Opto-Acoustic Imaging of Breast Masses: Correlation with Breast Biopsy Prognostic Indicators

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Overview

- Regulatory Status: The Imagio™ OA/US breast imaging system, a diagnostic opto-acoustic (OA) imaging device bearing the CE Mark, is in the U.S. FDA Premarket Approval process.
- Principle: OA/US provides both functional (relative oxygenation/de-oxygenation) and anatomic (angiogenesis) information that is co-registered and temporally interleaved in real time with gray-scale ultrasound improving discrimination between benign and malignant masses. We recently reported correlation studies demonstrating tumor-zone specific OA attributes in histopathologic grade I versus grade III malignancies.
- Purpose: To determine the relationship between OA attributes (individual feature scores or summed feature results) and prognostic subtypes of breast cancer (**PSBC**).

Materials and Methods

- HIPAA-compliant, IRB-approved prospective multi-center trial across 16 U.S. clinical sites.
- 1,808 masses in 1,739 subjects assessed as BI-RADS 3, 4 or 5 were imaged with OA/US. Of these, 678 lesions were malignant and the subject of this analysis.
- Each mass was scored by 7 blinded readers on 3 internal zone features of the tumor nidus and 2 external features of the tumor boundary and peripheral zones.
- Pathologic diagnoses were confirmed by an experienced central breast pathologist blinded to the OA assessment. Tumor histologic classification and grading were performed for all subjects.
- Tumor estrogen receptor (ER) and progesterone receptor (PR) were performed at each site by immunohistochemistry (IHC) and positive if 1% or greater.
- Tumor HER2-*neu* expression was reported by IHC as 0, 1+ (negative, not over-expressed), 2+ (indeterminate) and 3+ (over-expressed). All 2+ results reflexed to fluorescence in-situ hybridization (FISH) and reported as over-expressed or not over-expressed.

Materials and Methods - continued

- Tumor Ki-67 expression was evaluated with IHC and reported as positive (>20%), indeterminate or negative (<10%).
- **PSBC** were defined as :
  - Luminal A (LUM-A) if ER/PR (+), Ki-67 and Her2-*neu* (-)
  - Luminal B (LUM-B) if ER (+), PR (+/-), Ki-67 (+) and Her2-*neu* (+/-)
  - Triple-Negative Breast Cancer (TNBC) if ER/PR (-), Ki-67 (+/-) and Her2-*neu* (-).
  - Her 2-enriched (HER2) if ER/PR (-), Ki-67 (+/-) and Her2-*neu* (+).
  - Tumors not meeting any of these four categories were deemed unclassified.
- Statistical analysis of categorical measures of PSBC was performed using a two-way Analysis of Variance (ANOVA) and Least Squares Significant Difference test for pairwise comparisons. This ANOVA was repeated to test which specific PSBC sub-categories were related to OA attributes. Correlation coefficients were generated for PSBC that were continuous, not categorical. All statistical testing was done at a 5% significance level.

Results

- A total of 678 breast carcinomas (653 invasive and 25 DCIS) were scored for internal (tumor nidus) and external (boundary zone and periphery) OA attributes and compared with PSBC as defined by ER, PR, Her2-*neu* and Ki-67 expression. (2 lymphomas excluded)
- 292 subjects were not classified and an additional 25 with pure DCIS were excluded.

