

Breast cancer biomarkers in clinical testing: analysis of a UK NEQAS ICC & ISH database containing results from 199,300 patients.

Supplementary Tables and Figures

TABLE S1. Records entered. Records from centres entering fewer than 200 records in total were excluded from the main analysis (N = 1,600 centres), 11,866 records (6.0%).

<i>Records entered (N)</i>	<i>Count of Centres (N)</i>	<i>Sum of records (N)</i>	<i>Proportion of total records (%)</i>
<200	1600	11866	6.0
200-500	51	16897	8.5
500-1000	42	30670	15.4
1000-1500	23	27241	13.7
1500-2000	10	17012	8.5
2000-2500	13	28683	14.4
2500-3000	4	10428	5.2
>3000	12	56437	28.3

FIGURE S1. Tumour size at histological assessment.

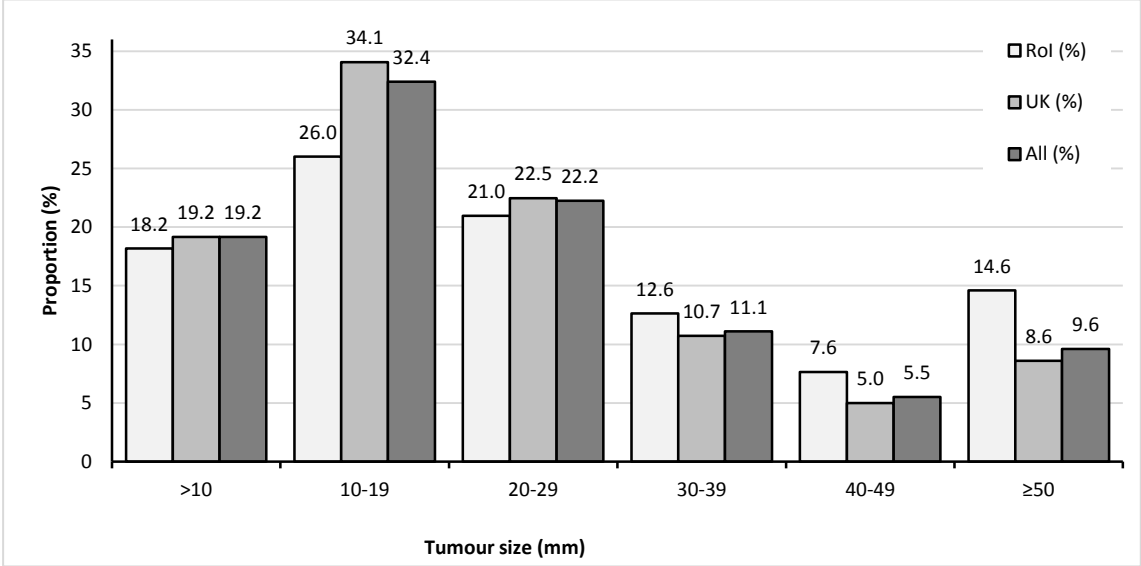


TABLE S2. HER2 positivity rates.

HER2 status reported by centres	RoI		UK		All	
	N	%	N	%	N	%
Negative	13217	86.0	125523	86.7	162754	86.9
Positive	2152	14.0	19210	13.3	24614	13.1
Total records	15369	100.0	144733	100.0	187368	100.0

TABLE S3. HER2 positivity rates by year. Datasets where less than 1000 records were available have been excluded.

Year of Test	RoI		UK		All	
	N	%	N	%	N	%
2008			289	13.1	289	13.1
2009	182	13.2	4166	13.5	4581	13.4
2010	286	13.6	4166	13.4	5130	13.6
2011	260	12.5	3099	13.5	3900	13.4
2012	418	14.1	2545	13.1	3430	13.2
2013	305	12.8	2185	13.3	2990	12.9
2014	377	16.0	1448	12.3	2225	12.1
2015	221	14.5	1099	12.8	1681	12.2
All records	2152	14.0	19210	13.3	24614	13.1

TABLE S4. HER2 positivity rates by ER status for: (A) Grade and (B) Nodal status.

A. Grade

N	ER Negative	ER Positive	All
Grade 1			
HER2 Negative	126	9473	12700
HER2 Positive	36	214	332
Sum	162	9687	13032
Grade 2			
HER2 Negative	1705	27913	38803
HER2 Positive	765	2843	4716
Sum	2470	30756	43519
Grade 3			
HER2 Negative	5582	8583	18603
HER2 Positive	1882	2646	5821
Sum	7464	11229	24424

%	ER Negative	ER Positive	All
Grade 1			
HER2 Negative	77.8	97.8	97.5
HER2 Positive	22.2	2.2	2.5
Sum	100.0	100.0	100.0
Grade 2			
HER2 Negative	69.0	90.8	89.2
HER2 Positive	31.0	9.2	10.8
Sum	100.0	100.0	100.0
Grade 3			
HER2 Negative	74.8	76.4	76.2
HER2 Positive	25.2	23.6	23.8
Sum	100.0	100.0	100.0

B. Nodal status

N	ER Negative	ER Positive	All
N0			
HER2 Negative	1023	4874	8132
HER2 Positive	284	485	1006
Sum	1307	5359	9138
N1			
HER2 Negative	392	1845	3131
HER2 Positive	135	244	482
Sum	527	2089	3613
N2			
HER2 Negative	136	503	882
HER2 Positive	56	78	172
Sum	192	581	1054
N3			
HER2 Negative	122	355	659
HER2 Positive	59	77	184
Sum	181	432	843

