**Table of contents**

Pages 2-3: Methods

Pages 4-8: Entire dataset

Pages 9-13: Cases from retrospective dataset

Pages 14-18: Cases from prospective value study
The cases included in this study are clinical cases on which onco
type DX® testing was requested by treating physicians. The data on tumor size, tumor grading and receptor studies were taken from Pathology reports for calculation of Magee Equation scores.

**NOTTINGHAM SCORE:** Breast tumor grading is performed using Nottingham grading system. The grading has 3 components; the tubule formation score, nuclear pleomorphism score, and mitotic activity score. Each component is scored from 1-3. Once individual scores for each component are assigned, the scores are added to get the Nottingham score that range from 3-9. The tumors with scores 3, 4, and 5 are grouped into grade I tumors, scores of 6 and 7 are grouped into grade II tumors, and the tumors with scores 8 and 9 are grade III tumors. Mitotic activity score is dependent on number of mitotic figures per 10 high power fields. Since the optical diameter of a high power field may vary between different scopes, Pathologists assign the mitotic activity scores based on field diameters. For a scope with field diameter of 0.55 mm, 0-8 mitotic figures per 10 high power field, the tumor gets a score of 1; for 9-18 mitotic figures per 10 high power fields, the tumor is assigned a score of 2; for >18 mitotic figures per 10 high power fields, the tumor is assigned a score of 3.

**ESTROGEN/PROGESTERONE RECEPTORS (ER/PR) TEST DETAILS:** The immunohistochemical staining was performed on clinical cases (mostly core biopsies) fixed in 10% neutral buffered formalin (8-72 hours) with appropriate positive and negative internal/external controls according to the 2010 American Society of Clinical Oncology-College of American Pathologist (ASCO-CAP) Guidelines. The case was classified as positive for Estrogen or Progesterone receptor when nuclear immunostaining is identified in greater than or equal to 1% of the tumor cells (i.e. and H-score of 1 or higher). The ER and PR Histologic Score (H-Score, or HS) is calculated as the sum of intensity of staining times the proportion of cells staining and has a dynamic range of 0 to 300.

Estrogen receptor antibody SP1, FDA approved, was performed using the IVIEW detection on the Benchmark ULTRA (Ventana, Tucson, AZ). Progesterone receptor antibody 1E2, FDA approved, was performed using the IVIEW detection on the Benchmark ULTRA (Ventana, Tucson, AZ).

**HER2 IMMUNOHISTOCHEMISTRY TEST DETAILS:** The immunohistochemical staining was performed on clinical cases (mostly core biopsies) fixed in 10% neutral buffered formalin (8-72 hours) with appropriate positive and negative external controls. The antibody clone, 4B5 was used as part of FDA approved Pathway on the Benchmark ULTRA (Ventana, Tucson, AZ) and interpreted according to the ASCO/CAP HER2 guidelines as follows: Score 0 (negative) = No staining is observed or incomplete faint/barely perceptible membranous staining is observed in ≤10% of the tumor cells. Score 1+ (negative) = A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane. Score 2+ (equivocal) = Circumferential weak to moderate membrane staining is observed in more than 10% of the tumor cells. Intense circumferential membrane staining in ≤10% of tumor cells is also interpreted as score of 2+. This score required reflex testing by fluorescence in-situ hybridization (FISH). Score 3+ (positive) = A strong complete membrane staining is observed in more than 10% of the tumor cells.

**Ki-67 IMMUNOHISTOCHEMISTRY TEST DETAILS:** The immunohistochemical staining was performed on clinical cases (mostly core biopsies) fixed in 10% neutral buffered formalin (8-72 hours)
hours) with appropriate controls. Ki-67 labeling index was calculated as percent positive tumor cells in the entire tumor section. The test was performed using antibody clone 30-9 (Ventana).

