to tumoural obstructive symptoms. His clinical history was unremarkable for malignancy. GNETs occur in young to middle aged adults without a gender predilection. They are composed of varying proportions of spindled and ovoid/epithelioid cells which can show a spectrum of histological architectural features in the epithelioid component. Multinucleate giant cells are variably present, clear cell change may be focal, and oncocytosis may rarely be seen. The immunophenotype is neural, melanocytic differentiation is absent, and there is consistent detection of *EWSR1* gene rearrangement with fusion partners *ATF1* or *CREB1* by *in situ* hybridisation. GNETs are postulated to arise from primitive neural crest progenitor cells of the gastrointestinal tract autonomic nervous system. The prognosis appears poor, although published data with follow-up information is scant, given the rarity of this disease.

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Androgen receptor immunoexpression in triple-negative breast cancers: is it a prognostic factor?

Sir,

Breast cancer is a heterogenous group of carcinomas with distinct clinical outcomes and therapeutic paradigms depending on their specific molecular profiles. Triple negative breast cancers (TNBCs) exhibit aggressive behaviour and are defined by the absence of oestrogen receptor, progesterone receptor and human epidermal growth factor 2 (HER2) overexpression. TNBCs display diverse molecular phenotypes with differing clinical characteristics and have recently been refined into four molecular subtypes: basal-like 1, basal-like 2, mesenchymal, and luminal androgen receptor-like (LAR).[1] Because TNBCs have less favourable outcomes and lack specific targeted therapy, there have been increasing efforts to identify potential prognostic and predictive markers in TNBCs.

Many breast cancers express androgen receptor (AR) and there has been recent interest in the role of AR as a potential biomarker in TNBCs. Approximately 10–35% of TNBCs express AR[2,3] when using a threshold of 10% nuclear staining by immunohistochemistry (IHC). AR expression has been demonstrated to confer an improved outcome in ER positive breast cancers and has been shown to be associated with smaller tumour size, lower clinical stage and lower mitotic score.[2,4–6] However, the role of AR in TNBCs is less clear. Some studies have suggested AR expression is associated with a favourable outcome in TNBCs[3,5,6] including response to anti-androgen therapy such as enzalutamide.[7] However, other studies have found no difference[8] or even a negative prognostic impact of AR expression, with increased mortality and poorer outcomes in ER negative breast cancers.[9] Therefore, we sought to investigate the clinical and pathological associations of AR expression in a large unselected cohort of Australian patients with TNBC.

A tissue microarray (TMA) of consecutive primary triple negative breast carcinoma cases reported at Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, was used. These cases had undergone resection between 2005 and 2015. Metastases, core biopsy specimens and cases occurring in males were excluded. The TMA contained two 1 mm cores from each tumour. Androgen receptor immunohistochemistry was performed using a commercially available rabbit polyclonal antibody (clone SP107; dilution 1/50, after heat induced antigen retrieval in an alkaline retrieval solution; Cell Marque, USA).

Greater than or equal to 10% nuclear staining was classified as positive (Fig. 1). The TMAs were scored by two independent observers and AR IHC was repeated on whole tissue sections in cases which demonstrated equivocal staining or when there was discordance between the two observers. Statistical analysis was performed using SPSS version 24.0 (Statistical Package for Social Sciences, USA). AR expression was correlated with other prognostic factors, using paired t-test and Pearson's Chi-square test.

AR expression was also correlated with disease free survival (DFS) and overall survival (OS). OS was measured from the date of surgery to the date of death or last available

