COMPARISON OF ONCOTYPE DX RECURRENCE SCORE TO MAGEE EQUATIONS RECURRENCE SCORE IN EARLY BREAST CANCER: USING ROUTINE HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMISTRY PARAMETERS.

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Declaration

I, Godson Obiora Uzonwa, declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master in medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

(Signature of candidature)

On this—31ST -----Day of -----August -----2021------in-----Johannesburg.

Dedication.

This dissertation is dedicated to my lovely wife, Dr N.M.P Mokone and my late mother-in-law Mrs Lorna Mokone.

Presentation arising from this research project.

This research result was presented on 21st February 2018 at the Wednesday Academic in-house meeting of Department of Surgery, University of the Witwatersrand.

Abstract

Objective: The main issue in planning for breast cancer treatment is the identification of the subgroup of patients who are most likely to develop disease recurrence so that the appropriate therapeutic regimen can be provided. The Oncotype DX assay has been clinically proven to reliably predict disease recurrence and help prognostication, however, the Oncotype DX assay costs about 4,000 US dollars and most medical aids or health insurance schemes may not cover its cost. Surrogate assays such as the new Magee equations have been observed in some studies to predict the Oncotype DX Recurrence Scores. However, the accuracy of the new Magee equation remains a matter for debate. Thus, the objective of this study was to compare the recurrence scores of the Oncotype DX, and Magee equations generated Recurrence Scores in early breast cancer using available Oncotype DX and histopathological report database.

Method: The histopathological reports from Drs Gritzman & Thatcher Inc. Laboratories and Oncotype Dx Breast Cancer Assay reports conducted by Genomic Health (Redwood City, CA) were reviewed from the database. The histopathological and immunohistochemistry parameters were extracted from the pathology reports and the estimation of Recurrence Score was calculated using the new Magee equations. Following that, descriptive statistics were calculated for the sample, and statistical tests such as Spearman rank-order correlation coefficient and Wilcoxon matched pairs test were performed. Observed frequencies were calculated and summarised in a Two-way contingency table, and differences between the Oncotype DX and Magee Recurrence Scores were plotted on the Bland-Alman plot.

Result: The Magee Equation Recurrence Scores 1, 2 and 3 were observed to show a statistically significant relationship with the Oncotype DX RS. However, only Magee RS 1 showed a non-statistically significant difference with the Oncotype DX RS. Observed frequencies indicate that the Magee RS 1 and 2 may be able to rule out high Oncotype DX RS, and Magee RS 3

may be better at predicting low Oncotype DX RS. The Magee Recurrence Scores all showed a fair level of agreement with few discrepancies.

Conclusion: While the Magee equation shows a certain level of agreement with the Oncotype DX, it seemed to show significant discordance with the intermediate Oncotype DX by reclassifying most of the Intermediate Oncotype DX RS as low RS. The prognostication of the Oncotype DX provides patients with intermediate recurrence risk a chance to benefit from adjuvant chemotherapy thus the Oncotype DX may not be totally omitted, but clinical trials with the Oncotype DX and Magee equations is advised, in other to achieve a better outcome.

Keywords: Oncotype DX, Magee Equation, Breast cancer, Recurrence score.

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CHAPTER ONE

INTRODUCTION

Breast cancer is the most common cancers in women[1] with estimated 2 million new cases diagnosed in 2018. This represents about 12% of all new cancer cases and 24.2% of all cancers in women.[1] Statistics South Africa, 2013 reported that breast cancer was the 9th leading cause of death among female 45 – 64 age groups in Gauteng Province. This accounts for 3.1% of deaths.[2]

Breast cancer is a heterogeneous disease with a wide range of clinical behaviour that is not fully predicted by the histological features of the tumours. About 70% of breast cancer cases are oestrogen receptor (ER)-positive.[3]

The main issue in planning for breast cancer treatment is the identification of the subgroup of patients who are most likely to develop disease recurrence so that the appropriate therapeutic regimen can be provided. As such, the rationale for this research is informed by the uncertainty in decision making with regards to the use and benefit of adjuvant chemotherapy in ER positive, node negative early breast cancer.

Breast cancer treatment approach

The current surgical approach for early breast cancer involves loco-regional control of the disease. The options available include mastectomy and breast conserving surgery (BCS) plus radiotherapy. Both approaches result in equivalent cancer-specific outcomes. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-06 trial showed no significant difference in overall survival among the treated groups.[4]

A randomized study by Veronesi et al, approved by the World Health Organization in 1969, with a 20year follow-up concludes: that long-term survival among women who undergo BCS plus radiotherapy is the same as that among the radical mastectomy group.[5] Oncological treatment of breast cancer patients includes adjuvant chemotherapy, endocrine and targeted therapies.

The concept for using adjuvant chemotherapy was supported by the discovery of haematogenous cancer cells and by animal studies that suggested that these could be eliminated with chemotherapy. The early trials in breast cancer i.e. NSABP B-01 and B-05 studies demonstrated a reduced risk of recurrence, improved survival and improved disease-free survival respectively.[6 7]

Adjuvant chemotherapy is offered to many patients with ER+, node negative breast cancer, but with few patients benefitting. Traditionally guidelines assume that all benefitted equally. This leads to under-treatment in few patients and over-treatment in many others.

The re-classification of breast cancer, as proposed by Veronesi et al in early 2000 [8], according to the biological profiles of the cancer provided a reasonable tool for adjuvant therapeutic decision making. Historically, predictive biomarkers of chemotherapy benefits are limited. The decision to use adjuvant chemotherapy was based on prognostic markers such as tumour size, age, histological grade and nodal status. Prognostic indices such Nottingham prognostic index (NPI) and Adjuvant! online are in use, but with varying predictive values.

Analysis of prospective trials suggests that adjuvant chemotherapy response may not be uniform across all the biological subtypes, particularly for those patients with low-grade, well-differentiated tumours and high expression of hormone receptors. Hence the flawed approach of one size fits all.

