Libyan Journal of Health, Science, and Development

Volume 2, Issue 2, 2026, Pages: 77 - 88

Journal homepage:

https://ljhsd.org.ly/index.php/ljhsd/index

Directory of Online Libyan Journals

(Article)

Immunohistochemical Profile and Molecular Subtype Distribution of Breast Cancer in Libya: A Multi-Laboratory Retrospective Study

Abdelmuhsen M. Abusneina[1*], Tawfeek A. Altwaty[2], Sarah M. Alemam[3], Mariam F. Bohdima[4], Hajir A. Abdulaziz[5], and Monia J. Bayou[6]

[1-6] Department of Molecular Diagnostics, Faculty of Biomedical Sciences, University of Benghazi, Benghazi, Libya.

النمط الكيميائي المناعي النسيجي وتوزيع الأنماط الجزيئية لسرطان الثدي في شرق وغرب وجنوب ووسط ليبيا: دراسة استعادية متعددة المختبرات

عبد المحسن محمد أبوسنينة[1*]، توفيق عبدالله التواتي[2]، سارة محمد الإمام[3]، مريم فرج بوهديمة[4]، هاجر عقيلة عبد 5 منية جمال بعيو[6]

[1-6]قسم التشخيص الجزيئي، كلية العلوم الطبية الحيوية، جامعة بنغازي، بنغازي، ليبيا.

Publication history: Received on 18 August 2026; Accepted on 01 September 2026; Published on 3 September 2026

Abstract: Immunohistochemistry (IHC) for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 is the routine surrogate for molecular subtyping of invasive breast cancer, but reference values from Libyan laboratories are scarce. We describe the IHC biomarker profile and molecular subtype distribution of 214 breast cancer reports compiled retrospectively from three Libyan sources (Saleem Laboratory, Benghazi Lab for Medical Analysis, and Benghazi National Cancer Center / other referring sources; 2010–2022, 88.8% dated 2019–2022). ER and PR were scored by the Allred system (0–8) and HER2 by IHC (0/1+/2+/3+); surrogate molecular subtypes were assigned using the post-2011 St Gallen definitions with a 15% Ki-67 cut-off. Patients were predominantly female (170/173 with known sex, 98.3%); known age (n=169) ranged 29–76 years (mean 50.1, median 49), and most reports originated from eastern Libya (172/200 with known location, 86.0%). Among scored reports, ER was strongly positive (Allred 7–8) in 54.1% and PR in 29.5%. HER2 (206 scored) was 0 in 51.9%, 1+ in 20.4%, 2+ (equivocal) in 5.8%, and 3+ (positive) in 21.8%. Among 191 reports classifiable by all four markers, luminal B-like was the most frequent subtype (46.1%), followed by luminal A-like (26.2%), triple-negative/basal-like (14.7%) and HER2-overexpression (13.1%); biomarker positivity was broadly consistent across laboratories. This multi-laboratory series provides local reference values for breast cancer IHC and molecular subtype distribution in Libya, dominated by luminal B-like disease and a marked geographic skew toward the east. Standardized IHC reporting and broader geographic sampling are needed for nationally representative estimates.

Keywords: Breast cancer; immunohistochemistry; molecular subtype; HER2; Libya.

الملخص

يُعدّ التحليل الكيميائي المناعي النسيجي (IHC) لمستقبلات الإستروجين (ER) والبروجستيرون (PR) ومستقبل عامل النمو البشروي البشري 2 (HER2) وKi-67 الوسيلة الروتينية البديلة لتصنيف الأنماط الجزيئية لسرطان الثدي الغازي، إلا أن القيم المرجعية من المختبرات الليبية نادرة. نصف في هذه الدراسة النمط الحيوي المناعي وتوزيع الأنماط الجزيئية لـ 214 تقريراً لسرطان الثدي جُمعت بأثر رجعي من ثلاثة مصادر ليبية (مختبر سليم، ومختبر بنغازي للتحاليل الطبية، والمركز الوطني للسرطان ببنغازي/مصادر إحالة أخرى؛ 2010–2022، و88.8% منها بتاريخ 2019–2022). صُنّفت ER وPR وفق نظام Allred (0–8) وHER2 بالتحليل المناعي (0/1+/2+/3+)؛ وحُدّدت الأنماط الجزيئية البديلة باستخدام تعريفات سانت غالن لما بعد عام 2011 مع حدٍّ فاصل لـ Ki-67 قدره 15%. كان المرضى في غالبيتهم من الإناث (173/170 ممن عُرف جنسهم، 98.3%)؛ وتراوحت الأعمار المعروفة (العدد=169) بين 29 و76 عاماً (المتوسط 50.1، الوسيط 49)، وورد معظم التقارير من شرق ليبيا (200/172، 86.0%). ومن بين التقارير المُقيَّمة، كانت ER إيجابية بقوة في 54.1% وPR في 29.5%. وكانت HER2 بدرجة 0 في 51.9%، و1+ في 20.4%، و2+ (غير حاسمة) في 5.8%، و3+ (إيجابية) في 21.8%. ومن بين 191 تقريراً قابلاً للتصنيف بالمؤشرات الأربعة جميعها، كان النمط اللمعي B هو الأكثر شيوعاً (46.1%)، يليه اللمعي A (26.2%)، ثم الثلاثي السلبي/القاعدي (14.7%)، وفرط تعبير HER2

(13.1%)؛ وكانت إيجابية المؤشرات متسقة عموماً بين المختبرات. توفّر هذه السلسلة متعددة المختبرات قيماً مرجعية محلية لتوزيع المؤشرات المناعية والأنماط الجزيئية لسرطان الثدي في ليبيا، يغلب عليها النمط اللمعي B مع تحيّز جغرافي واضح نحو الشرق. وثمة حاجة إلى توحيد معايير التقارير المناعية وتوسيع نطاق أخذ العينات جغرافياً للحصول على تقديرات ممثِّلة على المستوى الوطني.

الكلمات المفتاحية: سرطان الثدي؛ التحليل الكيميائي المناعي النسيجي؛ النمط الجزيئي؛ HER2؛ ليبيا.