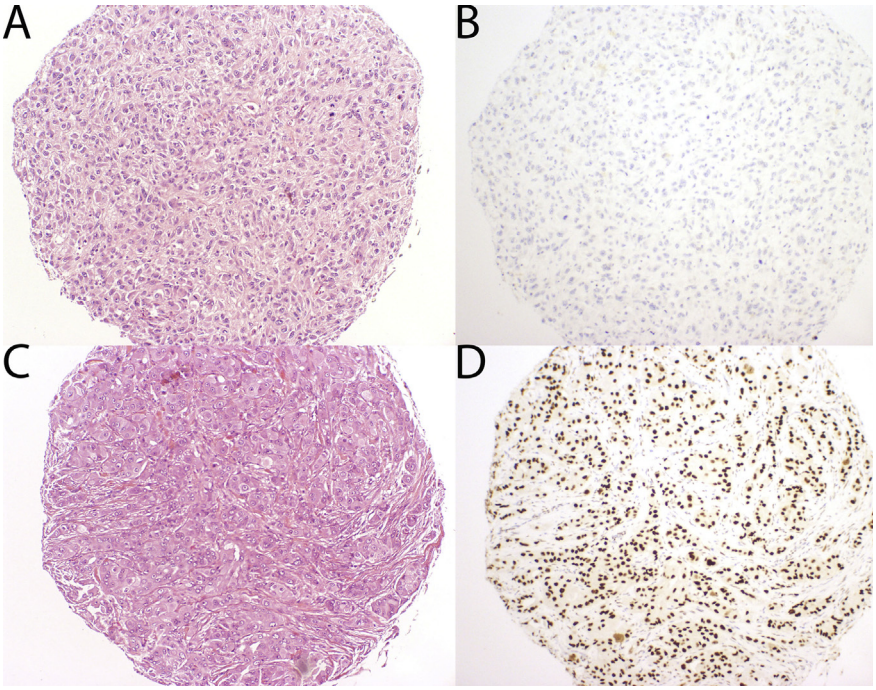

Fig. 1 Histology and immunohistochemistry of triple negative breast cancer (TNBC). H&E stain (left) and immunohistochemistry (right). (A,B) TNBC with <10% nuclei staining and (C,D) TNBC with positive staining in ≥10% nuclei.

follow up. DFS was measured from the date of surgery until the first local or distant recurrence. Kaplan−Meier survival curves were compared using the log-rank test. Multivariate cox regression tests were used to adjust for clinical and pathological variables. This study was approved by the Northern Sydney Local Health District review board.

There were 137 TNBCs with assessable material in the TMA slides. In nine cases IHC was performed on whole sections to resolve equivocal staining. Tumour and patient characteristics are presented in Table 1. Briefly, the median age was 51.0 (13.0−93.0). AR immunohistochemistry was positive in 29.2% (40/137) of the patients. At a median follow-up of 41.8 months, 21 local and/or distant relapse events were observed and 32 patients died.

The median DFS and OS were not reached at the end of the follow-up period. Kaplan−Meier survival curves did not show any statistically significant difference in the DFS and OS of TNBC cases categorised by AR expression (p=0.7 and

Table 1 Distribution of patient and tumour characteristics in triple negative carcinomas

	Entire cohort (N=137) Mean or *n* (%)	AR negative (*n*=97)	AR positive (*n*=40)	*p* value
Age, years, mean ± SD	52.2 ± 17.0	54.2 ± 15.6	47.5 ± 19.3	0.05[a]
Tumour size, cm	2.7 (3.0−15.0)	2.8 ± 2.1	2.6 ± 2.4	0.6
Mitotic count/10 HPF	35.0 ± 32.4	40.7 ± 33.7	21.0 ± 23.8	0.05[a]
Overall grade (modified Bloom & Richardson)				0.005[a]
1	1 (0.7%)	0 (0%)	1 (2.5%)	
2	28 (20.4%)	8 (28.6%)	20 (71.4%)	
3	108 (78.8%)	89 (82.4%)	19 (17.6%)	
Lymph node involvement				0.07
N0	77 (58.8%)	58 (75.3%)	19 (24.7%)	
N1-N3	46 (46.0%)	33 (71.7%)	13 (28.3%)	
Unknown	8 (6.1%)	3 (37.5%)	5 (62.5%)	
Overall pathological stage				0.1
1	47 (34.3%)	31 (33.0%)	13 (35.1%)	
2	66 (48.2%)	47 (50.0%)	17 (45.9%)	
3	22 (16.1%)	16 (17.0%)	5 (13.5%)	
Unknown	2 (1.5%)	0 (0.0%)	2 (5.4%)	
Tumour subtype				
Invasive (ductal) carcinoma NST	102 (74.5%)	80 (78.4%)	22 (21.6%)	<0.005[a]
Invasive lobular carcinoma	4 (2.9%)	2 (50%)	2 (50%)	
Invasive carcinoma NST with apocrine differentiation	11 (8.0%)	0 (0%)	11 (100%)	
Invasive carcinoma NST with basal-like features	3 (2.2%)	2 (66.7%)	1 (33.3%)	
Invasive carcinoma with medullary-like features	8 (5.8%)	8 (100%)	0 (0.0%)	
Other subtypes	9 (6.6%)	5 (55.6 %)	4 (44.4%)	