Perou et al[9], demonstrated by genetic array testing that breast cancer should not be considered as a single disease. They subdivided breast cancer into four distinct biological subtypes, which can have different epidemiological risk factors, natural histories, and systemic and local therapy responses. These intrinsic subtypes are designated as: luminal A (ER/PR +, HER2 negative, low Ki-67 <15%), luminal B (ER/PR +, HER2 negative, high Ki-67 >15%) / luminal B (ER/PR +, HER2 +, any Ki-67), HER2 enriched (HER2 +, ER/PR negative), and basal-like (triple negative: ER/PR negative, HER2 negative). This has created great interest in the development of clinical practical assay that can match these biological intrinsic subtypes and can be used to provide both prognostic and predictive information to guide patient care.

Several multigene assays are available that evaluate the likelihood of chemotherapy benefit and the probability of recurrence in breast cancer patients. These include: Oncotype DX, Mammaprint, PAM50-ROR, genomic grade index, Breast cancer index and Endopredict.

The 21-gene recurrence score assay (Oncotype DX Breast Cancer Assay; Genomic Health Inc, Redwood City, CA), has been validated to predict chemotherapy benefit and the likelihood of distant recurrence in patients with ER positive, node negative early breast cancer. The assay calculates recurrence score (RS) that group patients into: Low RS value (< 18) with little to no chemotherapy benefit. Intermediate RS value (18 – 30); with no substantial benefit. High RS value (≥ 31); with a significant chemotherapy benefit.

The 21-gene panel used to develop the Oncotype DX Recurrence score algorithm is as follows:[10]

Table 1.1: 21-gene panel Oncotype DX Recurrence score algorithm.

PROLIFERATION	ESTROGEN	INVASION	HER2	OTHERS	REFERENCE
Ki-67	ER	Stromelysin 3	GRB7	GSTM1	Beta-actin
STK15	PR	Cathepsin L2	HER2	CD68	GAPDH
Survivin	Bcl-2			BAG1	RPLPO
Cyclin B1	SCUBES2				GUS
MYBL2					TFRC

The panel is made up of 16 cancer genes – with a consistent and statistical association with breast cancer recurrence, and others with good predictive power for chemotherapy benefit. The 5 reference genes normalize gene expression and provide quality control.

The prognostic utility of Oncotype DX breast cancer assay was validated in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 and B-20 trials. The Oncotype DX breast cancer assay is the only multigene assay incorporated into the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) guidelines for the treatment of early breast cancer.[11]

Presently the listed price for Oncotype DX Breast cancer assay is more than $4,000 US dollars. The test is not offered to state patients obviously due to the exorbitant cost, and currently not covered by most medical aid funds, hence the need for less expensive comparative means of prognostication and prediction of chemotherapy benefits in hormone receptor positive, node negative early breast cancer.

Some studies have shown that using scoring systems and equations, that routinely make use of histopathological and immunohistochemistry parameters, can predict recurrence scores comparable to the Oncotype DX recurrence score at no additional cost to the patients and health system.[12]

It is important to note that the four biomarkers (ER, PR, HER-2 and Ki-67) that are given the highest weight in the algorithmic calculation of Oncotype DX Recurrence Score (ODX RS), is available as part of the standard histopathological report of breast cancer specimens.

One of the mathematical scoring systems in use is the **Magee equations**.[13] The equations use histopathological variables and semi-quantitative results of ER, PR, HER-2 and Ki-67 to calculate a recurrence score predictive or comparable to Oncotype DX Recurrence Score.

Flanagan et al (2008) [14] used linear regression analysis to propose a model equation (the original Magee equation) which showed that nuclear grade and mitotic counts in combination with semi-quantitative immunohistochemistry scores of ER, PR and HER-2 can predict outcome remarkably similar to that found in Oncotype DX Recurrence Score.

In a follow up study, Klein et al (2013)[12] used multiple linear regression analysis to create three new model equations, each using different combinations of Nottingham scores, Ki-67 or tumour size; in addition to semi-quantitative immunohistochemistry scores of ER, PR and HER-2 (**called the New Magee equations**) to predict the actual Oncotype DX Recurrence Score. Using a database of 800 cases that were sent for Oncotype DX testing to Genomic Health, they were able to accurately predict the Oncotype DX Recurrence Score. The variables used are the following: Nottingham score (3 – 9), Ki-67 index (0 – 100), tumour size in cm, H-scores (0 – 300) for ER and PR, and HER-2 status (negative, positive and equivocal determined based on FISH result). The equations were validated on a separate set of 255 cases sent for Oncotype DX testing.

The equations are as follows:

New Magee Equation 1: Recurrence score (RS) = 15.31385 + Nottingham score* 1.4055 + ERIHC* (- 0.01924) + PRIHC* (- 0.02925) + (0 for HER2 negative, 0.77681 for equivocal. 11.58134 for HER2 positive) + tumour size* 0.78677 + Ki-67 index* 0.13269.

New Magee Equation 2: RS = 18.8042 + NS* 2.34123 + ERIHC* (- 0.03749) + PRIHC* (- 0.03065) + (0 for HER2 negative, 1.82921 for equivocal, 11.51378 for HER positive) + tumour size* 0.04267.

New Magee Equation 3: RS = 24.30812 + ERIHC* (- 0.02177) + PRIHC* (- 0.02884) + (0 for HER2 negative, 1.46495 for HER2 equivocal, 12.75525 for HER2 positive) + Ki-67 index* 0.18649.

The concordance between ODX RS and the equations being 54.3%, 55.8%, 59.4% and 54.4% respectively for the original Magee equation and the new Magee equations 1,2 and 3 respectively. Upon elimination of the intermediate categories for both actual recurrence score and estimated recurrence score, the concordance increased to 96.9%, 100%, 98.6%, and 98.7% respectively.

Turner et al (2015), using a simple modification of Magee equations, predict ODX RS in a set of 283 patients with almost 100% concordance in the low and high recurrence scores. This study also indicated that the modified Magee recurrence scores along with histologic criteria may be a cost-effective alternative to Oncotype DX.[13]

In a comparative study of 416 patients with existing ODX RS, Khoury et al (2015)[15] found a concordance rate of 69.6% and 98.62% overall and after excluding the intermediate category. They conclude that their equation validated the Magee equation. Moreover, they postulate that consistent review of the histologic slides could improve the existing equations.