Introduction

Cancer is characterized by the proliferation of cells that have evaded the endogenous mechanisms that normally control growth, and cancers are increasingly classified not only by organ of origin but also by the molecular characteristics of the tumor cells themselves. Rapid advances in molecular diagnostics now allow the molecular make-up of a tumor to be characterized within clinically useful timeframes, with the long-term goal of tailoring treatment to the individual patient and tumor biology [1, 2]. Carcinogenesis involves the activation of oncogenes and/or inactivation of tumor suppressor genes, together with epigenetic changes, including histone modification, DNA methylation and hydroxymethylation, that further alter pathways governing apoptosis, autophagy and cell adhesion, ultimately enabling local invasion and distant metastasis [3]. Breast cancer is the most common cause of cancer-related death among women in many industrialized countries and the second or third most common cause in developing countries. Its incidence continues to rise globally, with wide geographic variation from low-incidence regions such as parts of Asia, Latin America and Africa to high-incidence regions such as North America, Western Europe and Australia [4, 5]. It is a histologically and molecularly heterogeneous disease: the WHO classification recognizes more than two dozen histological subtypes of invasive carcinoma, and gene-expression profiling has further shown that this histological diversity corresponds to distinct patterns of gene expression, broadly separating tumors into an estrogen-receptor (ER)-enriched “luminal” group, molecularly similar to normal luminal epithelium, and an ER-negative “basal” group [6]. In Libya, hospital-based cancer registries have historically reported total case counts without a clearly defined geographic catchment area, which has made it difficult to calculate population-based incidence rates or to compare biomarker prevalence meaningfully across regions [7]. A 2011 comparative study of 234 Libyan patients estimated a national breast cancer incidence of approximately 18.8 per 100,000 women and highlighted demographic differences, including a younger mean age at diagnosis, relative to European populations [4]. Beyond this and a small number of single-center studies, Libya-specific reference data on breast cancer biomarker expression remain limited, and, to our knowledge, no prior Libyan study has combined data from multiple independent laboratories to describe IHC-based molecular subtype distribution. A range of established and probable risk factors for breast cancer have been described, including a personal or family history of breast cancer, inherited mutations such as BRCA1/BRCA2, cumulative estrogen exposure (early menarche, late menopause, nulliparity, hormone therapy), and lifestyle factors such as obesity, alcohol use and sedentary behavior [8, 9]. While risk-factor data were not available for the present series, they provide important context for interpreting biomarker distributions across populations. The routine clinical assessment of invasive breast cancer relies on immunohistochemical evaluation of four markers. The estrogen receptor (ER) and progesterone receptor (PR) are ligand-activated nuclear transcription factors that mediate the proliferative and differentiating effects of estrogen and progesterone on breast epithelium, and their expression status guides the use of endocrine therapy [10–12]. PR expression is itself partly regulated by ER signaling and provides additional prognostic information beyond ER status alone [13]. Ki-67 is a nuclear protein expressed during all active phases of the cell cycle and is used as a surrogate marker of tumor proliferation, though its precise molecular function remains incompletely understood and inter-laboratory measurement is known to vary [14–16]. HER2 (human epidermal growth factor receptor 2) is a transmembrane receptor tyrosine kinase whose gene amplification or protein overexpression drives an aggressive tumor phenotype but also predicts response to HER2-targeted therapy; it is evaluated by IHC in around 80% of initial HER2 testing, with equivocal (2+) results referred for in-situ hybridization confirmation [17, 18]. Combined 4-marker IHC (“4-IHC”: ER, PR, HER2, Ki-67) has been adopted as a practical surrogate for gene-expression-based intrinsic subtyping since the 2011 St Gallen International Expert Consensus, because it is rapid and inexpensive relative to genomic platforms and shows reasonable, though imperfect, concordance with intrinsic subtypes defined by gene expression [19–23]. Using this approach, tumors are conventionally classified as luminal A-like (ER and/or PR positive, HER2-negative, low Ki-67), luminal B-like (ER and/or PR positive, with either HER2-positivity or high Ki-67), HER2-

overexpression/non-luminal (ER-negative, PR-negative, HER2-positive), or triple-negative/basal-like (ER-negative, PR-negative, HER2-negative); each subtype carries distinct prognostic and therapeutic implications, with luminal A-like disease generally managed with endocrine therapy alone and luminal B-like disease more often considered for additional chemotherapy [22, 23]. The Ki-67 cut-off used to separate luminal A-like from luminal B-like disease has varied between consensus statements (commonly 14–20%) and remains a recognized source of inter-laboratory and inter-study discordance [24]. This study aimed to describe the demographic profile, hormone receptor and HER2 status, and IHC-based molecular subtype distribution of a retrospective series of breast cancer reports collected from three Libyan laboratory sources, and to compare these findings with previously published Libyan and regional data.

Material and Methods

Study design and setting

This was a retrospective, descriptive, multi-source study of existing breast cancer IHC reports. No new tissue staining, sectioning, or laboratory testing was performed for this study; all biomarker results (ER, PR, HER2, Ki-67) had already been generated as part of routine diagnostic reporting by the source laboratories, using each laboratory's own standard clinical IHC workflow, and were subsequently compiled, cleaned and analyzed retrospectively for this manuscript. Because the underlying staining and interpretation were performed independently by each laboratory as part of routine care, no wet-laboratory protocol details or histological images are presented here; only the recorded biomarker results and case-level metadata generated during routine reporting are analyzed.

Data sources and case ascertainment

Two hundred and fourteen breast cancer IHC reports were compiled from three Libyan sources: Saleem Laboratory (Al-Fateh Street, Benghazi; n=53), Benghazi Lab for Medical Analysis (n=36), and Benghazi National Cancer Center together with other referring sources (n=125). Reports spanned 2010–2022, with the large majority (88.8%) dated 2019–2022. Inclusion required an available breast cancer IHC report from one of the three participating sources; no additional exclusion criteria were applied beyond the availability of a report, so cases with incomplete marker panels were retained and analyzed as missing data rather than excluded outright. For each report, the following variables were extracted: patient age, sex, city/locality of the reporting source, date of report, and the recorded ER, PR, HER2, and Ki-67 results.