%	ER Negative	ER Positive	All
N0			
HER2 Negative	78.3	90.9	89.0
HER2 Positive	21.7	9.1	11.0
Sum	100.0	100.0	100.0
N1			
HER2 Negative	74.4	88.3	86.7
HER2 Positive	25.6	11.7	13.3
Sum	100.0	100.0	100.0
N2			
HER2 Negative	70.8	86.6	83.7
HER2 Positive	29.2	13.4	16.3
Sum	100.0	100.0	100.0
N3			
HER2 Negative	67.4	82.2	78.2
HER2 Positive	32.6	17.8	21.8
Sum	100.0	100.0	100.0

TABLE S5A. *HER2* gene amplification rates distributed by *HER2* IHC category. The data are taken from user assigned categories and are for Republic of Ireland (RoI) patients. A small number of centres also used non-standard ‘1+/2+’ and ‘2+/3+’ categories; these are not recognised in any published *HER2* assessment guidelines and have been excluded (N = 134).

RoI						
HER2 IHC	HER2 ISH				Totals	
	Non-amplified		Amplified			
	N	%	N	%	N	%
0	102	99.0	1	1.0	103	2.9
0/1+	2	66.7	1	33.3	3	0.1
1+	439	95.9	19	4.1	458	13.0
2+	2347	84.5	429	15.5	2776	78.6
3+	24	12.5	168	87.5	192	5.4
Totals	2914	82.5	618	17.5	3532	100.0

TABLE S5B. *HER2* gene amplification rates distributed by *HER2* IHC category. The data are taken from user assigned categories and are for UK patients. A small number of centres also used non-standard ‘1+/2+’ and ‘2+/3+’ categories; these are not recognised in any published *HER2* assessment guidelines and have been excluded (N = 1,837).

UK						
HER2 IHC	HER2 ISH				Totals	
	Non-amplified		Amplified			
	N	%	N	%	N	%
0	68	81.0	16	19.0	84	0.3
0/1+	3	75.0	1	25.0	4	0.0
1+	193	89.8	22	10.2	215	0.9
2+	20212	82.7	4232	17.3	24444	97.7
3+	30	11.5	230	88.5	260	1.0
Totals	20506	82.0	4501	18.0	25007	100.0

TABLE S5C. *HER2* gene amplification rates distributed by HER2 IHC category. The data are taken from user assigned categories and are for all patients. A small number of centres also used non-standard ‘1+/2+’ and ‘2+/3+’ categories; these are not recognised in any published HER2 assessment guidelines and have been excluded (N = 2,000).

All						
HER2 IHC	HER2 ISH				Totals	
	Non-amplified		Amplified			
	N	%	N	%	N	%
0	179	90.9	18	9.1	197	0.6
0/1+	5	71.4	2	28.6	7	0.0
1+	645	93.9	42	6.1	687	2.1
2+	26342	83.7	5129	16.3	31471	95.9
3+	59	12.7	404	87.3	463	1.4
Totals	27230	83.0	5595	17.0	32825	100.0

TABLE S6A-J. HER2 status associations. This patient dataset includes only those cases where a HER2 IHC category of 0, 0/1+, 2+ or 3+ had been assigned (N = 169,725, 90.6% of total), and classifies as HER2 positive (ISH-amplified), 2+ cases with a *HER2*/CEP17 gene copy ratio ≥ 2.0 , or with a *HER2* copy ≥ 4.0 for cases where a ratio could not be calculated. The Tables shows the number and proportion of patients in each HER2 category. In each case the sub-table below the main table shows data for the number and proportion of patients classified as HER2-negative (defined as: 0, 0/1+, 1+ and 2+ (non-amplified)), or HER2-positive (defined as: 2+ (amplified) and 3+); HER2-equivocal cases (defined as: 2+ (unknown amplification status)) were excluded.

A. Patient age at testing

Age at test	RoI				UK				All			
	<56		≥56		<56		≥56		<56		≥56	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
0	580	15.2	812	15.0	10932	32.9	19468	32.4	13481	30.4	24240	30.1
0/1+	30	0.8	43	0.8	649	2.0	1376	2.3	741	1.7	1516	1.9
1+	1418	37.1	2143	39.5	10160	30.6	19501	32.4	14330	32.3	27889	34.6
2+ (non-amplified)	671	17.5	1040	19.2	3419	10.3	7237	12.0	5075	11.5	10614	13.2
2+ (amplified)	120	3.1	166	3.1	925	2.8	1420	2.4	1175	2.7	1867	2.3
2+ (equivocal)	285	7.5	458	8.4	2063	6.2	4796	8.0	2542	5.7	5744	7.1
3+	607	15.9	622	11.5	4590	13.8	5301	8.8	6339	14.3	7403	9.2
Grand Total	3711	100.0	5284	100.0	32738	100.0	59099	100.0	43683	100.0	79273	100.0

	RoI				UK				All			
	<56		≥56		<56		≥56		<56		≥56	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	2699	78.8	4038	83.7	25160	82.0	47582	87.6	33627	81.7	64259	87.4
HER2-positive	727	21.2	788	16.3	5515	18.0	6721	12.4	7514	18.3	9270	12.6
Grand Total	3426	100.0	4826	100.0	30675	100.0	54303	100.0	41141	100.0	73529	100.0