Conclusions

- This study reports highly significant pre-biopsy, OA differentiation of LUM-As from TNBC with summed internal external OA features
- Summed external OA Features differentiate Luminal-B from TNBC
- The Imagio™ OA/US breast imaging system may contribute significantly to the non-invasive diagnostic and prognostic evaluation of women with breast masses.
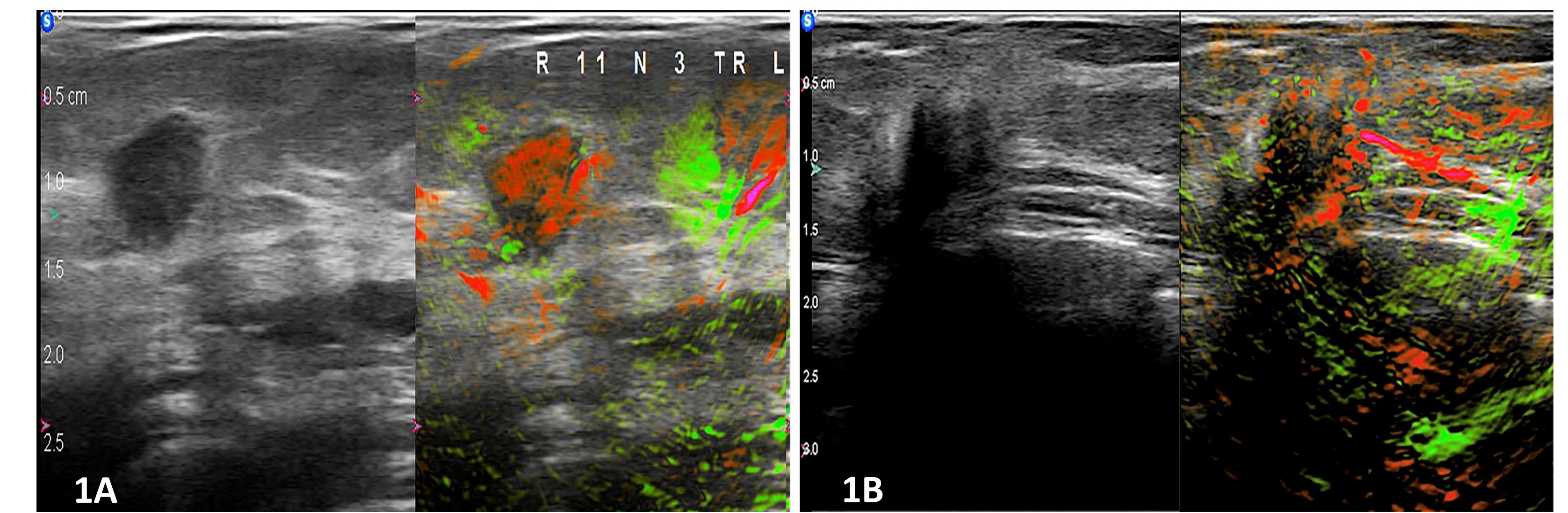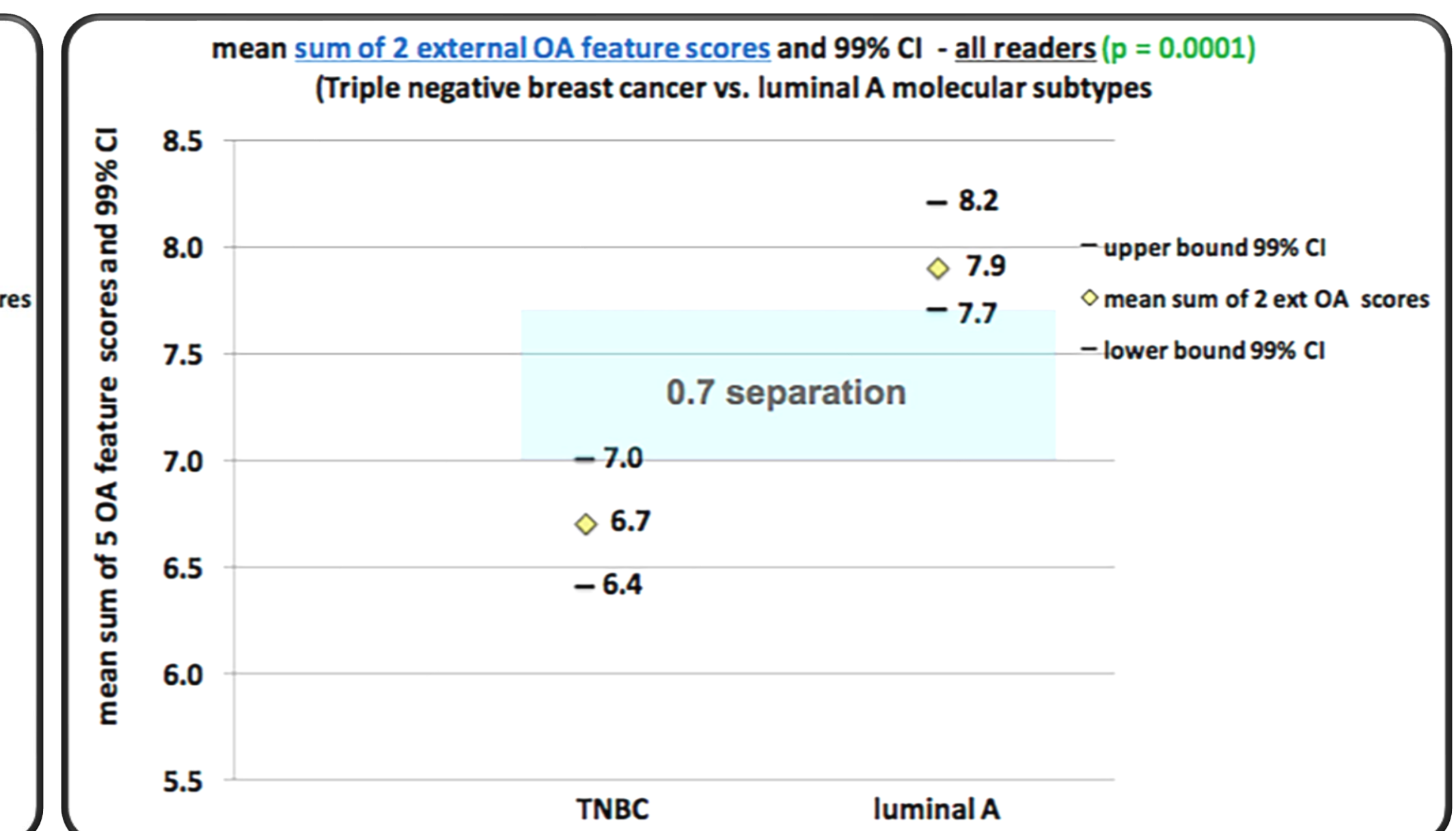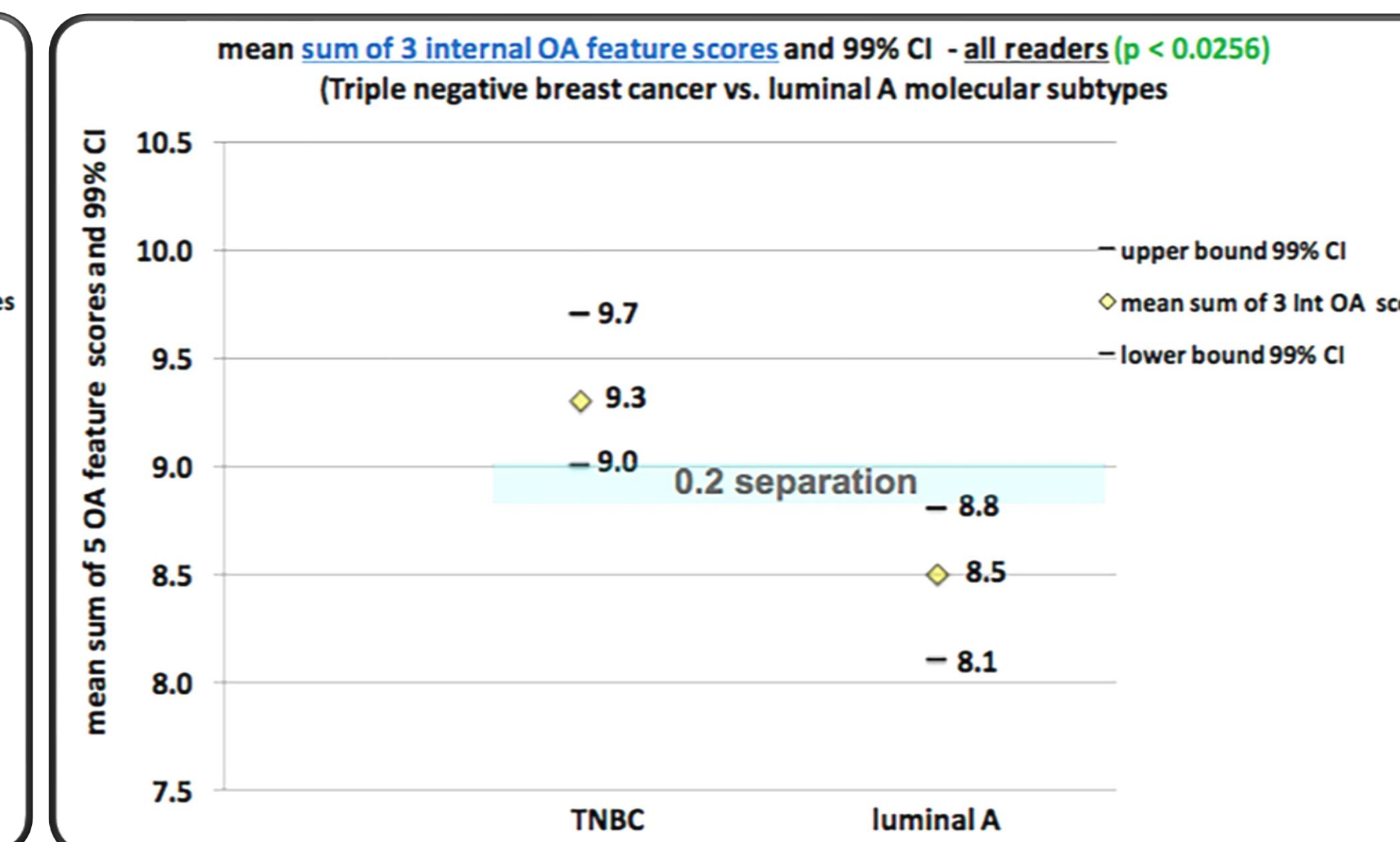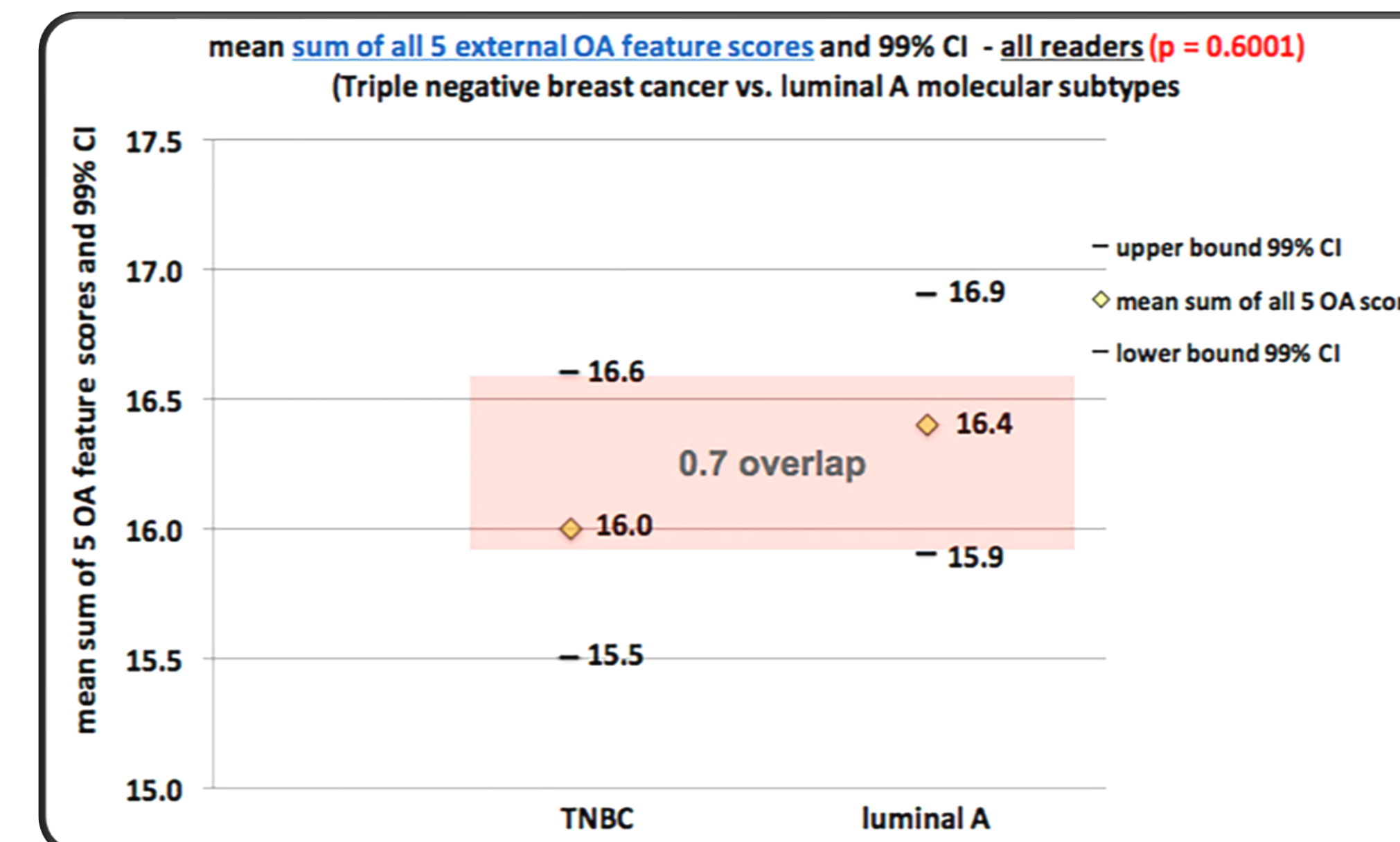