Biomarker scoring

ER and PR expression had been scored by the reporting laboratories using the Allred system, in which a proportion score (0–5, reflecting the estimated fraction of positively staining tumor cell nuclei) is added to an intensity score (0–3, reflecting average staining intensity) to give a combined score from 0 to 8 [25]. For this analysis, Allred scores were grouped into three categories: negative (0–2), moderately positive (3–6), and strongly positive (7–8). HER2 expression had been scored by IHC on the standard 0/1+/2+/3+ scale, in which 0 and 1+ are considered negative, 2+ is equivocal and requires in-situ hybridization confirmation, and 3+ is considered positive [18]. Ki-67 labeling index was recorded as reported in the free text of each original report (exact percentages, ranges, or qualitative terms such as "low" or "high") and was parsed into a numeric estimate for subtype classification; ranges were converted to their midpoint, and qualitative terms were assigned representative values consistent with the source laboratories' apparent usage.

Molecular subtype definitions

Reports were classified into IHC-based surrogate molecular subtypes using the definitions adopted following the 2011 St Gallen International Expert Consensus, applying a 15% Ki-67 cut-off to distinguish low from high proliferation: luminal A-like (ER and/or PR positive, HER2-negative, Ki-67 <15%); luminal B-like (ER and/or PR positive, with either HER2-positive status or Ki-67 ≥15%, encompassing both HER2-negative and HER2-positive luminal B-like tumors); HER2-overexpression/non-luminal (ER-negative, PR-negative, HER2-positive); and triple-negative/basal-like (ER-negative, PR-negative, HER2-negative) [20, 22, 23]. This four-category surrogate scheme approximates, but does not fully reproduce, the five intrinsic subtypes originally defined by gene-expression profiling (luminal A, luminal B, HER2-enriched, basal-like and normal-like), and concordance between IHC-based and genomic classification is imperfect. For example, only around 80% of triple-negative

tumors by IHC correspond to intrinsic basal-like tumors by gene expression [24]. Cases with a HER2 2+ (equivocal) IHC score were treated as equivocal for subtype assignment; in the absence of available confirmatory in-situ hybridization (ISH) data in these retrospective reports, 2+ cases were excluded from definitive HER2-positive or HER2-negative surrogate subtype assignment. Reports missing one or more of the four markers needed for full classification were similarly recorded as luminal-like-unspecified or unclassifiable, as appropriate, and were excluded from subtype percentage calculations.

Data cleaning and statistical analysis

The compiled dataset contained inconsistent notation for several variables (e.g., HER2 recorded as "+1", "1+" or "1"; Allred scores recorded as fractions such as "7/8" or as decimal equivalents such as "0.875"; mixed date formats), which were standardized before analysis. City/locality values were mapped to four regions of Libya (East, West, South, Central). Descriptive statistics (frequencies, percentages, means, medians, standard deviation and range) were computed using Python (pandas). No inferential statistical testing was performed, consistent with the descriptive aim of this study; formal comparison of biomarker distributions between source laboratories or regions would require adequately powered inferential analysis, which was beyond the scope of this descriptive report.

Ethical considerations

This study was a retrospective analysis of existing, de-identified diagnostic laboratory reports. No patient-identifying information was collected, accessed or used at any stage, and no new clinical intervention or contact with patients was involved. As the analysis used only anonymized, routinely generated pathology data and carried no risk to individuals, formal institutional review board approval and individual informed consent did not apply to this secondary use of non-identifiable data.

Results

Two hundred and fourteen breast cancer IHC reports were analyzed. Demographic and source characteristics are summarized in Table 1 and Figures 1 and 2. Known age (n=169) ranged from 29 to 76 years (mean 50.1 years, SD 10.2; median 49 years); age was most frequently in the 45–54-year band (62/169, 36.7%) and was not recorded in 45 reports (21.0% of the total). Among reports with known sex, 170/173 (98.3%) were female and 3/173 (1.7%) male; sex was not recorded for 41 reports (19.2%).

Table 1. Demographic and source characteristics of the study population (n = 214).

Characteristic	Category	n	%
Age group (years)	25–34	6	3.6%
	35–44	51	30.2%
	45–54	62	36.7%
	55–64	33	19.5%
	65+	17	10.1%
Sex	Female	170	98.3%
	Male	3	1.7%
Region of Libya	East	172	86.0%
	West	13	6.5%
	South	10	5.0%
	Central	5	2.5%
Source laboratory	Benghazi National Cancer Center / other sources	125	58.4%
	Saleem Laboratory	53	24.8%
	Benghazi Lab for Medical Analysis	36	16.8%

Percentages are calculated among reports with known data for each characteristic (age n = 169, sex n = 173, region n = 200, source laboratory n = 214). Data were missing for age in 45 reports, sex in 41, and region in 14. Known age (n = 169): mean 50.1 years (SD 10.2), median 49 years, range 29–76 years.

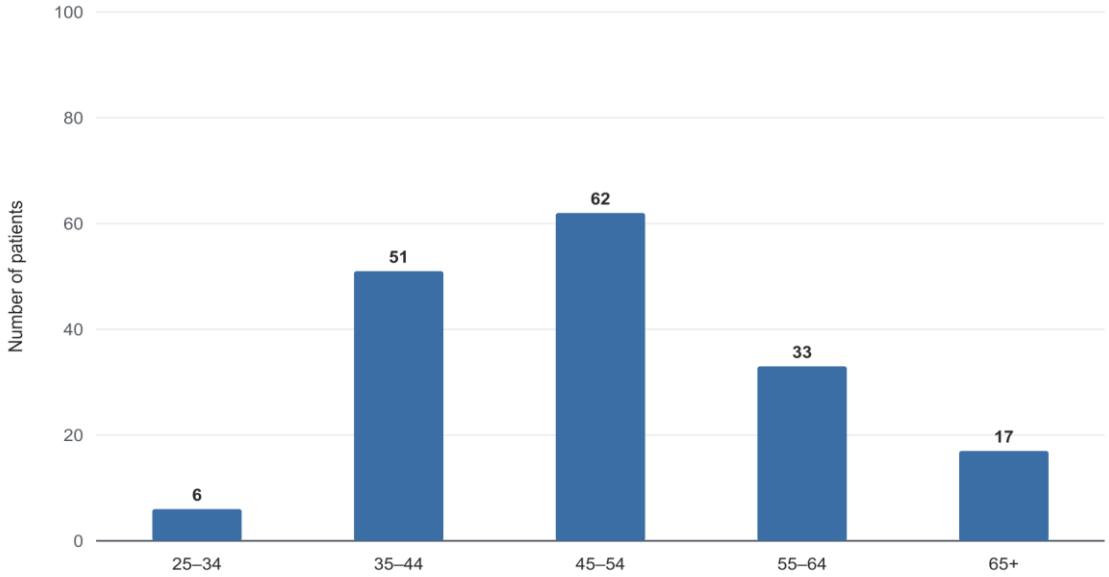

Figure 1: Age distribution of patients (n = 169 with known age).

