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PII: S1748-6815(21)00629-X
DOI: https://doi.org/10.1016/j.bjps.2021.11.088
Reference: PRAS 7512

To appear in: *Journal of Plastic, Reconstructive & Aesthetic Surgery*

Received date: 19 March 2021
Accepted date: 14 November 2021

Please cite this article as: Ryckie G Wade MBBS Msc MClinEd MRCS FHEA GradStat , Samuel Bailey BSc MRes , Alyss V Robinson MBChB MRes , Michelle C I Lo BSc MBChB MRCS , Howard Peach MBChB BSc FRCS(Plast) , Marc D S Moncrieff MBBS MD FRCS(Plast) , James Martin BSc PhD , MelRisk: Using the Neutrophil-to-Lymphocyte Ratio to Improve Risk Prediction Models for Metastatic Cutaneous Melanoma in the Sentinel Lymph Node, *Journal of Plastic, Reconstructive & Aesthetic Surgery* (2021), doi: https://doi.org/10.1016/j.bjps.2021.11.088

MelRisk: Using the Neutrophil-to-Lymphocyte Ratio to Improve Risk Prediction Models for Metastatic Cutaneous Melanoma in the Sentinel Lymph Node

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Abstract

Background: Identifying metastatic melanoma in the sentinel lymph node (SLN) is important. However, 80% of SLN biopsies are negative and 11% of patients develop complications. The neutrophil-lymphocyte ratio (NLR) is a biomarker of micrometastatic disease which could improve prediction models for SLN status. We externally validate existing models and developed 'MelRisk' to better predict SLN metastasis.

Methods: Models were externally validated using data from a multicentre cohort study of 1,251 adults. Additionally, we developed and internally validated a new prognostic score `MelRisk', using candidate predictors derived from the literature.

Results: The Karakousis model had a C-statistic of 0.58 (95% CI 0.54, 0.62). The Sondak model had a C-statistic of 0.57 (95% CI 0.53, 0.61). The MIA model had a C-statistic of 0.60 (95% CI 0.56, 0.64). Our 'MelRisk' model (which uses Breslow thickness, ulceration, age, anatomical site, and the NLR) had an adjusted C-statistic of 0.63 (95% CI 0.56, 0.64).

Conclusion: Our prediction tool is freely available in the Google Play Store and Apple App Store, and we invite colleagues to externally validate its performance.

Keywords

Melanoma; Neutrophils; Lymphocytes; Sentinel Lymph Node; Risk

Introduction

Cutaneous malignant melanoma is the fifth most prevalent cancer in the United Kingdom (UK) and over 90% of cases are diagnosed early. Regional lymph node status is the most important prognostic indicator for cutaneous melanoma[1] and so sentinel lymph node (SLN) biopsy is recommended for patients with high-risk primary tumours; however, over 80% of patients will have a negative SLN biopsy[2] and meta-data shows that 11% develop post-operative complications[3]. None-the-less, SLN biopsy is still of paramount importance for accurately staging disease and selecting patients for emergent adjuvant therapies[4].

Several institutions have developed models, nomograms, and algorithms[5–12] for predicting the presence of microscopic melanoma metastases in the SLN, although their external validity is variable[5,13–16]. In the UK, none of these prognostic models have been evaluated[17] or widely adopted and currently, SLN biopsy is offered on the basis of Breslow thickness and ulceration status alone[18]. Other factors such as mitotic rate and presence of angio-lymphatic invasion within the primary, can impact on this decision. This limited approach fails to identify 5% of patients with thin tumours and lymph node metastasis[7,19] and exposes the majority of recipients (80%, the node negative patients) to unbeneficial surgery. Therefore the development of better predictive tools, to aid patient selection for SLN biopsies, will reduce both patient morbidity from avoidable surgery[20] and the overall economic burden.