It has been suggested by several studies that mitotically active cellular stroma, or tumour-associated inflammatory cells, as part of the tumour microenvironment can be a contributory factor to intermediate or high ODX RS in low grade invasive breast cancers.[16 17] Many non-invasive tumour tissue components (such as stromal fibroblasts, adipose tissue, lymphocytes, macrophages etc) can be admixed during mRNA extraction for polymerase chain reaction analysis, despite gross macro-dissection or microdissection of tumour tissue, and may alter the RS[13].

The difference in correlation between estimated and actual recurrence score can be explained with a few concepts. First, estimated recurrence score and actual recurrence score measures two different parameters i.e. morphologic and immunohistochemistry and gene expression data respectively. Inter-observer variability for grading and semi-quantifying immunohistochemistry can lead to under or over-estimation[12].

The use of specific quantitative histopathologic variables by Allison et al (2012) indicates that Nottingham grade, PR status and Ki-67 index are strong predictors of ODX RS.[17] Low PR Allred scores, Ki-67 > 10% and Nottingham grade 3 cancers are more likely to have higher recurrence scores. Similarly, Tang et al (2010) in a study of 77 cases conclude that PR negativity, luminal B subtype, tubal formation and mitosis are all strongly correlated with high ODX RS.[18]

Several other authors have shown in their studies that ODX RS relies heavily on parameters already available from routine pathologic reports: Clark et al (2013)[19] showed a significant association between Nottingham grade and ODX RS; and an inverse relationship between PR H-score and ODX RS. Biroschak et al (2013)[20] showed a correlation between ODX RS and histological parameters in a small sample study of 60 cases.

Geradts et al (2010), in a study of 177 cases, incorporated histopathological features (ER, PR, HER2, TG, NG, MG) into an index: The Breast Cancer prognostic score (BCPS) showing a strong correlation with the ODX RS.[21]

Cuzick et al (2011)[22], in a study using 1,125 tumour tissue samples from the ATAC trial (Arimidex, Tamoxifen, Alone or in Combination), suggested that accurate quantitative immunohistochemistry for ER, PR, HER2 and Ki-67 (IHC4 score) constitutes an alternative and inexpensive test battery that can provide prognostic utility similar to that of the ODX RS.

Eden et al (2004)[23] suggest that clinical markers of the Nottingham Prognostic Index performed similarly to gene expression markers, in a study using a data set of 97 cases. Auerbach et al (2010), in a study of 138 cases, suggest that a mitotic count score greater than one, in combination with a negative PR result, could serve as a marker for intermediate or high Oncotype DX Recurrence Score.[24]

Other studies have found the ODX RS assay to be an accurate predictor of recurrence, more accurate than standard clinicopathological features individually and when integrated by an algorithm model – Goldstein et al (2008).[25] Wolf et al (2007) in a population, based study of 300 patients suggest that neither standard histopathologic features nor commonly used assessment tools can reliably predict ODX RS, outside a clinical trial population.[26]

Sparano et al (2008) summarized that following post marketing of Oncotype DX, fewer cases had high RS, probably because clinicians tend to order the assay for patients who have low or intermediate risk clinicopathologic features rather than high risk features. And the result altered treatment recommendation in approximately 25% of patients.[27]

Study objective

The objective of this study was to compare the Recurrence Scores of the Oncotype DX, and Magee equations generated Recurrence Scores in early breast cancer, using available Oncotype DX and histopathological report database.

This study may also help to determine the accuracy of our pathological reporting.

CHAPTER TWO

MATERIALS AND METHODS

Ethical considerations

Ethical approval for this study was obtained from The University of the Witwatersrand's Human Research and Ethics Committee (HREC) with clearance certificate number M170204. Permission to conduct this study at the Netcare Breast Care Centre and Drs Gritzman & Thatcher Incorporated Laboratories, and to use the Oncotype DX Recurrence Score report database was obtained from the respective institutions.

This study did not require informed consent as it was a retrospective study. However, to respect the patient's confidentiality, the datasheet did not contain any patient's details.

Study design

This was a retrospective study using the Oncotype DX Recurrence Score report database from the period 1st January 2010 – 31st December 2016, and available histopathology results from Drs Gritzman & Thatcher Incorporated Laboratories (Johannesburg). The Oncotype DX Breast Cancer assay recurrence scores are reported from samples sent to Genomic Health Inc, Redwood City, CA.

Study population

A total record of 233 breast cancer patients of all age groups with available Oncotype DX recurrence score reports on the database (from 1st January 2010 – 31st December 2016) was used in this study. This study was conducted at the Netcare Breast Care Centre, Milpark Hospital and Drs Gritzman & Thatcher Incorporated Laboratories, Johannesburg. Patients with

missing Oncotype DX Recurrence Score reports, missing or incomplete histopathological variables and immunohistochemistry parameters on the database were excluded from this study.

Study procedure

This study was a database record review, and no patient contact or intervention was undertaken. The histopathological reports from Drs Gritzman & Thatcher Inc. Laboratories and Oncotype Dx Breast Cancer Assay reports conducted by Genomic Health (Redwood City, CA) were reviewed from the database. The histopathological and immunohistochemistry parameters (i.e. tumour size in cm, Nottingham score, ER, PR, HER2 and Ki-67) were extracted from the pathology reports and entered into an excel spreadsheet. Following that, the estimation of recurrence score was calculated using the new Magee equations – these equations are available online at the University of Pittsburgh Medical Centre internet website (path.upmc.edu). These internet-based equations automatically calculated the Magee score upon entering the required parameters.

Statistical analysis

This study is a quantitative – comparative correlational study. Thus, in order to summarise the data, descriptive statistics including mean, median and standard deviation were calculated for all variables. Variables were further grouped into high, low and intermediate Oncotype DX RS groups, and the descriptive statistics calculated for each group. A Spearman rank-order correlation coefficient was performed to test for a monotonous relationship and a Wilcoxon matched pairs test was performed to check for difference in the population mean rank between Oncotype DX RS and Magee RS 1, 2 and 3. A value $P < .05$ was considered significant in both tests. Furthermore, observed frequencies were calculated and summarised in a Two-way

contingency table, and a Bland-Altman plot was used to visualise and further analyse the difference and agreement between Oncotype DX RS and Magee RS 1, 2 and 3 respectively. Data was collected using an excel spreadsheet. Histology generated Recurrence Scores were calculated using Magee equations, and the data were analysed with Microsoft Excel and SPSS V20 (IBM, Armonk, NY, USA).