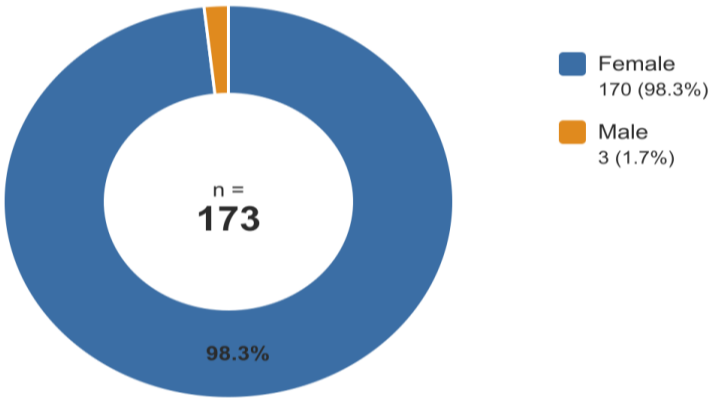

Figure 2: Sex distribution of patients (n = 173 with known sex).

Hormone receptor scoring was available for 133 reports (62.1%) for ER and 132 reports (61.7%) for PR (Table 2, Figure 3). Allred scores were not available for 81 reports (37.9%) for ER and 82 reports (38.3%) for PR. Among scored reports, ER was strongly positive in 54.1% and PR strongly positive in 29.5%; PR showed a higher proportion of negative scores (39.4%) than ER (27.8%), consistent with PR expression being lost more readily than ER expression in this cohort.

Table 2. Hormone receptor (ER and PR) Allred score distribution.

Allred score category	ER, n (%)	PR, n (%)
Negative (0–2)	37 (27.8%)	52 (39.4%)
Moderate positive (3–6)	24 (18.0%)	41 (31.1%)
Strong positive (7–8)	72 (54.1%)	39 (29.5%)
Total scored	**133**	**132**

Percentages calculated among reports with an available Allred score. An Allred score was not available for 81 reports for ER and 82 for PR.

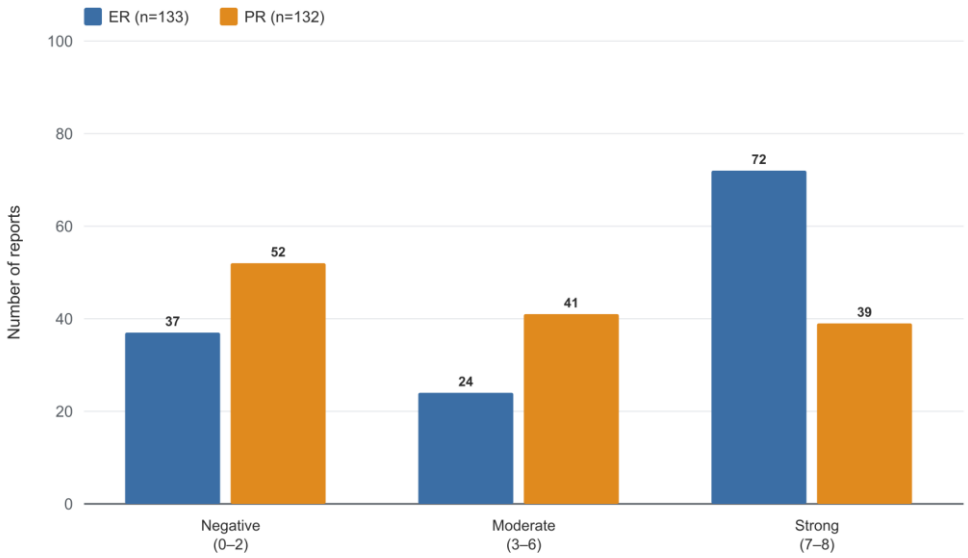

Figure 3: Allred score categories for ER and PR.

HER2 IHC scores were available for 206/214 reports (Table 3, Figure 4). Among these, 51.9% scored HER2 0 (negative) and 21.8% scored 3+ (positive); 5.8% were equivocal (2+) and would require in situ hybridization to definitively resolve HER2 status.

Table 3. HER2 immunohistochemical score distribution (n = 206).

HER2 IHC score	Interpretation	n	%
0	Negative	107	51.9%
1+	Negative	42	20.4%
2+	Equivocal (requires ISH confirmation)	12	5.8%
3+	Positive	45	21.8%

Percentages are calculated among the 206 reports with an available HER2 score; HER2 was not recorded for 8 reports.

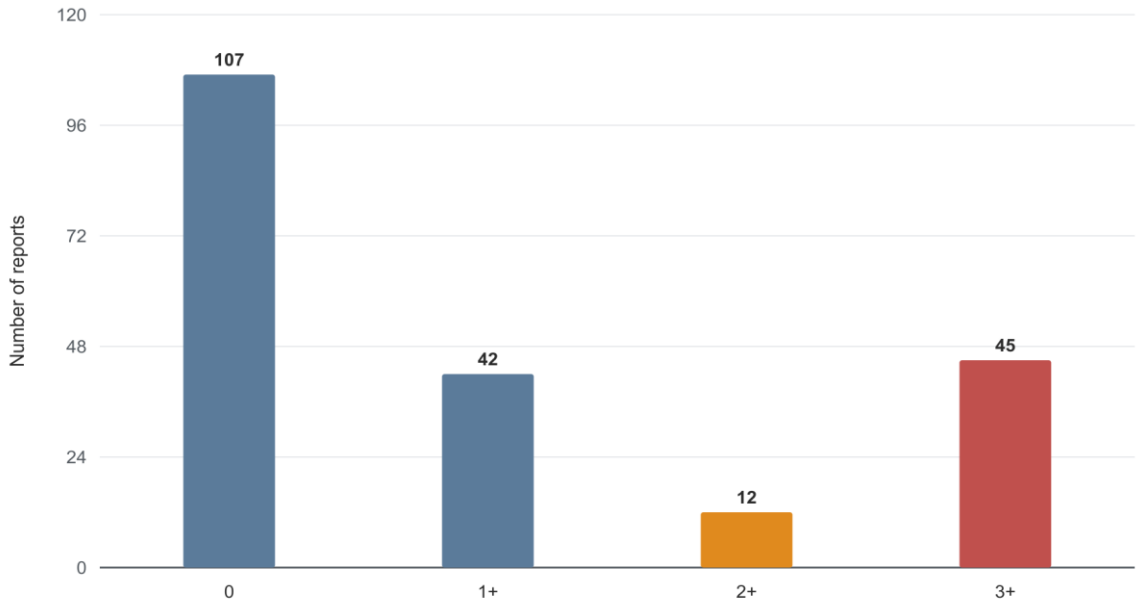

Figure 4: HER2 IHC score distribution (n = 206 with known HER2 status).