The host immune response to melanoma influences tumour growth, angiogenesis, and risk of regional and distant metastasis[21,22]. The association between the peripheral venous blood neutrophil-lymphocyte ratio (NLR) and cancer outcomes has been widely reported. More recently, the associations between the baseline NLR and survival in localised cutaneous[23], acral[24],

muscosal[25] and metastatic[26–29] melanoma have been described, as well as its association to microscopic SLN metastasis[30,31].

The aims of this study are to externally validate clinically applicable risk models for microscopic SLN metastasis in patients with primary cutaneous melanoma in the UK; and, to develop and internally validate a new risk prediction model 'MelRisk'.

Methods

Identification of existing models

In accordance with the Cochrane Handbook of Systematic Reviews[32] and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement[33,34], we systematically searched PubMed from inception to 22nd September 2020 for tools to predict SLN status (Appendix 1); 2,898 titles were screened by two independent authors and 12 full texts were retrieved for screening. We excluded three external validation studies[14–16]. Five primary studies reporting new risk prediction models[5–7,11,13,35] were also excluded. We excluded one study because osteopontin is not collected in routine practice[7], two studies[11,35] the intercept and coefficients of the model were not available and three studies[5,6,8] over construct concerns, given that the correlation between Breslow thickness and Clark level[36] may differ in the derivation and our test dataset, or indeed the wider population. Ultimately, we included three models by Karakousis et al., (2006), Sondak et al., (2004) and the Melanoma Institute Australia (MIA)[12] for external validation.

The Karakousis risk score is given by:

$$= -5.86 + 1.2(if\ mitotic\ rate > 0) + 2.03(if\ ulcerated) + 2.06(if\ in\ a\ vertical\ growth\ phase) + 0.91(if\ male)$$

The Sondak risk score is given by:

$$= -0.8832 + (-0.0387\ x\ age\ in\ years) + (1.2042\ x\ mitoses) + (-0.2862\ x\ Breslow\ thickness) + (-0.0165\ x\ age\ x\ mitoses) + (0.0131\ x\ age\ x\ Breslow\ thickness) + (-0.0509\ x\ mitoses\ x\ Breslow\ thickness)$$

The MIA risk score

$$= -1.9036 + (-0.0276\ x\ age\ in\ years) + (0.6376\ if\ 1\ mitosis, 0.7703\ if\ 2 - 3\ mitoses\ or\ 0.9042\ if > 3\ mitoses) + (0.5570\ x\ Breslow\ thickness) + (0.2696\ if\ ulcerated) + (0.7665\ if\ acral, -2.7533\ is\ desmoplastic, -0.6538\ if\ lentigo\ maligna\ or - 0.4704\ if\ nodular) + (1.462\ if\ lymphovascualr\ invasion\ present)$$

These risk scores are converted to a probability by

$$= 1 / (1 + exponential[-risk\ score])$$

External validation cohort

In accordance with the TRIPOD statement[37], we sought to test the external validity of available prediction tools using data from a retrospective multicenter cohort study of consecutive patients who underwent wider re-excision of biopsy-proven cutaneous melanoma and SLNB between 2006 and 2016. This cohort study was designed to examine haematological biomarkers in melanoma and details of the cohort are available elsewhere[30,31]. In brief, they were adults (of mean age 63 years [SD 13], 1:1 males:females) from Yorkshire and the East of England who underwent SLN biopsy for cutaneous melanoma, between 2006 and 2016. Ethical approval was gained from the local (reference PL15/368) and National Health Research Authority (IRAS ID: 234565).

Development of prediction model: Candidate predictors

Thirteen candidate variables (which are routinely collected in clinical practice) were identified from the literature as being useful in predicting SLN metastasis[5–11,30,31] which formed our candidate predictors (Table 1). These candidate predictors were selected a priori and were collected within the aforementioned multicenter cohort study[30,31]. Variables which were originally measured on a continuous scale (age in years, the neutrophil-to-lymphocyte ratio, Breslow thickness and mitoses per mm^2) were retained in this form and handled as continuous covariables.

