and the TBNC in 131 Australian subjects. Farinas *et al.*[23] suggested that the best body site in women for predicting TBNC was the leg. We analysed total leg (right and left) as well as leg above and below knee and found slightly lower correlation coefficients than with the arm. Echeverria et al.[11] more recently showed a higher association between TBNC and arm count (left or right), proposing interesting cut offs for the estimation of melanoma risk, but the authors analysed a different population from Spain with different skin types and sun exposure patterns who were recruited from dermatology clinics.

Quereux *et al*.[26] suggested a cut off of 20 naevi on the arm to detect patients at risk of melanoma, and more recently Argenziano *et al.*[12] also showed that having more than 20 naevi on the arms correlates with TBNC of more than 50 naevi although in this latter study the predictive number of naevi on the arms was much higher than for the study reported here.

Our data based on a large and healthy population unselected for skin cancer screening therefore support results from previous studies. The replication analyses on controls from a case control study in the UK confirmed that arm naevus count was the most predictive site for TBNC in males as well.

In a previous Australian case control study, the right arm has previously been considered as a strong predictor of melanoma risk with various cut offs of naevus counts at that site: Holman and

Armstrong[27] suggested that more than 10 naevi on the whole arm increased the risk of melanoma by 11 fold.

Clinically, Causasian populations tend to have more naevi on the upper arm compared to the lower arm and we report a different prediction for arm above compared to below elbow. The lower arm alone is a more difficult site as this site is more likely to include lentigines which could be miscounted for naevi. A limitation of the TwinsUK study is that all subjects were females. For historical reasons, the Twin Research Unit recruited more females than males and the number of male twins was not sufficient enough to be included in the current study. The difference for females is that they have a higher number of naevi on the legs and less on the trunk compared to males, but still females do not differ much from males regarding naevus counts on the arms as previously reported by Echeverria et al[11]. No differences were observed between males and females in the replication study so gender is unlikely to have had a major effect on the selection of the best site for predicting TBNC.

CONCLUSION

We demonstrated that arm naevus count of more than 11 is associated with a significant risk of having more than 100 naevi, that is in itself a strong predictor of risk for melanoma. This fast clinical evaluation should be used for a quick estimation of melanoma risk in general practices.

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