True positive small TNBC **(1A)** and small LUM-A cancer **(1B)**: The TNBC shows mainly internal findings consisting of polymorphic intensely deoxygenated vessels. The LUM-A cancer, on the other hand, shows a paucity of internal findings and abundant external findings consisting of short perpendicularly oriented tumor vessels in the boundary zone (BZ whiskers), partial BZ deoxygenated blush, and a peripheral radiating deoxygenated vessel on the right.
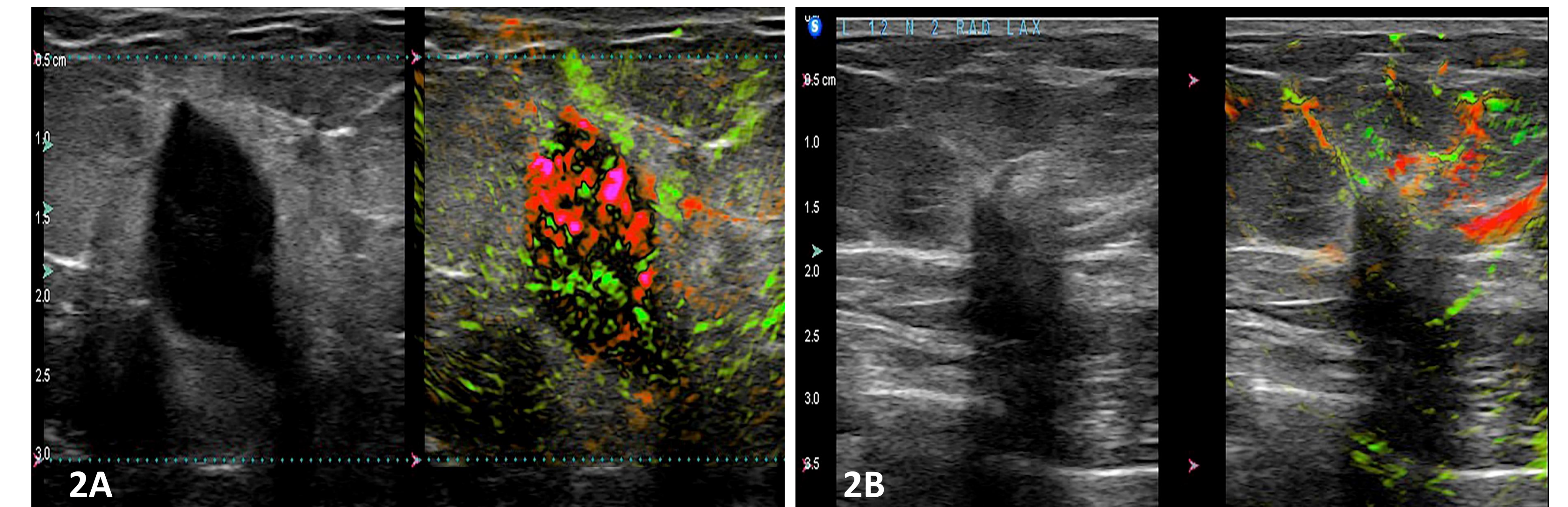
True positive medium-sized TNBC **(2A)** and medium sized LUM-A cancer **(2B)**: As in the smaller masses above, the TNBC has primarily internal findings consisting of numerous polymorphic intensely deoxygenated (red) internal vessels and a paucity of external findings. The LUM-A mass, on the other hand, has absent internal findings and abundant external findings consisting of a few BZ whiskers and multiple deoxygenated peripheral zone radiating vessels.