Developing the prediction model: Analysis

Using Stata v15, the primary outcome (SLN status) was modelled using a logistic regression. To begin, the full model was fitted with all candidate predictors included. Backwards elimination was performed, with a conservative p-value of 0.157 for retaining in the model[38]. For categorical variables, the category with the smallest p-value was used to determine significance. For continuous variables, we initially assumed a linear trend, but considered non-linear trends through fractional polynomials. A strict p-value ($p<0.001$) was used to indicate a non-linear trend over a linear trend[39]. Non-linear trends were considered for all continuous outcomes.

Multiple imputation using chained equations was used for all candidate predictors, with auxillary variables used to help the imputation. The primary outcome was not imputed. The number of imputed datasets was equal to the fraction of missing data (64 datasets because 64% of participants had at least one variable missing at random) of the 13 candidate predictors.

Assessment of performance of the prognostic model

We assessed the apparent predictive performance – that is the performance of the model in the dataset in which it was derived – by estimating the calibration and discrimination. We also produced the calibration slope and calibration in the large (an indicator of whether predictions are systematically too high or too low), alongside their respective 95% confidence intervals. Discrimination was measured using the c-statistic (area under the receiver operating curve).

Internal validation of the prognostic model

To account for overfitting in the predictive performance of the developed model, we used bootstrapping to internally validate the model. For each imputed dataset, we generated 100 bootstrapped datasets, in which each dataset was used to develop a prognostic model – in the same manner used to develop the original model. For each model developed in a bootstrapped dataset, estimates of the apparent performance (c-statistic and calibration slope) were obtained, as well as the test performance (c-statistic and calibration slope) by fitting the bootstrap model to the original dataset. The difference between the test performance and the apparent performance defines the optimism for that bootstrap. We averaged the optimism across all bootstraps from all imputed datasets. This optimism was used to obtain the optimism adjusted performance statistics, by subtracting the optimism from the apparent performance of the original model.

Final prognostic model

To adjust the original model for overfitting, we used the optimism adjusted calibration slope as a uniform shrinkage factor. By this, each beta coefficient from the original model is multiplied by the shrinkage factor to obtain an adjusted coefficient. To ensure calibration-in-the-large, the intercept was re-estimated using the adjusted coefficients.

Results

Our external validation cohort consisted of 1,351 participants of whom 274 (20%) had SLN metastases. Table 1 summarises the characteristics of the study population.

External validation of existing prognostic models

The performance of each model is shown in Figure 1. The Karakousis model had a C-statistic of 0.578 (95% CI 0.539 to 0.616) with a calibration slope of 0.025 (95% CI 0.010 to 0.040). The Sondak model had a C-statistic of 0.571 (95% CI 0.530 to 0.612) with a calibration slope of 0.137 (95% CI 0.062 to 0.212). The MIA model had a C-statistic of 0.602 (95% CI 0.563 to 0.640) with a calibration slope of 0.219 (95% CI 0.125 to 0.312).

MelRisk development

Of the 13 candidate predictors, 5 were retained in the final developed model (Breslow thickness, ulceration, age, anatomical site, and the neutrophil-lymphocyte ratio; Table 2). After adjusting for optimism (uniform shrinkage factor of 0.698) the prediction model was able to discriminate between SLN positive and SLN negative participants with a C-statistic of 0.63 (CI 0.56, 0.64; Table 3). The agreement between observed and predicted probabilities was good (Expected:Observed=0.999, calibration in the large=0.001, AUC=0.635; Supplementary Materials, eFigure 1). The final model and example calculations are available in the Supplementary Materials.