B. Patient referral pathway.

Patient pathway	RoI				UK				All			
	Screen detected		Symptomatic		Screen detected		Symptomatic		Screen detected		Symptomatic	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
0	184	10.7	344	10.2	247	13.4	523	16.0	431	12.1	867	13.1
0/1+	16	0.9	48	1.4	18	1.0	19	0.6	34	1.0	67	1.0
1+	757	44.2	1298	38.6	934	50.7	1397	42.7	1691	47.5	2695	40.6
2+ (non-amplified)	345	20.1	677	20.1	396	21.5	757	23.1	741	20.8	1434	21.6
2+ (amplified)	38	2.2	105	3.1	59	3.2	163	5.0	97	2.7	268	4.0
2+ (equivocal)	150	8.8	342	10.2	20	1.1	48	1.5	170	4.8	390	5.9
3+	162	9.5	466	13.9	141	7.6	347	10.6	303	8.5	813	12.3
Grand Total	1652	100.0	3280	100.0	1815	100.0	3254	100.0	3467	100.0	6534	100.0

	RoI				UK				All			
	Screen detected		Symptomatic		Screen detected		Symptomatic		Screen detected		Symptomatic	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	1302	86.7	2367	80.6	1595	88.9	2696	84.1	2897	87.9	5063	82.4
HER2-positive	200	13.3	571	19.4	200	11.1	510	15.9	400	12.1	1081	17.6
Grand Total	1502	100.0	2938	100.0	1795	100.0	3206	100.0	3297	100.0	6144	100.0

C. Specimen sample type.

Sample type	Rol						UK						All					
	Axilla/nodes		Core biopsy		Excision		Axilla/nodes		Core biopsy		Excision		Axilla/nodes		Core biopsy		Excision	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	26	10.7	1050	14.1	498	20.8	1037	19.8	17848	28.0	9106	39.3	1171	19.6	23116	26.2	10838	37.2
0/1+	3	1.2	42	0.6	49	2.0	43	0.8	1548	2.4	420	1.8	48	0.8	1690	1.9	511	1.8
1+	82	33.9	3013	40.5	929	38.8	1564	29.8	22774	35.7	6427	27.8	1801	30.2	33077	37.5	8466	29.1
2+ (non-amplified)	43	17.8	1466	19.7	323	13.5	228	4.3	8103	12.7	2317	10.0	375	6.3	12081	13.7	3092	10.6
2+ (amplified)	6	2.5	214	2.9	90	3.8	67	1.3	1569	2.5	583	2.5	78	1.3	2083	2.4	751	2.6
2+ (equivocal)	26	10.7	486	6.5	194	8.1	1573	30.0	3986	6.2	1561	6.7	1612	27.0	5155	5.8	1923	6.6
3+	52	21.5	952	12.8	271	11.3	691	13.2	6666	10.4	2499	10.8	837	14.0	9513	10.8	3217	11.1
Grand Total	238	100.0	7223	100.0	2354	100.0	5203	100.0	62494	100.0	22913	100.0	5922	100.0	86715	100.0	28798	100.0

	Rol						UK						All					
	Axilla/nodes		Core biopsy		Excision		Axilla/nodes		Core biopsy		Excision		Axilla/nodes		Core biopsy		Excision	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	154	72.6	5571	82.7	1799	83.3	2872	79.1	50273	85.9	18270	85.6	3395	78.8	69964	85.8	22907	85.2
HER2-positive	58	27.4	1166	17.3	361	16.7	758	20.9	8235	14.1	3082	14.4	915	21.2	11596	14.2	3968	14.8
Grand Total	212	100.0	6737	100.0	2160	100.0	3630	100.0	58508	100.0	21352	100.0	4310	100.0	81560	100.0	26875	100.0

D. Presentation site for metastatic cases.

Metastatic site	RoI						UK						All					
	Bone disease		Cutaneous		Visceral disease		Bone disease		Cutaneous		Visceral disease		Bone disease		Cutaneous		Visceral disease	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	23	19.7	32	19.5	72	30.4	82	44.8	291	28.9	412	37.9	130	33.9	388	28.3	610	33.7
0/1+	3	2.6	1	0.6	3	1.3	2	1.1	37	3.7	60	5.5	7	1.8	40	2.9	73	4.0
1+	49	41.9	51	31.1	56	23.6	58	31.7	341	33.9	230	21.1	144	37.6	482	35.1	415	22.9
2+ (non-amplified)	0	0.0	2	1.2	9	3.8	4	2.2	28	2.8	25	2.3	5	1.3	33	2.4	43	2.4
2+ (amplified)	11	9.4	10	6.1	13	5.5	3	1.6	40	4.0	38	3.5	18	4.7	53	3.9	122	6.7
2+ (equivocal)	17	14.5	29	17.7	39	16.5	17	9.3	122	12.1	155	14.2	41	10.7	172	12.5	261	14.4
3+	13	11.1	35	21.3	42	17.7	14	7.7	129	12.8	133	12.2	34	8.9	181	13.2	246	13.6
Grand Total	116	100.0	160	100.0	234	100.0	180	100.0	988	100.0	1053	100.0	379	100.0	1349	100.0	1770	100.0

	RoI						UK						All					
	Bone disease		Cutaneous		Visceral disease		Bone disease		Cutaneous		Visceral disease		Bone disease		Cutaneous		Visceral disease	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	75	75.8	86	65.6	140	71.8	146	89.6	697	80.5	727	81.0	286	84.6	943	80.1	1141	75.6
HER2-positive	24	24.2	45	34.4	55	28.2	17	10.4	169	19.5	171	19.0	52	15.4	234	19.9	368	24.4
Grand Total	99	100.0	131	100.0	195	100.0	163	100.0	866	100.0	898	100.0	338	100.0	1177	100.0	1509	100.0

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E. Histological tumour type.