Of the 214 reports, 191 (89.3%) had all four markers available and could be assigned to an IHC-based molecular subtype (Table 4, Figure 5). Luminal B-like was the most common subtype (46.1%), followed by luminal A-like (26.2%), triple-negative/basal-like (14.7%) and HER2-overexpression/non-luminal (13.1%). A further 16 reports were classifiable only as luminal-like (ER and/or PR positive, but HER2 and/or Ki-67 not both available), and 7 reports could not be classified due to missing markers; these 23 reports (10.7% of the total) are excluded from the subtype percentages.

Table 4. Molecular (intrinsic) subtype distribution among classifiable reports (n = 191).

IHC-based surrogate subtype	IHC profile	n	% of classified
Luminal A-like	ER+ and/or PR+, HER2−, low Ki-67	50	26.2%
Luminal B-like	ER+ and/or PR+, HER2− or HER2+, high Ki-67	88	46.1%
HER2 overexpression (non-luminal)	ER−, PR−, HER2+	25	13.1%
Triple-negative / basal-like	ER−, PR−, HER2−	28	14.7%

An additional 16 reports were classifiable only as luminal-like, and 7 could not be classified due to missing markers (23 reports, 10.7% of the total 214); these are excluded from the percentages above.

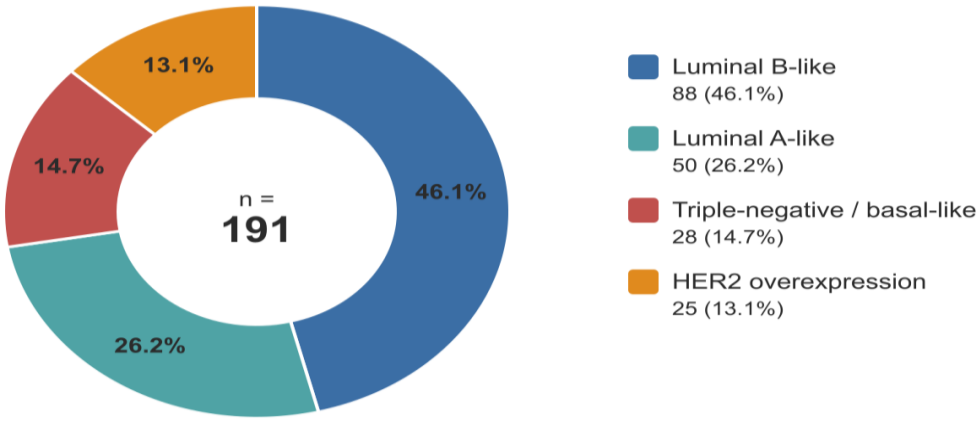

Figure 5: Molecular (intrinsic) subtype distribution among classified reports (n = 191).

Reports were geographically concentrated in eastern Libya (Figure 6) and were predominantly reported between 2019 and 2022 (Figure 7), reflecting the recruitment periods and referral patterns of the contributing laboratories rather than a true regional or temporal trend in disease incidence.

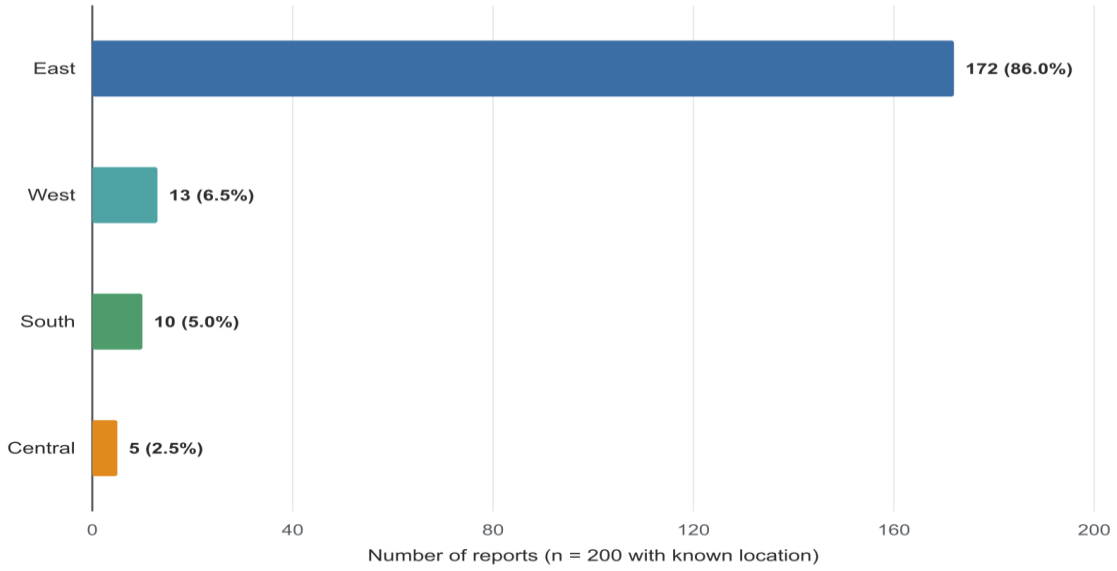

Figure 6: Geographic distribution of reports by region of Libya (n = 200 with known location).