Discussion

By incorporating the neutrophil-lymphocyte ratio (a biomarker of the hosts' responses to malignancy) the 'MelRisk' model appears to offer better overall accuracy for diagnosing sentinel lymph node metastases. However, the gains appear to be modest and we invite colleagues to test 'MelRisk' in different populations to determine its true validity. As applying a nomogram can be impractical in the clinical environment, we have created an app which generates a percentage probability of microscopic SLN metastasis (Figure 4). This app is compatible with both Apple (iOS) and Android devices, and (v1.0.0) is available for free in the Apple App Store and Google Play Store, respectively. The use of the calculator is supported by worked examples in the supplementary materials.

In melanoma, a number of studies have identified an association between a high NLR and poor prognosis[23–28,31,40,41] although the majority of the published evidence is skewed towards Stage 4 disease. Recent studies have shown the associations between NLR, survival, recurrence and nodal status in Stage I-III melanoma[30,31]. The relationship between systemic inflammation and cancer progression is well-established but complex[42]. Recent evidence suggests neutrophils may act as vectors for tumour extravasation and infiltration into tissues[43]. For these reasons, 'MelRisk' was designed to use a balance of known host- and tumour-specific characteristics which contribute to metastatic potential.

Modern clinical practice strives towards personalised, predictive tools which can supplement decision-making for patients and clinicians alike. The SLN biopsy remains the single most important staging criterion in melanoma and guides selection for emergent adjuvant therapies[4]. Ultimately, we feel that 'MelRisk' could be used counsel patients about their risk of metastasis, whilst balancing this probability against the (11%) prevalence of complications from SLN biopsy[3]. By identifying high-probability node-negative patients unbeneficial SLN biopsies could be avoided, whilst delivering cost-savings for health services. However, before new risk calculators are used clinically, external validation is necessary[44] to ensure acceptable performance in other populations. A tool which

delivers inaccurate risk scores could potentially cause harm and so we invite colleagues globally to test our tool in their populations, so that it may be improved. It is well known that prediction models perform best in the derivation dataset, that internal validation is insufficient and when tested on datasets from other populations, most risk scores underperform. To-date, several SLN metastasis calculators have been developed and externally validated, with highly variable performance. For example, the original study presenting the Memorial Sloan Kettering Cancer Centre (MSKCC) nomogram had a c-statistic of 69% [5] whereas external validation studies yielded c-statistics of 87%[14], 80%[15] and 68%[13]. The Mocellin model[6] reported a NPV of 94% which could not be replicated in the external validation study by Sabel et al., (2012). Equally, the 67.7% c-statistic of the original MIA model[12] is greater than the performance we observed. Thus, we must reiterate our view that 'MelRisk' should be externally validated before it is used clinically because it's true performance cannot be certain from this study alone[44].

In accordance with prior studies, 'MelRisk' uses clinicopathological factors which are known to predict the metastatic potential of cutaneous melanoma (age, Breslow thickness, ulceration, and anatomical site)[10,11,45]. The difference in performed between 'MelRisk' and other calculators may be due to several reasons. Firstly, unlike previous studies, we did not categorise continuous predictors (e.g. mitoses)[9,12] as this reduces statistical power, increases the risk of false-positive associations, underestimates the extent of variation in the outcome between groups, conceals information about the true distribution between exposure and outcome and undermines efforts to correct for confounders. Secondly, by incorporating the baseline neutrophil-lymphocyte ratio (NLR), a biomarker of metastatic potential in cutaneous melanoma[30,46], the discriminatory ability of the algorithm appears to be improved. The value-added by NLR is important since a peripheral venous blood count is easily (and often routinely) obtained, making the NLR readily available and easy to use, especially via the 'MelRisk' app.

There are a number of important limitations to our study. Missing data affects all real-world studies and whilst multiple imputation yields more precise estimates than other approaches (when the

assumptions of missing at random are met) our methods might have biased the findings[47]. The measurement of NLR was taken up to 28 days prior to SLN biopsy (median 19 days, IQR 3 to 28) meaning that blood samples taken outside this window may not be valid. The number of mitoses per mm^2 was excluded from the final 'MelRisk' model given that is was not (statistically) independently associated with the risk of SLN metastases – we decided not to force mitotic rate into the final 'MelRisk' model even though it is strongly associated with survival[48] and the prior models included it[10,12], because predicting survival is not the purpose of 'MelRisk'.