Histological type	Rol										UK										All									
	In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types		In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types		In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	19	13.6	1043	14.0	266	22.3	16	12.3	36	26.9	146	30.8	15429	31.6	2892	38.6	405	34.3	602	48.1	231	25.9	17880	29.2	3530	36.9	448	32.7	693	45.8
0/1+	0	0.0	66	0.9	16	1.3	1	0.8	2	1.5	3	0.6	1508	3.1	268	3.6	47	4.0	40	3.2	3	0.3	1687	2.8	306	3.2	49	3.6	50	3.3
1+	40	28.6	2831	38.0	583	48.8	70	53.8	57	42.5	83	17.5	14833	30.4	2696	36.0	394	33.3	392	31.3	178	19.9	19530	31.9	3609	37.8	488	35.6	493	32.6
2+ (non-amplified)	2	1.4	1477	19.8	164	13.7	26	20.0	21	15.7	11	2.3	6937	14.2	889	11.9	169	14.3	122	9.7	17	1.9	8883	14.5	1119	11.7	197	14.4	150	9.9
2+ (amplified)	2	1.4	265	3.6	18	1.5	1	0.8	3	2.2	3	0.6	1487	3.0	134	1.8	35	3.0	20	1.6	5	0.6	1849	3.0	158	1.7	37	2.7	27	1.8
2+ (equivocal)	17	12.1	516	6.9	65	5.4	5	3.8	7	5.2	94	19.8	1661	3.4	193	2.6	24	2.0	23	1.8	152	17.0	2447	4.0	299	3.1	29	2.1	32	2.1
3+	57	40.7	1040	14.0	51	4.3	4	3.1	6	4.5	130	27.4	5904	12.1	265	3.5	64	5.4	40	3.2	297	33.3	7649	12.5	358	3.7	70	5.1	50	3.3
Grand Total	137	100.0	7238	100.0	1163	100.0	123	100.0	132	100.0	470	100.0	47759	100.0	7337	100.0	1138	100.0	1239	100.0	883	100.0	59925	100.0	9379	100.0	1318	100.0	1495	100.0

HER2 category	Rol										UK										All									
	In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types		In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types		In-situ disease		Invasive ductal		Invasive lobular		Mixed IDC/ILC		Special types	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	61	50.8	5417	80.6	1029	93.7	113	95.8	116	92.8	243	64.6	38707	84.0	6745	94.4	1015	91.1	1156	95.1	429	58.7	47980	83.5	8564	94.3	1182	91.7	1386	94.7
HER2-positive	59	49.2	1305	19.4	69	6.3	5	4.2	9	7.2	133	35.4	7391	16.0	399	5.6	99	8.9	60	4.9	302	41.3	9498	16.5	516	5.7	107	8.3	77	5.3
Grand Total	120	100.0	6722	100.0	1098	100.0	118	100.0	125	100.0	376	100.0	46098	100.0	7144	100.0	1114	100.0	1216	100.0	731	100.0	57478	100.0	9080	100.0	1289	100.0	1463	100.0

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F. Histological Grade.

Histological Grade	RoI						UK						All					
	Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	177	14.9	779	14.7	372	18.0	3551	35.3	10822	32.6	6336	33.2	3980	33.4	12138	30.0	7053	31.5
0/1+	9	0.8	59	1.1	24	1.2	409	4.1	1019	3.1	500	2.6	453	3.8	1142	2.8	570	2.5
1+	625	52.7	2192	41.2	614	29.8	4238	42.1	10976	33.0	4300	22.6	5124	43.1	13913	34.4	5247	23.5
2+ (non-amplified)	232	19.6	1043	19.6	337	16.3	1201	11.9	4879	14.7	2193	11.5	1481	12.4	6144	15.2	2654	11.9
2+ (amplified)	11	0.9	174	3.3	95	4.6	75	0.7	886	2.7	755	4.0	89	0.7	1086	2.7	883	3.9
2+ (equivocal)	84	7.1	363	6.8	113	5.5	228	2.3	1334	4.0	741	3.9	336	2.8	1773	4.4	902	4.0
3+	27	2.3	550	10.3	447	21.7	164	1.6	2524	7.6	3874	20.3	207	1.7	3255	8.1	4604	20.6
Grand Total	1165	100.0	5160	100.0	2002	100.0	9866	100.0	32440	100.0	18699	100.0	11670	100.0	39451	100.0	21913	100.0

HER2 category	RoI						UK						All					
	Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3		Grade 1		Grade 2		Grade 3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	1043	96.5	4073	84.9	1347	71.3	9399	97.5	27696	89.0	13329	74.2	11038	97.4	33337	88.5	15524	73.9
HER2-positive	38	3.5	724	15.1	542	28.7	239	2.5	3410	11.0	4629	25.8	296	2.6	4341	11.5	5487	26.1
Grand Total	1081	100.0	4797	100.0	1889	100.0	9638	100.0	31106	100.0	17958	100.0	11334	100.0	37678	100.0	21011	100.0