**HER2 STATUS CLASSIFICATION (for Magee Equation score calculation):** Although HER2 cases are currently classified according to the ASCO/CAP 2018 update at our institution, a slightly modified approach is taken for Magee Equation score calculation. It is to be noted that the 2007 ASCO/CAP HER2 guideline criteria were utilized for classification of cases for initial development and validation of Magee Equations. However, the ASCO/CAP HER2 classification criteria have changed over the years. In the neoadjuvant study assessing the chemopredictiveness of Magee Equation 3 (Mod Pathol. 2017;30:1078-1085. PMID: 28548119), following criteria were used for determination of HER2 status:

HER2 negative: HER2 IHC score 0, IHC score 1+, and IHC score 2+ with HER2 copies per cell less than 4 by FISH

HER2 positive: HER2 IHC score of 3+, and HER2 IHC score of 2+ with HER2 copies per cell of 6 or more by FISH

HER2 equivocal: HER2 IHC score of 2+ with HER2 copies per cell of 4 to less than 6 by FISH

We have used the same criteria for HER2 status classification in the current study. Only HER2 negative and HER2 “equivocal” cases were included in the current study.
**Entire dataset**
ER+/HER2-neg/eq cases with all 3 Magee Equation scores: 2196

Based on all 3 equations (Magee Decision Algorithm):

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send  | Grand Total |
|----------------|-------------------------|-----------------------|-------|-------------|
| >25            | 19                      | 75                    | 191   | 285         |
| 25 or less     | 1                       | 1443                  | 467   | 1911        |
| **Grand Total**| **20**                  | **1518**              | **658** | **2196**   |

%age classified as do not send: 20+1518/2196 = 1538/2196 = 70%
%age classified as send: 658/2196 = 30%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|-----------------------|-------------|
| >25            | 19                      | 75                    | 94          |
| 25 or less     | 1                       | 1443                  | 1444        |
| **Grand Total**| **20**                  | **1518**              | **1538**    |

Accuracy of do not send: 19+1443/1538 = 95.1%
Ability to predict low-risk (≤25): 1443/1518 = 95.1%
Ability to predict high-risk (>25): 19/20 = 95%
Based on ME1 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|------------------------|------------------------|------|-------------|
| >25            | 41                     | 92                     | 152  | 285         |
| 25 or less     | 9                      | 1512                   | 390  | 1911        |
| **Grand Total**| **50**                 | **1604**               | **542** | **2196**  |

%age classified as do not send: 50+1604/2196=75.3%
%age classified as send: 542/2196=24.7%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|------------------------|------------------------|-------------|
| >25            | 41                     | 92                     | 133         |
| 25 or less     | 9                      | 1512                   | 1521        |
| **Grand Total**| **50**                 | **1604**               | **1654**    |

Accuracy of do not send: 41+1512/1654=93.9%
Accuracy of do not send (expect low): 1512/1604=94.3%
Accuracy of do not send (expect high): 41/50=82%
Based on ME2 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 30                      | 95                     | 160  | 285         |
| 25 or less     | 7                       | 1500                   | 404  | 1911        |
| **Grand Total**| **37**                  | **1595**               | **564** | **2196**   |

%age classified as do not send: 37+1595/2196=74.3%
%age classified as send: 564/2196=25.7%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 30                      | 95                     | 125         |
| 25 or less     | 7                       | 1500                   | 1507        |
| **Grand Total**| **37**                  | **1595**               | **1632**    |

Accuracy of do not send: 30+1500/1632=93.8%
Accuracy of do not send (expect low): 1500/1595=94%
Accuracy of do not send (expect high): 30/37=81.1%
Based on ME3 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 31                      | 92                     | 162  | 285         |
| 25 or less     | 9                       | 1599                   | 303  | 1911        |
| **Grand Total**| **40**                  | **1691**               | **465** | **2196**   |

%age classified as do not send: 40+1691/2196=78.8%
%age classified as send: 465/2196=21.2%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 31                      | 92                     | 123         |
| 25 or less     | 9                       | 1599                   | 1608        |
| Grand Total    | **40**                  | **1691**               | **1731**    |

Accuracy of do not send: 31+1599/1731=94.2%
Accuracy of do not send (expect low): 1599/1691=94.6%
Accuracy of do not send (expect high): 31/40=77.5%
Based on ME-mean:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 31                      | 93                     | 161  | 285         |
| 25 or less     | 2                       | 1538                   | 371  | 1911        |
| **Grand Total**| **33**                  | **1631**               | **532** | **2196** |