Conclusions

By incorporating the neutrophil-lymphocyte ratio (a biomarker of the hosts' responses to malignancy) the 'MelRisk' model appears to offer better overall accuracy for diagnosing sentinel lymph node metastases than prior models. 'MelRisk' is available for iOS and Android devices via the app stores and could be used as an adjunct to the risk-benefit discussion with patients who are candidates for SLN biopsy. Specifically, 'MelRisk' could be used to identify very-low risk patients who could reasonably avoid SLN biopsy but before this tool is deployed in clinical practice, we invite external validation studies to determine the real-world accuracy of 'MelRisk' and its potential utility.

Ethical Approval

Ethical approval was gained from the local (reference PL15/368) and United Kingdom National Health Research Authority (HRA, IRAS ID: 234565). There was no requirement to take consent from patients for this study, as determined by the UK HRA given that the study used historical data that was already captured and there was no need to interface with patients.

Funding

Ryckie Wade is a Doctoral Research Fellow funded by the National Institute for Health Research (NIHR, DRF-2018-11-ST2-028). The views expressed are those of the author(s) and not necessarily those of the United Kingdom's National Health Service, NIHR or Department of Health.

Competing Interests

None declared.

Acknowledgements

We thank James Smith, Donald J Dewar and Jenny Goodenough for testing the app at the alpha and beta stages.

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Table 1: Baseline characteristics of study participants by SLN metastasis positive or negative.

Values are Number (percentage) unless specified

Variable		All (*N* = 1,351)	SLN Negative (*n* = 1,077)	SLN Positive (n = 274)	P-value
Age (years), median (IQR)		64.5 (54.1, 71.8)	65.0 (55.5, 72.2)	62.5 (50.1, 69.7)	0.002
Male sex		678 (50.2%)	542 (50.3%)	136 (49.6%)	0.84
Median Neutrophil-lymphocyte ratio (IQR)		2.30 (1.77, 3.08)	2.27 (1.76, 3.02)	2.38 (1.80, 3.19)	0.063
Breslow thickness, median (IQR)		1.9 (1.2, 3)	1.8 (1.15, 3)	2.2 (1.5, 3.5)	<0.001
Ulceration		323 (25.5%)	251 (24.9%)	72 (27.7%)	0.36
Vascular Invasion		33 (5.7%)	14 (3.3%)	19 (12.3%)	<0.001
Mitotic rate (mm2), median (IQR)		3 (1.5, 8)	3 (1, 7)	4 (2, 9)	<0.001
Microsatellites		29 (5.0%)	15 (3.5%)	14 (9.2%)	0.006
Vertical growth		529 (97.8%)	386 (97.0%)	143 (100.0%)	0.036
Regression		93 (16.0%)	71 (16.6%)	22 (14.4%)	0.52
TILs	Absent	92 (16.3%)	59 (14.3%)	33 (21.9%)	<0.001
	Non-brisk	374 (66.3%)	268 (64.9%)	106 (70.2%)	
	Brisk	98 (17.4%)	86 (20.8%)	12 (7.9%)	
Anatomical Location	Trunk	530 (39.3%)	404 (37.6%)	126 (46.0%)	0.001
	Upper Limb	294 (21.8%)	246 (22.9%)	48 (17.5%)	
	Lower Limb	364 (27.0%)	282 (26.3%)	82 (29.9%)	
	Head & Neck	160 (11.9%)	142 (13.2%)	18 (6.6%)	
Histology of	SSM	409 (66.1%)	305 (67.0%)	104 (63.4%)	0.18

tumour	Nodular	144 (23.3%)	98 (21.5%)	46 (28.0%)	
	Other	66 (10.7%)	52 (11.4%)	14 (8.5%)	