NST, no special type.
[a] Statistically significant.

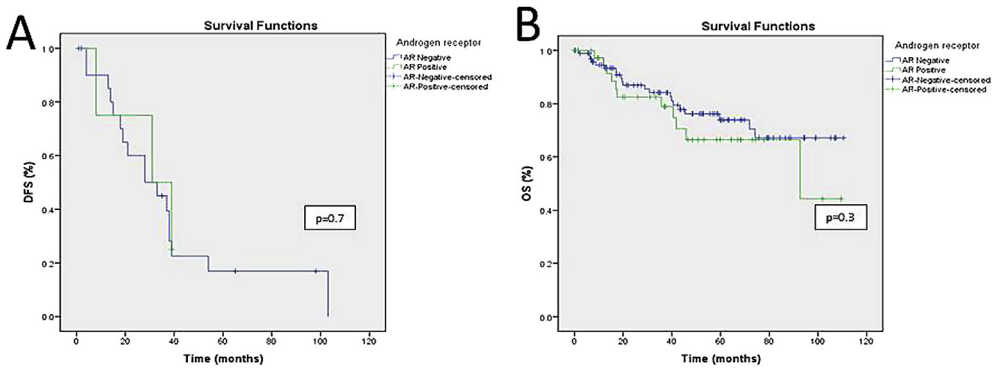

Fig. 2 Kaplan–Meier curves of estimated disease free survival (A) and overall survival (B) for TNBC patients grouped based on androgen receptor immunoexpression.

p=0.3, respectively). This finding remained valid after adjusting for confounders such as age, tumour size, grade and overall pathological stage using a Cox regression model (Fig. 2).

Expression of AR was more frequent in younger patients and in tumours with apocrine differentiation and less common in tumours with medullary-like features ($p<0.005$). Positive AR immunostaining was also shown to be correlated with mitotic rate ($p<0.05$) and overall tumour grade (G2>G3) ($p<0.001$).

We accept that this study was based on TMA sections rather than whole sections (WS) and heterogeneous expression may be a confounding factor. Indeed, we did find occasional cases where one core was positive for AR and the other negative which were resolved by staining whole sections (n=9). Søiland *et al.*[10] demonstrated that there was generally very good agreement between IHC-WS and IHC-TMA when the percent of AR positive nuclei using IHC was >80%, however discrepancies occurred when AR positive nuclei were <80%.

Another potential limitation is the lack of detailed treatment data for this cohort, including the fact that treatment algorithms are likely to have been inconsistent over the 10 year time frame of this study. Of note, AR expression was not routinely performed in our department during this time and therefore knowledge of AR expression status would not have altered treatment.

In conclusion, in our large unselected TMA-based cohort of 137 TNBCs, we found a rate of AR expression of 29.2% and no significant association between AR expression and disease free survival or overall survival. We hope that this information informs the use of AR immunohistochemistry in routine clinical practice.

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Digital evaluation of proliferative 'hotspots' of more than 16,000 cells negatively impacts Ki-67 assessment in breast carcinoma

Sir,

Ki-67 is a nuclear protein expressed during all phases of cell proliferation, which can be detected by a rapid and affordable immunohistochemical assay, widely available in pathology laboratories. Ki-67 proliferative index (PI) has strong prognostic and predictive value in invasive breast carcinoma (IBC). High Ki-67 levels are associated with poorer outcomes