%age classified as do not send: $33 + 1631 / 2196 = 75.8\%$

%age classified as send: $532 / 2196 = 24.2\%$

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 31                      | 93                     | 124         |
| 25 or less     | 2                       | 1538                   | 1540        |
| **Grand Total**| **33**                  | **1631**               | **1664**    |

Accuracy of do not send: $31 + 1538 / 1664 = 94.3\%$

Accuracy of do not send (expect low): $1538 / 1631 = 94.3\%$

Accuracy of do not send (expect high): $31 / 33 = 93.9\%$
**Cases from retrospective dataset**

ER+/HER2-neg/eq cases with all 3 Magee Equation scores: 1824

Based on all 3 equations (Magee Algorithm):

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 16                      | 67                     | 166  | 249         |
| 25 or less     | 1                       | 1182                   | 392  | 1575        |
| Grand Total    | 17                      | 1249                   | 558  | 1824        |

%age classified as do not send: 17+1249/1824 = 1266/1824 = 69.4%

%age classified as send: 558/1824 = 30.6%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 16                      | 67                     | 83          |
| 25 or less     | 1                       | 1182                   | 1183        |
| Grand Total    | 17                      | 1249                   | 1266        |

Accuracy of do not send: 16+1182/1266 = 94.6%

Accuracy of do not send (expect low): 1182/1249 = 94.6%

Accuracy of do not send (expect high): 16/17 = 94.1%
Based on ME1 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 35                      | 81                     | 133  | 249         |
| 25 or less     | 7                       | 1240                   | 328  | 1575        |
| **Grand Total**| **42**                  | **1321**               | **461** | **1824** |

%age classified as do not send: 42 + 1321 / 1824 = 74.7%
%age classified as send: 461 / 1824 = 25.3%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 35                      | 81                     | 116         |
| 25 or less     | 7                       | 1240                   | 1247        |
| **Grand Total**| **42**                  | **1321**               | **1363**    |

Accuracy of do not send: 35 + 1240 / 1363 = 93.5%
Accuracy of do not send (expect low): 1240 / 1321 = 93.9%
Accuracy of do not send (expect high): 35 / 42 = 83.3%
Based on ME2 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 26                      | 86                     | 137  | 249         |
| 25 or less     | 5                       | 1234                   | 336  | 1575        |
| Grand Total    | 31                      | 1320                   | 473  | 1824        |

%age classified as do not send: 31+1320/1824=74.1%
%age classified as send: 473/1824=25.9%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 26                      | 86                     | 112         |
| 25 or less     | 5                       | 1234                   | 1239        |
| Grand Total    | 31                      | 1320                   | 1351        |

Accuracy of do not send: 26+1234/1351=93.3%
Accuracy of do not send (expect low): 1234/1320=93.5%
Accuracy of do not send (expect high): 26/31=83.8%
Based on ME3 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 26                      | 81                     | 142  | 249         |
| 25 or less     | 8                       | 1313                   | 254  | 1575        |
| Grand Total    | 34                      | 1394                   | 396  | 1824        |

% age classified as do not send: 34+1394/1824=78.3%
% age classified as send: 396/1824=21.7%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 26                      | 81                     | 107         |
| 25 or less     | 8                       | 1313                   | 1321        |
| Grand Total    | 34                      | 1394                   | 1428        |

Accuracy of do not send: 26+1313/1428=93.7%
Accuracy of do not send (expect low): 1313/1394=94.2%
Accuracy of do not send (expect high): 26/34=76.5%
Based on ME-mean:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 26                      | 84                     | 139  | 249         |
| 25 or less     | 2                       | 1264                   | 309  | 1575        |
| Grand Total    | 28                      | 1348                   | 448  | 1824        |

%age classified as do not send: 28+1348/1824=75.4%
%age classified as send: 448/1824=24.6%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 26                      | 84                     | 110         |
| 25 or less     | 2                       | 1264                   | 1266        |
| Grand Total    | 28                      | 1348                   | 1376        |