Table 2: Final multivariable model for MelRisk

Variable		OR (95%CI)	β coefficients
Age		0.989 (0.982 - 0.996)	-0.01147063
Neutrophil-lymphocyte ratio		1.105 (1.030 - 1.187)	0.10026050
Breslow thickness (mm)		1.095 (1.045 - 1.148)	0.09110591
Ulceration		0.945 (0.740 - 1.206)	-0.05695661
Site	Trunk	reference	reference
	Upper limb	0.729 (0.561 - 0.947)	-0.31624484
	Lower limb	0.967 (0.772 - 1.210)	-0.03401549
	Head and Neck	0.543 (0.373 - 0.790)	-0.61051715
Constant (intercept)			-1.00788800

Table 3: Model diagnostics (with 95% CI)

Measure	Apparent Performance	Test Performance	Average Optimism	Optimism corrected
C-Statistic	0.63 (0.60 to 0.67)	0.64 (0.61 to 0.66)	0.033	0.60 (0.56 to 0.64)
Calibration slope	0.98 (0.68 to 1.27)	0.70 (0.45 to 0.92)	0.242	0.73 (0.44 to 1.03)

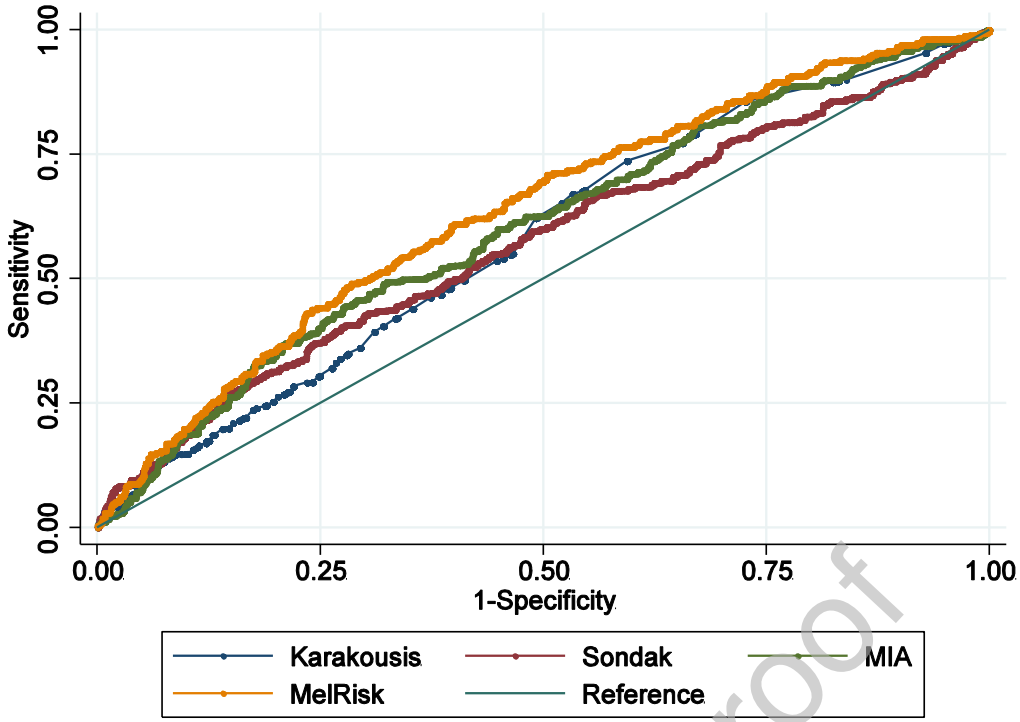

Figure 1. The performance of the Karakousis, Sondak and MIA risk prediction models on our external validation cohort compared to the optimism-adjusted performance of MelRisk, visualised in ROC space