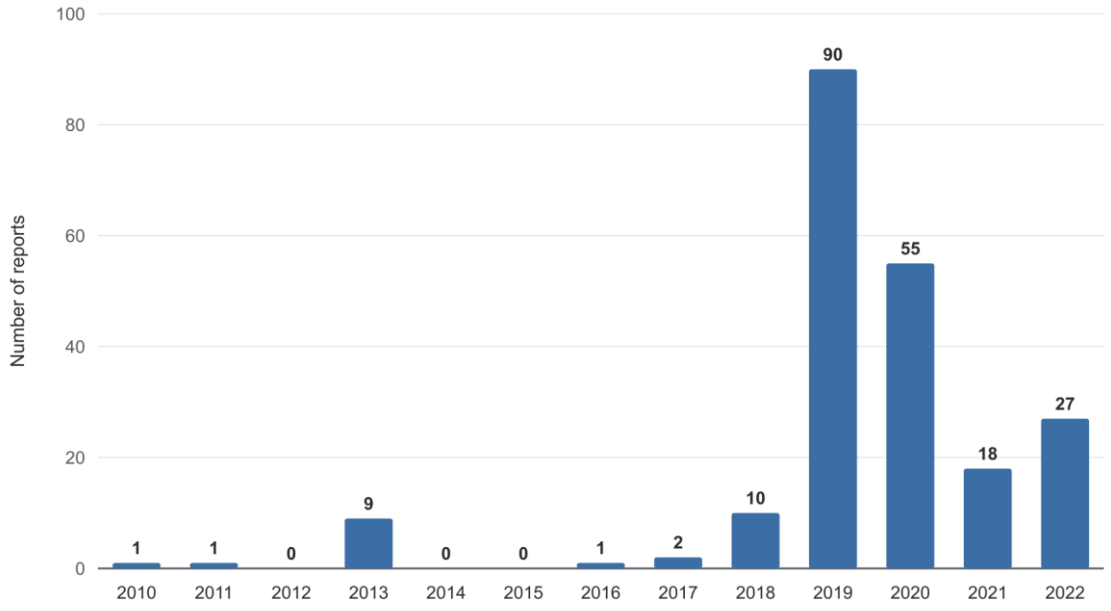

Figure 7: Distribution of reports by year (n = 214).

Biomarker positivity also varied modestly by source laboratory (Table 5, Figure 8). Overall, ER was positive (by qualitative report status) in 153/214 reports (71.5%) and PR in 135/214 (63.1%); HER2 was 2+ or 3+ in 57/214 reports (26.6%). The proportional patterns of ER, PR, and HER2 positivity were broadly similar across the three sources, with Benghazi National Cancer Center/other sources contributing both the largest share of cases and the highest proportion of ER/PR-positive results.

Table 5. ER, PR and HER2 positivity by source laboratory.

Source laboratory	n	ER+, n (%)	PR+, n (%)	HER2+ (2+/3+), n (%)
Saleem Laboratory	53	31 (58.5%)	26 (49.1%)	13 (24.5%)
Benghazi Lab for Medical Analysis	36	24 (66.7%)	19 (52.8%)	12 (33.3%)
Benghazi National Cancer Center / other sources	125	98 (78.4%)	90 (72.0%)	32 (25.6%)
Total	**214**	**153 (71.5%)**	**135 (63.1%)**	**57 (26.6%)**

ER/PR positivity determined from the qualitative status recorded in the original report text; HER2 positivity defined as IHC score 2+ or 3+. Denominators for positivity rates are the total 214 reports, including those with missing HER2 scores.

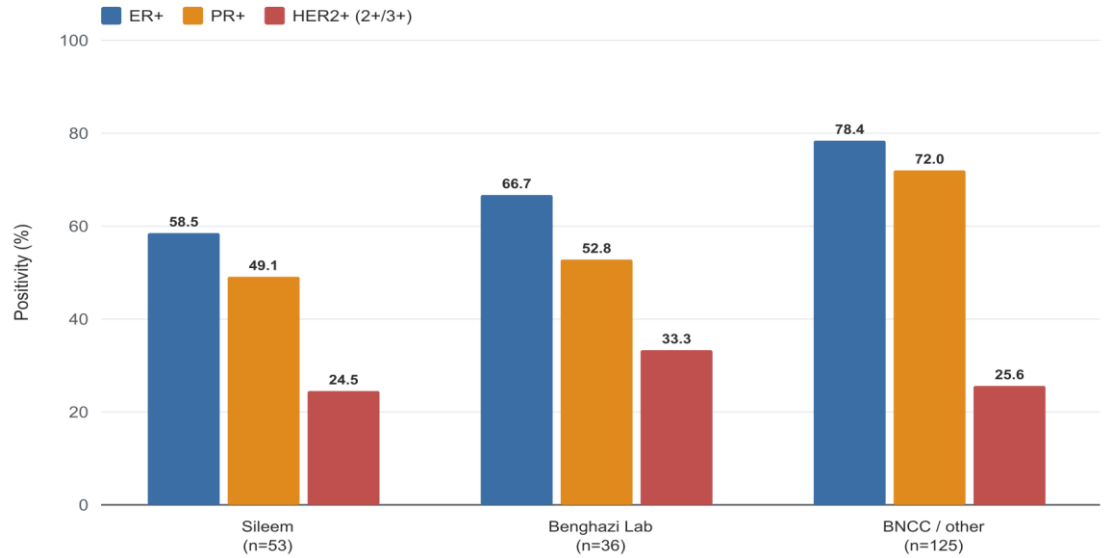

Figure 8: ER, PR and HER2 positivity by source laboratory.

Discussion

This retrospective series of 214 breast cancer IHC reports, compiled from three independent Libyan laboratory sources, provides a set of local reference values for hormone receptor, HER2 and molecular subtype distribution that, to our knowledge, has not previously been reported at this scale for Libya. Luminal B-like disease was the most common subtype (46.1%), followed by luminal A-like (26.2%), triple-negative/basal-like (14.7%) and HER2-overexpression (13.1%).

This distribution differs from the pattern most often reported in Western, genomic-platform-based series, where luminal A-like disease is typically the largest single group, accounting for roughly 40–50% of invasive breast cancers, with HER2-overexpressing tumors comprising around 15% [5]. In the present series, luminal A-like disease was proportionally smaller and luminal B-like proportionally larger than these benchmark ranges. Because the luminal A-like/B-like boundary in this analysis depends entirely on the Ki-67 cut-off applied (15%, per the 2011 St Gallen consensus) and on Ki-67 values that were extracted from heterogeneous free-text report fields rather than a single standardized assay, some of this apparent shift toward luminal B-like disease may reflect cut-off and measurement variability across the three contributing laboratories rather than a true difference in underlying tumor biology [24]. A referral-pattern effect is also plausible: Benghazi National Cancer Center, which contributed the largest single share of reports in this series, is likely to receive a disproportionate share of more advanced or higher-grade referrals, which could skew the pooled subtype distribution toward higher-proliferation, luminal B-like disease relative to a truly population-representative sample.