Accuracy of do not send: 26+1264/1376=93.8%
Accuracy of do not send (expect low): 1264/1348=93.8%
Accuracy of do not send (expect high): 26/28=92.9%
**Cases from prospective value study**
ER+/HER2-neg/eq cases with all 3 Magee Equation scores: 372

Based on all 3 equations (Magee Algorithm):

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 3                       | 8                      | 25   | 36          |
| 25 or less     |                         | 261                    | 75   | 336         |
| Grand Total    | 3                       | 269                    | 100  | 372         |

%age classified as do not send: 3+269/372 = 272/372 = 73.1%
%age classified as send: 100/372 = 26.9%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 3                       | 8                      | 11          |
| 25 or less     |                         | 261                    | 261         |
| Grand Total    | 3                       | 269                    | 272         |

Accuracy of do not send: 3+261/272 = 97.1%
Accuracy of do not send (expect low): 261/269 = 97%
Accuracy of do not send (expect high): 3/3 = 100%
Based on ME1 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 6                       | 11                     | 19   | 36          |
| 25 or less     | 2                       | 272                    | 62   | 336         |
| **Grand Total**| **8**                   | **283**                | **81**| **372**     |

%age classified as do not send: 8+283/372=78.3%
%age classified as send: 81/372=21.7%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 6                       | 11                     | 17          |
| 25 or less     | 2                       | 272                    | 274         |
| **Grand Total**| **8**                   | **283**                | **291**     |

Accuracy of do not send: 6+272/291=95.5%
Accuracy of do not send (expect low): 272/283=96.1%
Accuracy of do not send (expect high): 6/8=75%
Based on ME2 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 4                       | 9                      | 23   | 36          |
| 25 or less     | 2                       | 266                    | 68   | 336         |
| Grand Total    | 6                       | 275                    | 91   | 372         |

%age classified as do not send: 6+275/372=75.5%
%age classified as send: 91/372=24.5%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 4                       | 9                      | 13          |
| 25 or less     | 2                       | 266                    | 268         |
| Grand Total    | 6                       | 275                    | 281         |

Accuracy of do not send: 4+266/281=96%
Accuracy of do not send (expect low): 266/275=96.7%
Accuracy of do not send (expect high): 4/6=66.7%
Based on ME3 ONLY:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|-----------------------|------|-------------|
| >25            | 5                       | 11                    | 20   | 36          |
| 25 or less     | 1                       | 286                   | 49   | 336         |
| Grand Total    | 6                       | 297                   | 69   | 372         |

%age classified as do not send: 6+297/372=81.5%
%age classified as send: 69/372=18.5%

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|-----------------------|-------------|
| >25            | 5                       | 11                    | 16          |
| 25 or less     | 1                       | 286                   | 287         |
| Grand Total    | 6                       | 297                   | 303         |

Accuracy of do not send: 5+286/303=96%
Accuracy of do not send (expect low): 286/297=96.3%
Accuracy of do not send (expect high): 5/6=83.3%
Based on ME-mean:

| Oncotype Score | Do not send-expect high | Do not send-expect low | Send | Grand Total |
|----------------|-------------------------|------------------------|------|-------------|
| >25            | 5                       | 9                      | 22   | 36          |
| 25 or less     |                         | 274                    | 62   | 336         |
| Grand Total    | 5                       | 283                    | 84   | 372         |

%age classified as do not send: \(5+283/372=77.4\%\)
%age classified as send: \(84/372=22.6\%\)

| Oncotype Score | Do not send-expect high | Do not send-expect low | Grand Total |
|----------------|-------------------------|------------------------|-------------|
| >25            | 5                       | 9                      | 14          |
| 25 or less     |                         | 274                    | 274         |
| Grand Total    | 5                       | 283                    | 288         |

Accuracy of do not send: \(5+274/288=96.9\%\)
Accuracy of do not send (expect low): \(274/283=96.8\%\)
Accuracy of do not send (expect high): \(5/5=100\%\)