CHAPTER THREE

RESULTS

A total of 223 individuals met the study criteria and were included for this study. Magee equation 1, 2 and 3 were calculated for on all cases having the needed information available. Descriptive statistics were computed and is shown in Table 3.1a and 3.1b.

Table 3.1a: Descriptive statistics including mean and median of the total population and all variables.

Variable	Valid N	Mean	Confidence -95.000%	Confidence 95.000%	Median	Minimum	Maximum	Lower Quartile
Tumour Size (cm)	223	1.7623	1.6265	1.8982	1.6000	0.16000	8.0000	1.2000
Nottingham score	223	6.7803	6.6117	6.9488	7.0000	3.00000	9.0000	6.0000
Ki67%_cleaned	223	16.4915	14.5604	18.4226	12.0000	1.00000	80.0000	7.5000
ER H-Score	223	228.0448	220.3888	235.7009	240.0000	25.00000	300.0000	200.0000
PR H-score	223	171.4170	159.7398	183.0943	190.0000	0.00000	295.0000	120.0000
Oncotype DX Recurrence Score	223	19.8834	18.6540	21.1128	19.0000	1.00000	62.0000	13.0000
Magee RS1	223	19.0192	18.3921	19.6463	18.6430	8.80200	34.4210	15.2160
Magee RS2	223	20.9848	20.3094	21.6601	20.4360	8.32500	34.3620	16.8010
Magee RS3	223	17.4737	16.9020	18.0454	16.5320	10.39400	31.9240	14.0970

Table 3.1b: Upper Quartile and Standard Deviation of all variables on the total population (Descriptive statistics)

Variable	Upper Quartile	Std.Dev.
Tumour Size (cm)	2.2000	1.02942
Nottingham score	8.0000	1.27737
Ki67%_cleaned	20.0000	14.63314
ER H-Score	270.0000	58.01404
PR H-score	250.0000	88.48495
Oncotype DX Recurrence Score	24.0000	9.31602
Magee RS1	22.3630	4.75211
Magee RS2	25.1460	5.11758
Magee RS3	20.0890	4.33218

The data were further grouped based on the Oncotype DX RS into three category as high RS (≥ 31) n = 31, low RS (<18) n =96 and intermediate RS (18 – 30) n = 96; and the descriptive statistics were computed and recorded for each group as shown in Table 3.2 – 3.4.

Table 3.2a: Descriptive statistics of high Oncotype DX RS (≥ 31)

Variable	Valid N	Mean	Confidence -95.000%	Confidence 95.000%	Median	Minimum	Maximum	Lower Quartile
Tumour Size (cm)	31	1.7629	1.4990	2.0268	1.7000	0.3500	3.4800	1.2000
Nottingham score	31	8.0645	7.6985	8.4305	8.0000	6.0000	9.0000	7.0000
Ki67%_cleaned	31	34.0290	27.2382	40.8198	29.2000	7.5000	80.0000	24.0000
ER H-Score	31	227.6129	210.6552	244.5706	240.0000	150.0000	291.0000	180.0000
PR H-score	31	101.3548	71.5399	131.1698	115.0000	0.0000	240.0000	10.0000
Oncotype DX Recurrence Score	31	36.5484	34.0537	39.0431	34.0000	31.0000	62.0000	31.0000
Magee RS1	31	25.2298	23.7186	26.7411	24.4470	18.8620	34.4210	22.4120
Magee RS2	31	26.1791	24.8535	27.5047	26.5950	18.5520	34.3620	24.0830
Magee RS3	31	22.8205	21.1295	24.5115	22.6570	14.0880	31.9240	19.6110

Table 3.2b: Upper Quartile and Standard Deviation of high Oncotype DX RS (≥ 31)

(Descriptive statistics)

Variable	Upper Quartile	Std.Dev.
Tumour Size (cm)	2.3000	0.71941
Nottingham score	9.0000	0.99785
Ki67%_cleaned	40.0000	18.51349
ER H-Score	265.0000	46.23107
PR H-score	175.0000	81.28327
Oncotype DX Recurrence Score	42.0000	6.80117
Magee RS1	27.5640	4.12013
Magee RS2	28.2830	3.61394
Magee RS3	25.4530	4.61012

Table 3.3a: Descriptive statistics of low Oncotype DX RX (<18)

Variable	Valid N	Mean	Confidence -95.000%	Confidence 95.000%	Median	Minimum	Maximum	Lower Quartile
Tumour Size (cm)	96	1.8109	1.5759	2.0459	1.6000	0.30000	8.0000	1.2000
Nottingham score	96	6.3125	6.0842	6.5408	6.0000	3.00000	9.0000	6.0000
Ki67%_cleaned	96	11.8656	9.9644	13.7668	10.0000	1.00000	56.4000	5.0000
ER H-Score	96	231.4271	219.6364	243.2178	250.0000	40.00000	300.0000	200.0000
PR H-score	96	206.1250	192.1543	220.0957	225.0000	0.00000	295.0000	170.0000
Oncotype DX Recurrence Score	96	11.9896	11.1542	12.8250	13.0000	1.00000	17.0000	9.0000
Magee RS1	96	16.6966	15.9652	17.4280	16.5095	8.80200	28.1920	13.9585
Magee RS2	96	18.6989	17.7876	19.6102	17.6070	8.32500	32.6280	15.8380
Magee RS3	96	15.5348	14.8771	16.1924	14.6520	10.39400	27.1610	13.4630

Table 3.3b: Upper Quartile and Standard Deviation of low Oncotype DX RX (<18)

(Descriptive statistics)

Variable	Upper Quartile	Std.Dev.
Tumour Size (cm)	2.1500	1.15979
Nottingham score	7.0000	1.12683
Ki67%_cleaned	15.0000	9.38302
ER H-Score	275.0000	58.19147
PR H-score	260.0000	68.95067
Oncotype DX Recurrence Score	15.0000	4.12309
Magee RS1	18.5245	3.60977
Magee RS2	21.1405	4.49755
Magee RS3	16.5845	3.24570