G. Tumour size.

Tumour size (mm)	RoI						UK						All					
	<20mm		20-49mm		≥50mm		<20mm		20-49mm		≥50mm		<20mm		20-49mm		≥50mm	
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	217	21.3	153	19.3	44	18.3	2660	39.1	1988	40.8	443	40.8	3247	37.6	2360	37.9	525	36.6
0/1+	24	2.4	20	2.5	4	1.7	114	1.7	65	1.3	18	1.7	157	1.8	102	1.6	25	1.7
1+	408	40.0	285	35.9	85	35.4	2112	31.0	1238	25.4	245	22.6	2757	31.9	1675	26.9	352	24.5
2+ (non-amplified)	140	13.7	132	16.6	36	15.0	855	12.6	627	12.9	163	15.0	1050	12.2	804	12.9	209	14.6
2+ (amplified)	29	2.8	41	5.2	10	4.2	189	2.8	176	3.6	35	3.2	225	2.6	229	3.7	47	3.3
2+ (equivocal)	60	5.9	78	9.8	32	13.3	137	2.0	143	2.9	29	2.7	230	2.7	249	4.0	71	5.0
3+	125	12.2	68	8.6	28	11.7	674	9.9	578	11.9	146	13.5	890	10.3	730	11.7	197	13.7
Grand Total	1003	100.0	777	100.0	239	100.0	6741	100.0	4815	100.0	1079	100.0	8556	100.0	6149	100.0	1426	100.0

HER2 category	RoI						UK						All					
	<20mm		20-49mm		≥50mm		<20mm		20-49mm		≥50mm		<20mm		20-49mm		≥50mm	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	789	83.7	590	84.4	169	81.6	5741	86.9	3918	83.9	869	82.8	7211	86.6	4941	83.7	1111	82.0
HER2-positive	154	16.3	109	15.6	38	18.4	863	13.1	754	16.1	181	17.2	1115	13.4	959	16.3	244	18.0
Grand Total	943	100.0	699	100.0	207	100.0	6604	100.0	4672	100.0	1050	100.0	8326	100.0	5900	100.0	1355	100.0

UK NEQAS breast biomarker supplementary tables and figures: J Pathol Clin Res
 Dodson et al

H. Nodal status.

Nodal status	RoI								UK								All							
	N0		N1		N2		N3		N0		N1		N2		N3		N0		N1		N2		N3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	200	20.3	92	21.7	20	18.2	13	14.9	2257	37.1	877	36.6	260	35.0	221	36.1	2594	34.5	1028	34.5	292	32.7	243	33.2
0/1+	21	2.1	6	1.4	1	0.9	3	3.4	96	1.6	52	2.2	18	2.4	13	2.1	141	1.9	69	2.3	20	2.2	16	2.2
1+	376	38.2	156	36.9	45	40.9	26	29.9	1955	32.1	681	28.4	177	23.9	126	20.6	2502	33.3	887	29.8	234	26.2	161	22.0
2+ (non-amplified)	149	15.1	74	17.5	12	10.9	13	14.9	844	13.9	337	14.1	114	15.4	67	10.9	1033	13.7	421	14.1	132	14.8	85	11.6
2+ (amplified)	26	2.6	16	3.8	9	8.2	7	8.0	180	3.0	72	3.0	25	3.4	19	3.1	210	2.8	94	3.2	34	3.8	26	3.6
2+ (equivocal)	79	8.0	31	7.3	8	7.3	15	17.2	138	2.3	77	3.2	28	3.8	21	3.4	235	3.1	115	3.9	40	4.5	41	5.6
3+	121	12.3	43	10.2	11	10.0	8	9.2	561	9.2	279	11.6	105	14.2	133	21.7	735	9.8	336	11.3	121	13.5	145	19.8
Grand Total	972	100.0	418	100.0	106	100.0	85	100.0	6031	100.0	2375	100.0	727	100.0	600	100.0	7450	100.0	2950	100.0	873	100.0	717	100.0

HER2 category	RoI								UK								All							
	N0		N1		N2		N3		N0		N1		N2		N3		N0		N1		N2		N3	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	746	83.5	328	84.8	78	79.6	55	78.6	5152	87.4	1947	84.7	569	81.4	427	73.7	6270	86.9	2405	84.8	678	81.4	505	74.7
HER2-positive	147	16.5	59	15.2	20	20.4	15	21.4	741	12.6	351	15.3	130	18.6	152	26.3	945	13.1	430	15.2	155	18.6	171	25.3
Grand Total	893	100.0	387	100.0	98	100.0	70	100.0	5893	100.0	2298	100.0	699	100.0	579	100.0	7215	100.0	2835	100.0	833	100.0	676	100.0

I. ER status.

ER status	RoI				UK				All			
	Negative		Positive		Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%	N	%	N	%
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
0	412	23.2	1259	13.8	4524	40.1	17198	31.2	5363	37.8	20386	28.9
0/1+	21	1.2	81	0.9	248	2.2	1620	2.9	287	2.0	1832	2.6
1+	427	24.1	3920	43.1	2047	18.2	19445	35.3	2716	19.1	25790	36.6
2+ (non-amplified)	185	10.4	1848	20.3	805	7.1	7379	13.4	1050	7.4	9791	13.9
2+ (amplified)	49	2.8	261	2.9	292	2.6	1433	2.6	360	2.5	1793	2.5
2+ (equivocal)	80	4.5	645	7.1	443	3.9	2682	4.9	566	4.0	3706	5.3
3+	559	31.5	858	9.4	2747	24.4	4236	7.7	3655	25.7	5750	8.2
Grand Total	1733	100.0	8872	100.0	11106	100.0	53993	100.0	13997	100.0	69048	100.0

HER2 category	RoI				UK				All			
	Negative		Positive		Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	1045	63.2	7108	86.4	7624	71.5	45642	89.0	9416	70.1	57799	88.5
HER2-positive	608	36.8	1119	13.6	3039	28.5	5669	11.0	4015	29.9	7543	11.5
Grand Total	1653	100.0	8227	100.0	10663	100.0	51311	100.0	13431	100.0	65342	100.0