		Molecular Subtype				p-value					
Significance to p < 0.05		LUM-A (n=108)	LUM-B (n=153)	TNBC (n=80)	HER2 (n=23)	LUM-A v. LUM-B	LUM-A v. TNBC	LUM-A v. HER2	LUM-B v. TNBC	LUM-B v. HER2	TNBC v. HER2
Sum of 3 Internal OA Features	Mean (SD)	8.5 (3.60)	9.1 (3.54)	9.3 (3.30)	9.4 (3.08)	0.0831	0.0256	0.0707	0.4632	0.4684	0.8385
	99% CI	8.1, 8.8	8.8, 9.3	9.0, 9.7	8.8, 10.1						
Sum of 2 External OA Features	Mean (SD)	7.9 (2.82)	7.6 (2.76)	6.7 (2.82)	7.2 (2.61)	0.2301	0.0001	0.0617	0.0020	0.2724	0.2516
	99% CI	7.7, 8.2	7.4, 7.8	6.4, 7.0	6.6, 7.7						
Sum of all 5 OA Features	Mean (SD)	16.4 (5.63)	16.6 (5.63)	16.0 (5.37)	16.6 (4.87)	0.6500	0.6001	0.7781	0.3146	0.9724	0.4990
	99%CI	15.9, 16.9	16.2, 17.1	15.5, 16.6	15.5, 16.6						