Table 3.4a: Descriptive statistics of intermediate Oncotype DX RX (18 – 30)

Variable	Valid N	Mean	Confidence -95.000%	Confidence 95.000%	Median	Minimum	Maximum	Lower Quartile
Tumour Size (cm)	96	1.7136	1.5147	1.9124	1.5500	0.16000	7.0000	1.1000
Nottingham score	96	6.8333	6.5879	7.0787	7.0000	3.00000	9.0000	6.0000
Ki67%_cleaned	96	15.4542	12.7280	18.1804	10.0000	1.00000	66.0000	7.5000
ER H-Score	96	224.8021	212.3422	237.2619	250.0000	25.00000	297.0000	190.0000
PR H-score	96	159.3333	140.6646	178.0020	180.0000	0.00000	295.0000	97.5000
Oncotype DX Recurrence Score	96	22.3958	21.7168	23.0749	22.0000	18.00000	30.0000	20.0000
Magee RS1	96	19.3363	18.5197	20.1528	19.4805	10.78500	33.0360	16.5560
Magee RS2	96	21.5933	20.6345	22.5521	21.2840	12.41400	34.1480	17.8390
Magee RS3	96	17.6862	16.9434	18.4289	17.3675	10.68700	31.7080	14.9560

Table 3.4b: Upper Quartile and Standard Deviation of intermediate Oncotype DX RX (18 – 30) (Descriptive statistics)

Variable	Upper Quartile	Std.Dev.
Tumour Size (cm)	2.2000	0.98134
Nottingham score	8.0000	1.21106
Ki67%_cleaned	20.0000	13.45484
ER H-Score	270.0000	61.49397
PR H-score	237.5000	92.13705
Oncotype DX Recurrence Score	24.5000	3.35129
Magee RS1	22.4015	4.03001
Magee RS2	25.4795	4.73182
Magee RS3	19.8705	3.66574

In order to test for correlation significant between the Oncotype DX RS and the Magee RS 1, 2 and 3, the Spearman rank-order correlation coefficient was performed on the Oncotype DX RS and the Magee RS 1, 2 and 3 respectively and a value of $p < .05$ was considered to be statistically significant. The results are shown in Table 3.5 – 3.7.

Table 3.5: Spearman rank-order correlation coefficient between Oncotype DX RS and Magee RS 1

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Oncotype DX Recurrence Score & Magee RS1	223	0.568879	10.28304	0.000000

Table 3.5 shows that the correlations of the paired variables of the Oncotype DX RS and Magee RS 1 are statistically significant at $p = .000000$

Table 3.6: Spearman rank – order correlation coefficient between Oncotype DX RS and Magee RS 2

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Oncotype DX Recurrence Score & Magee RS2	223	0.517816	8.998194	0.000000

Table 3.6 shows that the correlations of the paired variables of the Oncotype DX RS and Magee RS 2 are statistically significant at $p = .000000$

Table 3.7: Spearman rank – order correlation coefficient between Oncotype DX RS and Magee RS 3

Pair of Variables	Valid N	Spearman R	t(N-2)	p-value
Oncotype DX Recurrence Score & Magee RS3	223	0.552274	9.848276	0.000000

Table 3.7 shows that the correlations of the paired variables of the Oncotype DX RS and Magee RS 3 are statistically significant at $p = .000000$

The difference between the distribution of the Oncotype DX RS and the Magee RS 1, 2 and 3 respectively cannot be assumed to be a normally distributed sample. Thus, the Wilcoxon matched pairs test which is a non-parametric statistical test was used to test the mean rank difference between the Oncotype DX RS and Magee RS 1, 2 and 3 respectively and a value of $p < .05$ was considered to be statistically significant. The obtained values and results are shown in Table 3.8 – 3.10.

Table 3.8: Wilcoxon Matched Pairs Test between Oncotype DX RS and Magee RS 1

Pair of Variables	Valid N	T	Z	p-value
Oncotype DX Recurrence Score & Magee RS1	223	10932.00	1.613188	0.106705

Table 3.8 showing a statistically nonsignificant difference between the paired variable of Oncotype DX RS and Magee RS 1 as $p = 0.1060705$

Table 3.9: Wilcoxon Matched Pairs Test between Oncotype DX RS and Magee RS 2

Pair of Variables	Valid N	T	Z	p-value
Oncotype DX Recurrence Score & Magee RS2	223	10137.00	2.437407	0.014794

Table 3.9 showing a statistically significant difference between the paired variable of Oncotype DX RS and Magee RS 2 as $p = 0.014794$

Table 3.10: Wilcoxon Matched Pairs Test between Oncotype DX RS and Magee RS 3

Pair of Variables	Valid N	T	Z	p-value
Oncotype DX Recurrence Score & Magee RS3	223	8279.000	4.363695	0.000013

Table 3.10 showing a statistically significant difference between the paired variable of Oncotype DX RS and Magee RS 3 as $p = 0.000013$.

The observed frequencies of the high, low and intermediate of each Magee RS (1,2 and 3) and the Oncotype DX RS were computed and tabulated in a 2-way summary table as shown in Table 3.11 – 3.13.

Table 3.11: 2-way summary table of observed frequencies (Magee RS 1).

DX_cat	M_RS1Cat Intermediate	M_RS1Cat Low	M_RS1Cat High	Row Totals
High	27	0	4	31
Column %	22.69%	0.00%	80.00%	
Row %	87.10%	0.00%	12.90%	
Total %	12.11%	0.00%	1.79%	13.90%
Low	32	64	0	96
Column %	26.89%	64.65%	0.00%	
Row %	33.33%	66.67%	0.00%	
Total %	14.35%	28.70%	0.00%	43.05%
Intermediate	60	35	1	96
Column %	50.42%	35.35%	20.00%	
Row %	62.50%	36.46%	1.04%	
Total %	26.91%	15.70%	0.45%	43.05%
Totals	119	99	5	223
Total %	53.36%	44.39%	2.24%	100.00%

Table 3.11 showing observed frequencies of Magee RS 1 with marked cells indicated in RED of the intermediate and low Magee RS 1 category having counts > 10.

Table 3.12: 2-way summary table of observed frequencies (Magee RS 2).