J. PR status.

PR status	RoI				UK				All			
	Negative		Positive		Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%	N	%	N	%
HER2 category	N	%	N	%	N	%	N	%	N	%	N	%
0	639	21.7	719	16.2	5756	34.5	10792	33.0	7012	32.7	12828	31.3
0/1+	34	1.2	36	0.8	446	2.7	1161	3.5	508	2.4	1239	3.0
1+	815	27.7	2021	45.7	3870	23.2	12101	36.9	5123	23.9	15612	38.1
2+ (non-amplified)	388	13.2	872	19.7	1552	9.3	3640	11.1	2057	9.6	4819	11.8
2+ (amplified)	89	3.0	119	2.7	444	2.7	689	2.1	566	2.6	865	2.1
2+ (equivocal)	134	4.6	215	4.9	930	5.6	1860	5.7	1151	5.4	2348	5.7
3+	775	26.4	343	7.7	3413	20.4	1876	5.7	4696	21.9	2549	6.2
Grand Total	2874	100.0	4325	100.0	16411	100.0	32119	100.0	21113	100.0	40260	100.0

HER2 category	RoI				UK				All			
	Negative		Positive		Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%	N	%	N	%
HER2-negative	1876	68.5	3648	88.8	11624	75.1	27694	91.5	14700	73.6	34498	91.0
HER2-positive	864	31.5	462	11.2	3857	24.9	2565	8.5	5262	26.4	3414	9.0
Grand Total	2740	100.0	4110	100.0	15481	100.0	30259	100.0	19962	100.0	37912	100.0

TABLE S7A-C. *HER2* gene amplification, by copy number, *HER2*/CEP17 ratio and combined. For all cases where data were available, regardless of *HER2* IHC category.

A. All cases.

All cases				
Category	Amplification status (1)	Amplification status (2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			9901	82.2
Copy number <4.0	Non-amplified	Non-amplified	8689	72.1
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	945	7.8
Copy number ≥6.0	Amplified	Amplified	267	2.2
Ratio ≥2.0			2148	17.8
HER2 copy number <4.0	Non-amplified	Amplified	397	3.3
HER2 copy number ≥4.0 but <6.0	Borderline non-amplified/Equivocal	Amplified	690	5.7
HER2 copy number ≥6.0	Amplified	Amplified	1061	8.8
Total cases			12049	100.0

All cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	9086	75.4	8689	72.1
Borderline non-amplified/Equivocal	1635	13.6	945	7.8
Amplified	1328	11.0	2415	20.0
Total	12049	100.0	12049	100.0

B. ER negative cases.

ER negative cases				
Category	Amplification status (1)	Amplification status (2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			661	72.9
Copy number <4.0	Non-amplified	Non-amplified	577	63.6
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	56	6.2
Copy number ≥6.0	Amplified	Amplified	28	3.1
Ratio ≥2.0			246	27.1
HER2 copy number <4.0	Non-amplified	Amplified	44	4.9
HER2 copy number ≥4.0 but <6.0	Borderline non-amplified/Equivocal	Amplified	68	7.5
HER2 copy number ≥6.0	Amplified	Amplified	134	14.8
Total cases			907	100.0

ER negative cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	621	68.5	577	63.6
Borderline non-amplified /Equivocal	124	13.7	56	6.2
Amplified	162	17.9	274	30.2
Total	907	100.0	907	100.0

C. ER positive cases.

ER positive cases				
Category	Amplification status (1)	Amplification status (2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			6014	83.0
Copy number <4.0	Non-amplified	Non-amplified	5270	72.7
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	565	7.8
Copy number ≥6.0	Amplified	Amplified	179	2.5
Ratio ≥2.0			1235	17.0
HER2 copy number <4.0	Non-amplified	Amplified	254	3.5
HER2 copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Amplified	422	5.8
HER2 copy number ≥6.0	Amplified	Amplified	559	7.7
Total cases			7249	100.0

ER positive cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	5524	76.2	5270	72.7
Borderline non-amplified/ Equivocal	987	13.6	565	7.8
Amplified	738	10.2	1414	19.5
Total	7249	100.0	7249	100.0

(1). Defined using HER2 copy number alone

(2). Defined using dual probe ratio and HER2 copy number algorithm

TABLE S7D-F. *HER2* gene amplification, by copy number, *HER2/CEP17* ratio and combined. For all cases where data were available, and *HER2* IHC category was 2+.

D. All *HER2* IHC 2+ cases

All <i>HER2</i> IHC 2+ cases				
Category	Amplification status (1)	Amplification status (2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			8681	82.5
Copy number <4.0	Non-amplified	Non-amplified	7629	72.5
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	844	8.0
Copy number ≥6.0	Amplified	Amplified	208	2.0
Ratio ≥2.0			1842	17.5
<i>HER2</i> copy number <4.0	Non-amplified	Amplified	361	3.4
<i>HER2</i> copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Amplified	623	5.9
<i>HER2</i> copy number ≥6.0	Amplified	Amplified	858	8.2
Total cases			10523	100.0

All <i>HER2</i> IHC 2+ cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	7990	75.9	7629	72.5
Borderline non-amplified/ Equivocal	1467	13.9	844	8.0
Amplified	1066	10.1	2050	19.5
Total	10523	100.0	10523	100.0

E. HER2 IHC 2+, ER negative cases

HER2 IHC 2+/ER negative cases				
Category	Amplification status (1)	Amplification status (2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			565	73.9
Copy number <4.0	Non-amplified	Non-amplified	492	64.3
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	48	6.3
Copy number ≥6.0	Amplified	Amplified	25	3.3
Ratio ≥2.0			200	26.1
HER2 copy number <4.0	Non-amplified	Amplified	38	5.0
HER2 copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Amplified	64	8.4
HER2 copy number ≥6.0	Amplified	Amplified	98	12.8
Total cases			765	100.0