Borderline Significance

Sum of 3 Internal Score (Range 0-15), Sum of 2 External Score (Range 0-11), Sum of all 5 Scores (Range 0-26)

mean sum of all 5 external OA feature scores and 99% CI - all readers (p = 0.6001)
(Triple negative breast cancer vs. luminal A molecular subtypes)
- upper bound 99% CI
- mean sum of all 5 OA scores
- lower bound 99% CI
- 16.9
- 16.6
- 16.4
- 16.0
- 15.9
- 15.5
0.7 overlap
TNBC   luminal A

mean sum of 3 internal OA feature scores and 99% CI - all readers (p < 0.0256)
(Triple negative breast cancer vs. luminal A molecular subtypes)
- upper bound 99% CI
- mean sum of 3 int OA scores
- lower bound 99% CI
- 9.7
- 9.3
- 9.0
- 8.8
- 8.5
- 8.1
0.2 separation
TNBC   luminal A

mean sum of 2 external OA feature scores and 99% CI - all readers (p = 0.0001)
(Triple negative breast cancer vs. luminal A molecular subtypes)
- upper bound 99% CI
- mean sum of 2 ext OA scores
- lower bound 99% CI
- 8.2
- 7.9
- 7.7
- 7.0
- 6.7
- 6.4
0.7 separation
TNBC   luminal A

Both TNBCs and LUM-A cancers have abundant neovascularity and deoxygenation, but the sum of all 5 OA feature scores shows overlap in their 99% confidence intervals and non-significant p-value. It is the difference in distribution of neovascularity and deoxygenation in the tumor zones that distinguishes the OA findings of TNBC and LUM-A breast cancers, as shown by significant p-values and separation of confidence intervals. TNBCs have higher internal OA feature scores, and LUM-A breast cancers have higher external OA feature scores, particularly within the external peripheral zone. P-values and separation of confidence intervals show external OA features to be more significant.