DX_cat	M_RS2Cat Intermediate	M_RS2Cat Low	M_RS2Cat High	Row Totals
High	27	0	4	31
Column %	19.85%	0.00%	44.44%	
Row %	87.10%	0.00%	12.90%	
Total %	12.11%	0.00%	1.79%	13.90%
Low	41	52	3	96
Column %	30.15%	66.67%	33.33%	
Row %	42.71%	54.17%	3.13%	
Total %	18.39%	23.32%	1.35%	43.05%
Intermediate	68	26	2	96
Column %	50.00%	33.33%	22.22%	
Row %	70.83%	27.08%	2.08%	
Total %	30.49%	11.66%	0.90%	43.05%
Totals	136	78	9	223
Total %	60.99%	34.98%	4.04%	100.00%

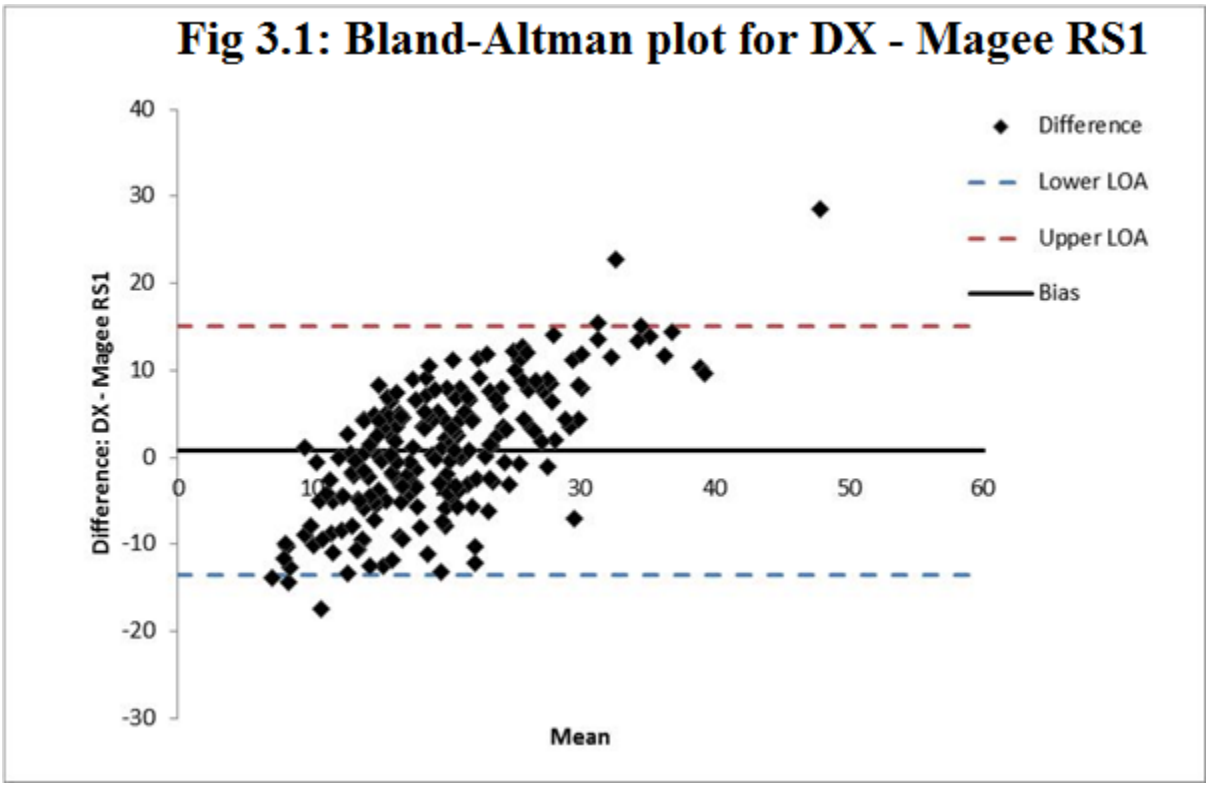
Table 3.12 showing observed frequencies of Magee RS 2 with marked cells indicated in RED of the intermediate and low Magee RS 2 category having counts > 10.

Table 3.13: 2-way summary table of observed frequencies (Magee RS 3).

DX_cat	M_RS3Cat Intermediate	M_RS3Cat Low	M_RS3Cat High	Row Totals
High	23	4	4	31
Column %	28.05%	2.94%	80.00%	
Row %	74.19%	12.90%	12.90%	
Total %	10.31%	1.79%	1.79%	13.90%
Low	16	80	0	96
Column %	19.51%	58.82%	0.00%	
Row %	16.67%	83.33%	0.00%	
Total %	7.17%	35.87%	0.00%	43.05%
Intermediate	43	52	1	96
Column %	52.44%	38.24%	20.00%	
Row %	44.79%	54.17%	1.04%	
Total %	19.28%	23.32%	0.45%	43.05%
Totals	82	136	5	223
Total %	36.77%	60.99%	2.24%	100.00%

Table 3.13 showing observed frequencies of Magee RS 3 with marked cells indicated in RED of the intermediate and low Magee RS 3 category having counts > 10.

In order to visualise the difference and agreement between the compared variables of the Oncotype DX RS and the Magee RS 1,2 and 3 respectively, the mean scores and the difference in the variables were plotted on the Bland-Altman plot as shown in Fig 3.1 – 3.3.