HER2 IHC 2+/ER negative cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	530	69.3	492	64.3
Borderline non-amplified/ Equivocal	112	14.6	48	6.3
Amplified	123	16.1	225	29.4
Total	765	100.0	765	100.0

F. HER2 IHC 2+, ER positive cases

HER2 IHC 2+/ER positive cases				
Category	Amplification status(1)	Amplification status(2)	Count of cases (N)	Proportion of cases (%)
Ratio <2.0			5298	83.4
Copy number <4.0	Non-amplified	Non-amplified	4627	72.8
Copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Borderline non-amplified/ Equivocal	521	8.2
Copy number ≥6.0	Amplified	Amplified	150	2.4
Ratio ≥2.0			1054	16.6
HER2 copy number <4.0	Non-amplified	Amplified	234	3.7
HER2 copy number ≥4.0 but <6.0	Borderline non-amplified/ Equivocal	Amplified	376	5.9
HER2 copy number ≥6.0	Amplified	Amplified	444	7.0
Total cases			6352	100.0

HER2 IHC 2+/ER positive cases	Status by copy number		Status by ratio and copy number	
	Count of cases (N)	Proportion of cases (%)	Count of cases (N)	Proportion of cases (%)
Non-amplified	4861	76.5	4627	72.8
Borderline non-amplified/ Equivocal	897	14.1	521	8.2
Amplified	594	9.4	1204	19.0
Total	6352	100.0	6352	100.0

(1). Defined using HER2 copy number alone

(2). Defined using dual probe ratio and HER2 copy number algorithm

TABLE S8. ER status

ER status (N)	Centre assigned			Allred score			H-Score			% positivity			Combined all data		
	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All
Negative	1864	10892	14610	269	8907	10711	124	481	606	154	1390	1544	1954	12601	16422
Positive	9217	50777	68798	1787	50020	59539	343	2540	2905	2253	9045	11305	9678	59909	78465
Totals	11081	61669	83408	2056	58927	70250	467	3021	3511	2407	10435	12849	11632	72510	94887

ER status (%)	Centre assigned			Allred score			H-Score			% positivity			Combined all data		
	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All
Negative	16.8	17.7	17.5	13.1	15.1	15.2	26.6	15.9	17.3	6.4	13.3	12.0	16.8	17.4	17.3
Positive	83.2	82.3	82.5	86.9	84.9	84.8	73.4	84.1	82.7	93.6	86.7	88.0	83.2	82.6	82.7
Proportion (1)	95.3	85.0	87.9	17.7	81.3	74.0	4.0	4.2	3.7	20.7	14.4	13.5	100.0	100.0	100.0

(1) Proportion of all available data within category (RoI, UK or All) to indicate depth of coverage

TABLE S9. ER positive rates by patient age. Using finalised ER status, which is a combined categorisation, based on centre assigned category, Allred score, % positive score and H-Score (in that order of precedence).

ER positive rate (finalised category)										
Age range (years)	RoI			UK			All			Mean
	ER+ (N)	Total (N)	ER+ (%)	ER+ (N)	Total (N)	ER+ (%)	ER+ (N)	Total (N)	ER+ (%)	
<35	143	212	67.5	743	1118	66.5	1014	1523	66.6	82.5
35-39	315	407	77.4	1311	1809	72.5	1782	2449	72.8	
40-44	582	723	80.5	2931	3694	79.3	3943	4968	79.4	
45-49	849	1034	82.1	5076	6119	83.0	6740	8159	82.6	
50-54	1115	1308	85.2	6422	7709	83.3	8721	10427	83.6	
55-59	914	1100	83.1	5492	6659	82.5	7427	8995	82.6	
60-64	998	1214	82.2	6495	7835	82.9	8795	10584	83.1	
65-69	753	911	82.7	6102	7262	84.0	8126	9674	84.0	
70-74	723	877	82.4	4119	5001	82.4	5557	6748	82.4	
75-79	553	679	81.4	3619	4252	85.1	4716	5598	84.2	
80-84	349	417	83.7	2892	3386	85.4	3705	4370	84.8	
85-89	228	264	86.4	1768	2087	84.7	2262	2679	84.4	
≥90	90	105	85.7	887	1028	86.3	1128	1301	86.7	82.5
Totals	7612	9251	82.3	47857	57959	82.6	63916	77475	82.5	

TABLE S10. ER status by age, categorised by Allred score.

	Patient age at test (years)														Mean	
	<40		40-49		50-59		60-69		70-79		80-89		≥90			
Allred score	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	621	23.0	1389	14.6	1831	13.5	1973	13.1	1182	13.1	655	12.1	96	9.3	7747	13.8
2	89	3.3	169	1.8	198	1.5	190	1.3	88	1.0	63	1.2	9	0.9	806	1.4
3	81	3.0	171	1.8	237	1.8	204	1.4	132	1.5	67	1.2	12	1.2	904	1.6
4	77	2.9	199	2.1	231	1.7	190	1.3	121	1.3	49	0.9	11	1.1	878	1.6
5	96	3.6	245	2.6	254	1.9	210	1.4	129	1.4	86	1.6	11	1.1	1031	1.8
6	138	5.1	536	5.6	507	3.7	383	2.5	215	2.4	122	2.3	18	1.7	1919	3.4
7	481	17.8	1856	19.5	1780	13.1	1249	8.3	687	7.6	341	6.3	73	7.1	6467	11.5
8	1114	41.3	4930	51.9	8504	62.8	10649	70.8	6499	71.8	4031	74.5	803	77.7	36530	64.9
All cases*	2697	4.8	9495	16.9	13542	24.1	15048	26.7	9053	16.1	5414	9.6	1033	1.8	56282	100.0

* percentage is for proportion of all cases

TABLE S11. PR status using two different cut-points.