The mean age in this series (50.1 years) was notably higher than the 46 years reported by Boder et al. in a series of 234 Libyan breast cancer patients treated in Sabratha between 2002 and 2006, and that same study estimated a Libyan breast cancer incidence of approximately 18.8 per 100,000 women, underscoring the historical lack of population-based incidence data noted for Libya more broadly [4, 7]. The reason for the age difference between the two series cannot be determined from the present data, but plausible contributors include differences in referral source (this series draws in part from a national cancer center that may see an older, more advanced-disease population), changes in diagnostic practice or screening uptake over the roughly 15 years separating the two cohorts, or differences in how missing age data (21.0% of reports here) were distributed.

Abdalla et al. previously examined ER and PR expression in a smaller series of 62 Libyan patients and found that ER- and PR-positive tumors were associated with better survival than receptor-negative tumors, consistent with the prognostic relevance of hormone receptor status observed more broadly in the literature; that study did not report an overall ER/PR positivity proportion in a form directly comparable with the Allred-based categories used here, so a direct numerical comparison was not attempted [26]. HER2 status in Libyan breast cancer has also been examined in relation to clinicopathological features and survival by Jebriel et al., and, in an older population (≥70 years), by Al-Zawi et al. in a multicenter series of 150 cases; the 21.8% HER2 3+ positivity rate observed in the present series falls within the broad range reported across such regional studies [27, 28].

The pronounced geographic skew toward eastern Libya (86.0% of reports with known location) reflects the location of the contributing laboratories (all based in or referring through Benghazi) rather than a true regional difference in disease burden, and mirrors a limitation previously highlighted for Libyan hospital-based cancer registries more generally, which typically lack a defined geographic catchment area and therefore cannot be used to estimate population-based incidence or make valid regional comparisons [7].

Biomarker positivity was broadly similar across the three contributing laboratories (Table 5), which is reassuring for the internal consistency of this pooled analysis, although the modestly higher ER/PR positivity proportion at Benghazi National Cancer Center compared with the other two sources could reflect either a genuine case-mix difference or subtle differences in antibody clones, staining protocols or scoring thresholds between laboratories, a distinction that cannot be resolved without prospective, standardized comparison.

Differentiating luminal A-like from luminal B-like disease has direct therapeutic relevance, since luminal A-like tumors are generally managed with endocrine therapy alone, while luminal B-like tumors are more often considered for additional chemotherapy [22]. Because the Ki-67 cut-off used to make this distinction varies between guidelines and laboratories (commonly 14–20%), and because inter-laboratory variation in Ki-67 measurement is a recognized problem even when a common cut-off is nominally applied, the subtype proportions reported here should be interpreted as indicative rather than as a precise, guideline-independent estimate of the true underlying subtype distribution in this population [24]. Standardizing Ki-67 scoring and reporting conventions across Libyan laboratories, for example, by adopting a single explicit cut-off and requiring

quantitative rather than qualitative ("low"/"high") reporting, would improve the comparability of future multi-center Libyan series such as this one.

Strengths and limitations

The principal strength of this study is its multi-source design, providing the first combined, IHC-based molecular subtype distribution for breast cancer in Libya. Limitations include its retrospective design without access to clinical outcomes, and the pooling of data from three laboratories without standardized staining protocols, risking inter-laboratory variability. Missing data (age 21.0%, sex 19.2%, ER 37.9%, PR 38.3%) and geographic concentration in eastern Libya may bias estimates and limit national generalizability. Additionally, extracting Ki-67 from heterogeneous free-text fields may misclassify luminal A/B subtypes, and the modest sample size (n=214) precludes inferential statistical analysis.

Conclusion

This multi-laboratory retrospective series describes the hormone receptor, HER2 and IHC-based molecular subtype distribution of breast cancer reports from Libya, showing a predominance of luminal B-like disease and a strong geographic concentration in the east of the country. These findings offer local reference values that may assist Libyan pathology laboratories and oncologists in benchmarking their reporting practices. Standardized inter-laboratory IHC protocols, more complete biomarker reporting and broader geographic sampling will be needed to obtain nationally representative estimates of breast cancer molecular subtype distribution in Libya and, ultimately, to support equitable access to biomarker-guided treatment across the country.

Acknowledgements

The authors thank Saleem Laboratory (Benghazi), Benghazi Lab for Medical Analysis, and Benghazi National Cancer Center for access to the anonymized immunohistochemistry reports used in this study.

Conflict of interest

The authors declare no conflict of interest.

Funding

No external funding was received for this study.

References

1. Kim, S. W., Roh, J., & Park, C. S. (2016). Immunohistochemistry for pathologists: Protocols, pitfalls, and tips. *Journal of Pathology and Translational Medicine*, *50*(6), 411–418. https://doi.org/10.4132/jptm.2016.08.08

2. Krieghoff-Henning, E., Folkerts, J., Penzkofer, A., & Weg-Remers, S. (2017). Cancer – An overview. *Medizinische Monatsschrift für Pharmazeuten*, *40*(2), 48–54.

3. Sarkar, S., Horn, G., Moulton, K., Oza, A., Byler, S., Kokolus, S., & Longacre, M. (2013). Cancer development, progression, and therapy: An epigenetic overview. *International Journal of Molecular Sciences*, *14*(10), 21087–21113. https://doi.org/10.3390/ijms141021087

4. Boder, J. M. E., Abdalla, F. B. E., Elfageih, M. A., Abusaa, A., Buhmeida, A., & Collan, Y. (2011). Breast cancer patients in Libya: Comparison with European and central African patients. *Oncology Letters*, *2*(2), 323–330. https://doi.org/10.3892/ol.2011.245

5. Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T. C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & Diseases*, *5*(2), 77–106. https://doi.org/10.1016/j.gendis.2018.05.001

6. Rakha, E. A., & Green, A. R. (2017). Molecular classification of breast cancer: What the pathologist needs to know. *Pathology*, *49*(2), 111–119. https://doi.org/10.1016/j.pathol.2016.10.012

7. Zarmouh, A., Almalti, A., Alzedam, A., Hamad, M., Elmughrabi, H., Alnajjar, L., Elmugassabi, M., Kashbour, M., & Elsaghayer, W. (2022). Cancer incidence in the middle region of Libya: Data from the cancer epidemiology study in Misurata. *Cancer Reports*, *5*(1), e1448. https://doi.org/10.1002/cnr2.1448

8. Sharma, G. N., Dave, R., Sanadya, J., Sharma, P., & Sharma, K. K. (2010). Various types and management of breast cancer: An overview. *Journal of Advanced Pharmaceutical Technology & Research*, *1*(2), 109–126.