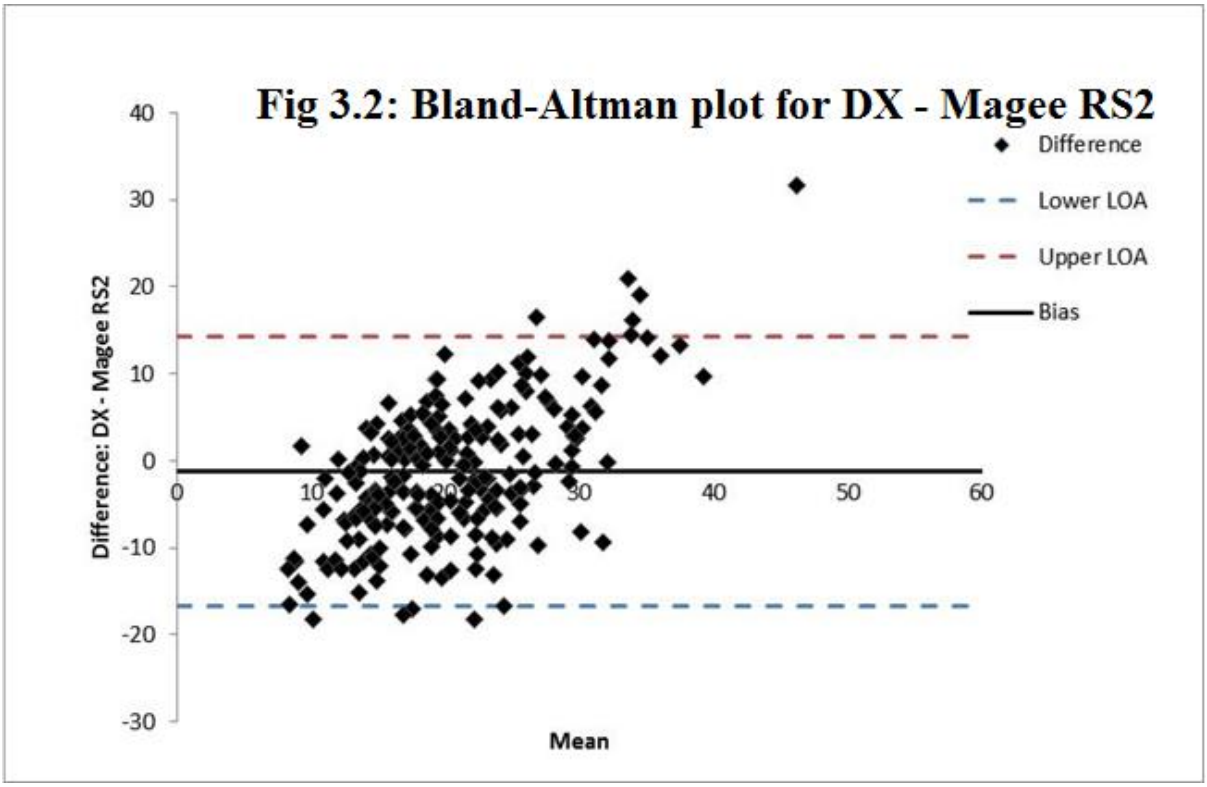


Fig 3.2: Bland-Altman plot for DX - Magee RS2
Difference
Lower LOA
Upper LOA
Bias
Difference: DX - Magee RS2
Mean
40
30
20
10
0
-10
-20
-30
0
10
20
30
40
50
60

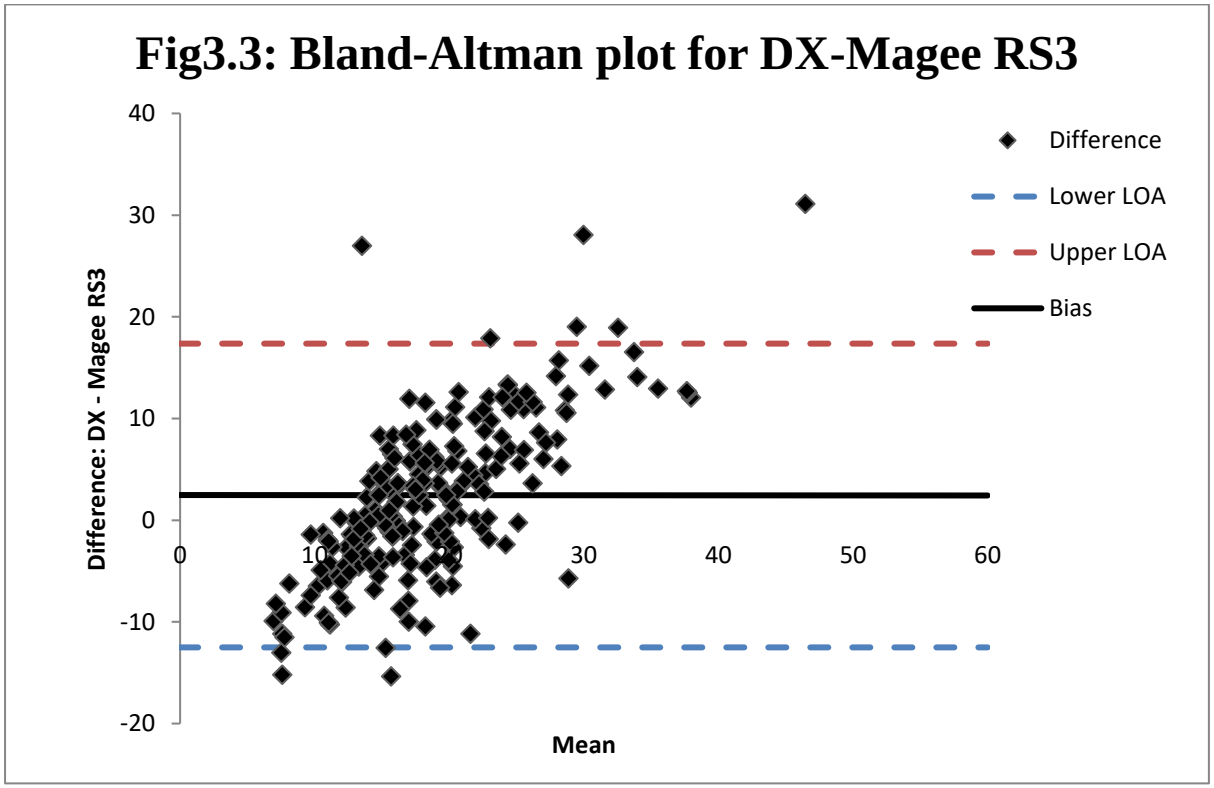


Fig3.3: Bland-Altman plot for DX-Magee RS3
Difference
Lower LOA
Upper LOA
Bias
Difference: DX - Magee RS3
Mean
40
30
20
10
0
-10
-20
0
10
20
30
40
50
60

The bland-Altman plot for Magee RS 1, 2 and 3 shows a small measurement error, however, thereis no proportional bias and no obvious systematic difference. The differences are fairly constant though they contain a few large discrepancies particularly in Magee RS 2 and 3.