Cut-point = Allred >2	RoI		UK		All	
	N	%	N	%	N	%
Allred Negative	572	27.9	11559	28.6	14482	29.2
Allred Positive	1480	72.1	28854	71.4	35063	70.8
Grand Total	2052	100.0	40413	100.0	49545	100.0

Cut-point = Allred >3	RoI		UK		All	
	N	%	N	%	N	%
Allred Negative	652	31.8	13162	32.6	16403	33.1
Allred Positive	1400	68.2	27251	67.4	33142	66.9
Grand Total	2052	100.0	40413	100.0	49545	100.0

Cut-point = H-Score ≥1	RoI		UK		All	
	N	%	N	%	N	%
H-Score Negative	2	100.0	11559	28.6	14482	29.2
H-Score Positive	0	0.0	28854	71.4	35063	70.8
Grand Total	2	0.1	40413	100.0	49545	100.0

Cut-point = H-Score ≥10	RoI		UK		All	
	N	%	N	%	N	%
H-Score Negative	2	100.0	657	33.3	714	35.1
H-Score Positive	0	0.0	1318	66.7	1318	64.9
Grand Total	2	0.1	1975	100.0	2032	100.0

Cut-point = % ≥1%	RoI		UK		All	
	N	%	N	%	N	%

UK NEQAS breast biomarker supplementary tables and figures: J Pathol Clin Res
 Dodson et al

% Negative	354	16.8	1949	29.8	2303	26.5
% Positive	1755	85.5	4585	70.2	6386	73.5
Grand Total	2109	102.8	6534	100.0	8689	100.0

Cut-point = % \geq 10%	RoI		UK		All	
	N	%	N	%	N	%
% Negative	614	29.1	2315	35.4	2975	34.2
% Positive	1495	72.9	4219	64.6	5714	65.8
Grand Total	2109	102.8	6534	100.0	8689	100.0

Centre assigned	RoI		UK		All	
	N	%	N	%	N	%
Negative	3067	41.3	14710	34.0	20201	34.9
Positive	4365	58.7	28548	66.0	37757	65.1
Grand Total	7432	100.0	43258	100.0	57958	100.0

TABLE S12. PR status by different scoring methods.

PR status (N)	Centre assigned			Allred score			H-Score			% positivity			Combined all data		
	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All
Negative	3067	14710	20201	652	13162	16403	2	657	714	614	2315	2975	3257	18065	23808
Positive	4365	28548	37757	1400	27251	33142	0	1318	1318	1495	4219	5714	4738	34460	44070
Totals	7432	43258	57958	2052	40413	49545	2	1975	2032	2109	6534	8689	7995	52525	67878

PR status (%)	Centre assigned			Allred score			H-Score			% positivity			Combined all data		
	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All	RoI	UK	All
Negative	41.3	34.0	34.9	31.8	32.6	33.1	100.0	33.3	35.1	29.1	35.4	34.2	40.7	34.4	35.1
Positive	58.7	66.0	65.1	68.2	67.4	66.9	0.0	66.7	64.9	70.9	64.6	65.8	59.3	65.6	64.9
Proportion (1)	93.0	82.4	85.4	25.7	76.9	73.0	0.0	3.8	3.0	26.4	12.4	12.8	100.0	100.0	100.0

(1) Proportion of all available data within category (RoI, UK or All) to indicate depth of coverage

TABLE S13. PR positive rates, using finalised ER status, which is a combined categorisation, based on centre assigned category, Allred score, % positive score and H-Score (in that order of precedence).

PR positive rate (finalised category)										
Age range (years)	RoI			UK			All			Mean
	PR+ (N)	Total (N)	PR+ (%)	PR+ (N)	Total (N)	PR+ (%)	PR+ (N)	Total (N)	PR+ (%)	
<35	76	164	46.3	415	827	50.2	557	1116	49.9	64.9
35-39	153	280	54.6	830	1392	59.6	1051	1821	57.7	
40-44	311	521	59.7	1802	2686	67.1	2326	3563	65.3	
45-49	443	702	63.1	3134	4427	70.8	4047	5804	69.7	
50-54	337	651	51.8	3841	5566	69.0	4880	7211	67.7	
55-59	230	523	44.0	3098	4781	64.8	3903	6185	63.1	
60-64	292	616	47.4	3637	5568	65.3	4693	7318	64.1	
65-69	342	608	56.3	3322	5080	65.4	4343	6681	65.0	
70-74	318	593	53.6	2310	3601	64.1	2983	4754	62.7	
75-79	259	456	56.8	2023	3062	66.1	2559	3957	64.7	
80-84	161	280	57.5	1620	2429	66.7	2006	3039	66.0	
85-89	108	187	57.8	1004	1533	65.5	1245	1924	64.7	
≥90	42	68	61.8	499	734	68.0	613	899	68.2	64.9
Totals	3072	5649	54.4	27535	41686	66.1	35206	54272	64.9	

TABLE S14. Combined ER and PR status case distribution.

N	ER neg	ER pos	SUM
PR neg	12978	10733	23711
PR pos	762	43229	43991
SUM	13740	53962	67702

%	ER neg	ER pos	SUM
PR neg	19.2	15.9	35.0
PR pos	1.1	63.9	65.0
SUM	20.3	79.7	100.0