9. Hankinson, S. E., Colditz, G. A., & Willett, W. C. (2004). Towards an integrated model for breast cancer etiology: The lifelong interplay of genes, lifestyle, and hormones. *Breast Cancer Research*, *6*(5), 213–218. https://doi.org/10.1186/bcr921

10. Pakdel, F. (2018). Molecular pathways of estrogen receptor action. *International Journal of Molecular Sciences*, *19*(9), 2591. https://doi.org/10.3390/ijms19092591

11. Millas, I., & Duarte Barros, M. (2021). Estrogen receptors and their roles in the immune and respiratory systems. *The Anatomical Record*, *304*(6), 1185–1193. https://doi.org/10.1002/ar.24612

12. Esteva, F. J., & Hortobagyi, G. N. (2004). Prognostic molecular markers in early breast cancer. *Breast Cancer Research*, *6*(3), 109–118. https://doi.org/10.1186/bcr777

13. Grimm, S. L., Hartig, S. M., & Edwards, D. P. (2016). Progesterone receptor signaling mechanisms. *Journal of Molecular Biology*, *428*(19), 3831–3849. https://doi.org/10.1016/j.jmb.2016.06.020

14. Scholzen, T., & Gerdes, J. (2000). The Ki-67 protein: From the known and the unknown. *Journal of Cellular Physiology*, *182*(3), 311–322.

15. Wang, D., Xu, J., Shi, G., & Yin, G. (2015). Molecular markers' progress of breast cancer treatment efficacy. *Journal of Cancer Research and Therapeutics*, *11*(Suppl 1), C11–C15.

16. Remnant, L., Kochanova, N. Y., Reid, C., Cisneros-Soberanis, F., & Earnshaw, W. C. (2021). The intrinsically disorderly story of Ki-67. *Open Biology*, *11*(8), 210120. https://doi.org/10.1098/rsob.210120

17. Krishnamurti, U., & Silverman, J. F. (2014). HER2 in breast cancer: A review and update. *Advances in Anatomic Pathology*, *21*(2), 100–107.

18. Wolff, A. C., Hammond, M. E. H., Allison, K. H., Harvey, B. E., Mangu, P. B., Bartlett, J. M. S., Bilous, M., Ellis, I. O., Fitzgibbons, P. L., Hanna, W., Jenkins, R. B., Press, M. F., Spears, P. A., Vance, G. H., Viale, G., McShane, L. M., & Dowsett, M. (2018). Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO/CAP Clinical Practice Guideline Focused Update. Journal of Clinical Oncology, 36(20), 2105–2122. https://doi.org/10.1200/JCO.2018.77.8738

19. Gándara-Cortés, M., Vázquez-Boquete, Á., Fernández-Rodríguez, B., Viaño, P., Ínsua, D., Seoane-Seoane, A., Gude, F., Gallego, R., Fraga, M., Antúnez, J. R., Curiel, T., Pérez-López, E., & García-Caballero, T. (2018). Breast cancer subtype discrimination using standardized 4-IHC and digital image analysis. *Virchows Archiv*, *472*(2), 195–203. https://doi.org/10.1007/s00428-017-2194-z

20. Cheang, M. C. U., Chia, S. K., Voduc, D., Gao, D., Leung, S., Snider, J., Watson, M., Davies, S., Bernard, P. S., Parker, J. S., Perou, C. M., Ellis, M. J., & Nielsen, T. O. (2009). Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *Journal of the National Cancer Institute*, *101*(10), 736–750. https://doi.org/10.1093/jnci/djp082

21. Maisonneuve, P., Disalvatore, D., Rotmensz, N., Curigliano, G., Colleoni, M., Dellapasqua, S., Pruneri, G., Mastropasqua, M. G., Luini, A., Bassi, F., Pagani, G., Viale, G., & Goldhirsch, A. (2014). Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. *Breast Cancer Research*, *16*(3), R65. https://doi.org/10.1186/bcr3679

22. Goldhirsch, A., Winer, E. P., Coates, A. S., Gelber, R. D., Piccart-Gebhart, M., Thürlimann, B., & Senn, H. J. (2013). Personalizing the treatment of women with early breast cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Annals of Oncology*, *24*(9), 2206–2223. https://doi.org/10.1093/annonc/mdt303

23. Goldhirsch, A., Wood, W. C., Coates, A. S., Gelber, R. D., Thürlimann, B., & Senn, H. J. (2011). Strategies for subtypes: Dealing with the diversity of breast cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Annals of Oncology*, *22*(8), 1736–1747. https://doi.org/10.1093/annonc/mdr304

24. Tsang, J. Y. S., & Tse, G. M. (2020). Molecular classification of breast cancer. *Advances in Anatomic Pathology*, *27*(1), 27–35.

25. Allred, D. C., Harvey, J. M., Berardo, M., & Clark, G. M. (1998). Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Modern Pathology*, *11*(2), 155–168.

26. Elmabrouk Abdalla, F. B., Markus, R., Buhmeida, A., Boder, J., Syrjänen, K., & Collan, Y. (2012). Estrogen receptor, progesterone receptor, and nuclear size features in female breast cancer in Libya: Correlation with clinical features and survival. *Anticancer Research*, *32*(8), 3485–3493.

27. Jebriel, A. O., Huober, J., & Abdalla, F. B. (2021). HER2/neu distribution in female breast cancer in Libya: Correlation with clinicopathological features and survival. *International Journal of Cancer and Clinical Research*, *8*, 149.

28. Al-Zawi, A. S. A., Omer, M., Tan, E., Adamczyk, B., English, J., Elamass, M., & Eldruki, S. (2019). Human epidermal growth factor-2 receptor (HER2) status in patients aged 70 years or more with operable early breast cancer: Multicentre based study with review of 150 cases. *Medical Research Journal*, *4*(3), 157–162.