CHAPTER FOUR

DISCUSSION

This study presents an independent comparative analysis with a quantitative approach of each Magee RS with the Oncotype DX RS. In the study cohort, it was observed that the Magee RS 1, 2 and 3 were all strongly correlated with the Oncotype DX RS, thus, the correlation was statistically significant as the value of p = .000000 for all three Magee RS with the Oncotype DX RS, thus, indicating a positive relationship (table 3.5 – 3.7). However, only Magee RS 1 was observed to show a statistically nonsignificant difference with the Oncotype DX RS in a Wilcoxon matched pairs test performed between the Oncotype DX RS and Magee RS, as the value of p = 0.1060705 (table 3.8), as Magee RS 2 and 3 respectively showed statistical significance with the Oncotype DX RS as the value of p = 0.014794 for Magee RS 2 and p = 0.000013 for Magee RS 3 (table 3.9 and 3.10). Of the 31 patients classified as high recurrence risk by the Oncotype DX RS the Magee equation 1 and 2 both classified 87.10% (27) as intermediate and 12.90% (4) as high recurrence risk, however, Magee equation 3 while classifying 74.19% (23) as intermediate and 12.90% (4) as high recurrence risk, also classified 12.90% as low recurrence risk (table 3.11 – 3.13). Of the 96 patients classified low recurrence risk by the Oncotype DX RS, Magee RS 1 classified 33.33% (32) as intermediate and 66.67% (64) as low recurrence risk, Magee RS 2 classified 42.71% (41) as intermediate, 54.17% (52) as low and 3.13% (3) as high recurrence risk, while Magee RS 3 classified 16.67% (16) as intermediate, and was able to classify a larger percentage of 83.33% (80) as low recurrence risk. Of the 96 patients classified intermediate recurrence risk by the Oncotype DX RS, Magee RS 1 classified 62.50% (60) as intermediate 36.46% (35) as low and 1.04% (1) as high recurrence risk, Magee RS 2 classified 70.83% (68) as intermediate 27.08% (26) as low and 2.08% (20) as high recurrence risk, Magee RS 3 classified 44.79% (43) as intermediate 54,17% (52) as low and 1.04% (1) as high recurrence risk. Considering that Magee equation 1 and 2

were able to reclassify the 31 patients classified by the Oncotype DX as high recurrence risk into intermediate and high recurrence risk with similar values, and had a high concordance 62.50% and 70.83% with the intermediate Oncotype DX RS may indicate that Magee equation 1 and 2 are able to rule out cases considered to be high recurrence risk by the Oncotype DX and predict the intermediate RS. The Magee equation 3 may be best suited for predicting low RS, as observed frequencies show a high concordance of 83.33% with low Oncotype DX RS.

No one assay can be considered perfect or better than the other, as such, variability and discordance in the observed frequencies may result from histological reporting.[28] Tumours with marked inflammation may show a false high Oncotype DX RS.[29] Studies have also shown that RT-qPCR assay utilised by Oncotype DX in determining HER-2 status may be unreliable, as it was observed to have less than 40% concordance between Oncotype DX and IHC/FISH gold standard.[28 30] Necrosis and changes at the tissue biopsy site characterised by fibrosis and marked inflammation may also be responsible for a false Oncotype DX RS.[29] However, this present study lack these information as such, the Oncotype DX RS could not be determined as false or inaccurate.

In studies of this nature that requires a comparison of two methods designed to measure the same variable, a conclusion cannot be drawn from the test of correlation of both methods. The correlation coefficient may be inadequate sometimes and, thus, misleading in assessing the agreement of the Oncotype DX RS and Magee RS. Thus, a statistically significant correlation between the Oncotype DX RS and Magee RS 1, 2 and 3 respectively only indicates a good relationship between the Oncotype DX RS and Magee RSs. Therefore, in assessing the agreement between the Oncotype DX RS and Magee RS 1, 2 and 3, the Bland-Altman plot may have shown a fair level of agreement in this study cohort. However, the Oncotype DX is clinically proven to aid accurate prognostic decision in prospective studies of large sample sizes. Chemotherapy is to be considered in the proper management of patients classified as

intermediate Oncotype DX RS, as such, the complete exclusion of the Oncotype DX may not be admissible as intermediate recurrence risk patients stand to benefit from adjuvant chemotherapy. Thus, it is critical to ascertain the true recurrence risk of patients classified as low recurrence risk by the Magee RS 1, 2 and 3, but classified as intermediate recurrence risk by Oncotype DX. Identifying the true risk of diseases recurrence in such patients is pertinent in the decision of clinical adoption of the Magee equations as accurate prognostic biomarkers, as excluding chemotherapy in the management of this category of patients based on the Magee RS alone may lead to bad patient outcome, given the significant benefit of adjuvant chemotherapy in such a category of patients.

Limitation

The retrospective nature of this study in itself is considered a limitation, as the data is analysed without information of follow up on the patient or patient's outcome. This study was conducted on what may be considered a small sample size from just two institutions, and only a few high Oncotype DX RS patients of 13.90% of the entire population was recorded. It is possible that the precision of the Magee equations as recorded in this study may differ in other studies with larger sample size and more individuals with a high Oncotype DX RS.

CHAPTER FIVE

CONCLUSION

Based on the results provided by the analysis of the data in this study, the Magee equation 1, 2 and 3 are observed to show a satisfiable level of concordance with the Oncotype DX. It also cannot be determined from this study that one assay is better than the other. The Magee equations may be comparable, however, performing all three Magee equations on a patient may further aid the decision to exclude the Oncotype DX testing, thereby reducing cost. However, totally excluding the Oncotype DX testing in patients based on the Magee RS may be dangerous to the patients' health as the discordance between Oncotype DX RS and Magee RS can only be determined to be correct based on the patient's outcome.

This study may serve as a foundation to further studies, as it encourages continuous validation of the Magee equations in more clinical populations particularly those of high and intermediate risk. It may also be beneficial to compare the Magee RS with Oncotype DX RS in terms of the patient's outcomes in order to ascertain if the discordance in Magee RS with Oncotype DX RS results in reduced accuracy of prognostication.

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