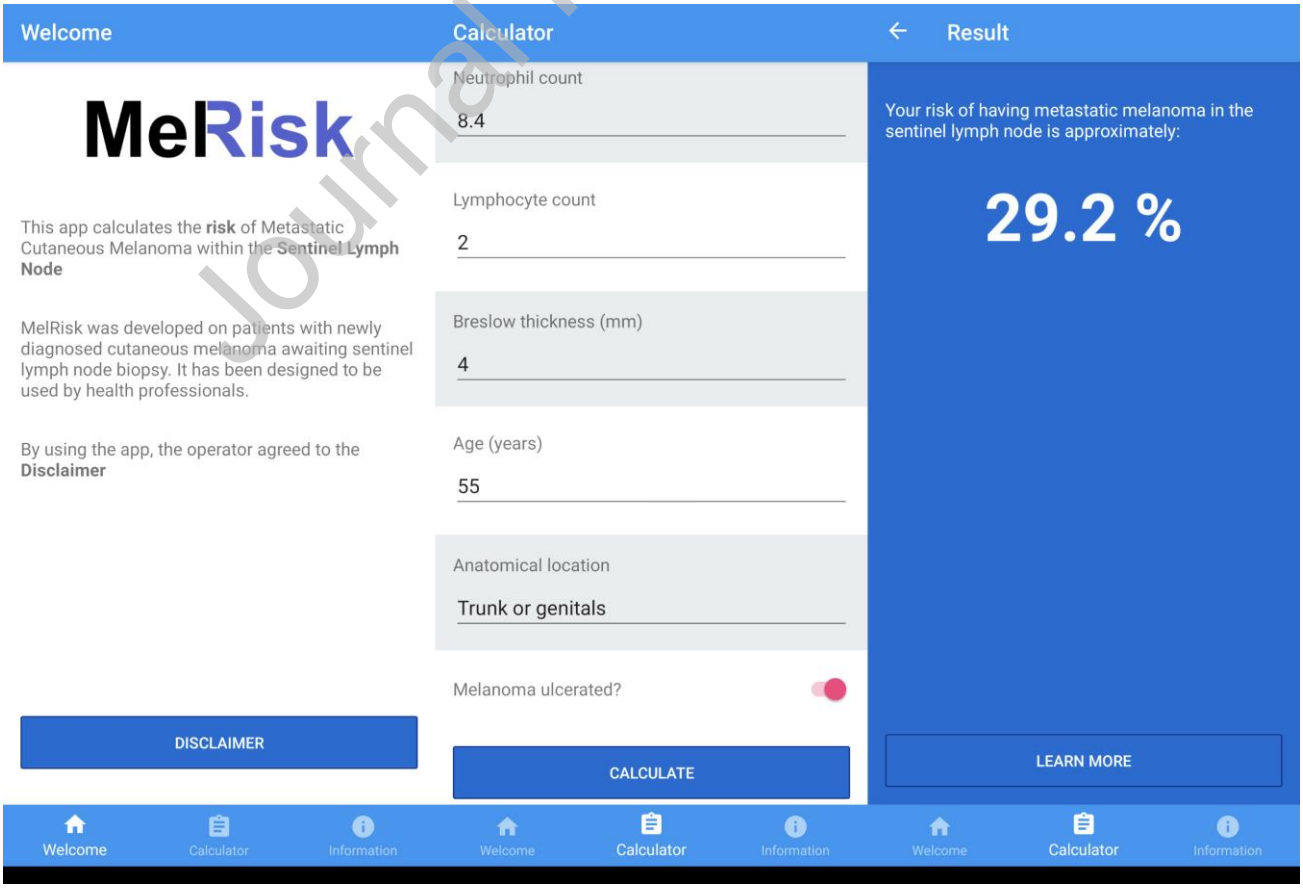

Figure 2: The MelRisk app welcome screen, calculator and results pages