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Characterising the adipose-inflammatory microenvironment in male breast cancer

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Abstract

Male breast cancer (MBC) incidence seems to parallel global increases in obesity. The stromal microenvironment contributes to carcinogenesis, yet the role of adipocytes in this is understudied in MBC. We identified 4 cohorts of male breast tissues diagnosed when obesity was rare (archival cohort) and more common (contemporary cohort). We examined the microenvironment of archival and contemporary cohorts of MBC, diagnosed 1940 - 1970 and 1998 - 2006, respectively, with 2 cohorts of, archival and contemporary gynaecomastia, diagnosed 1940 - 1979 and 1996 – 2011, respectively serving as controls. We quantified adipocytes, crown-like structures (CLS) and the presence of CD8, αSMA and CD68+ macrophages in both cohorts, and determined how these affected survival, in the contemporary MBC cohort. In both MBC cohorts, mean adipocyte diameter was larger in the distant stroma compared with stroma close to the invading tumour (92.2 μm vs 66.7 μm). This was not seen in gynaecomastia. CLS were more frequent in both MBC cohorts than gynaecomastia (44/55 [80%] vs 11/18 [61%], $p < 0.001$). No relationship was found between CLS number and adipocyte size, although there were greater numbers of CLS in contemporary MBC > archival MBC > gynaecomastia. CD8 and CD68 expression in the stroma was significantly associated with reduced survival, with no effects seen with αSMA. Changes in the adipose-inflammatory microenvironment may be a contributing factor to the increase seen in MBC diagnosis.

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Introduction

Breast cancer (BC) affects both genders, although it is rare in men with a global incidence of around 8000 (Humphries, et al. 2015). Anecdotal evidence that the number of men receiving a BC diagnosis is growing is supported by population-based evidence informed from data collected from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database. This showed a significant increase in male BC from 0.86 to 1.08 per 100,000 population in the US over 25 years (1973–1998 (SEER. 2014) . Our group confirmed this in an interrogation of the 2014 SEER dataset (Humphries et al. 2015) and have previously shown parallel rises using data obtained in the UK, Canada and Australia (Speirs and Shaaban 2009). Reasons for the gradual rise in incidence are not known, however we have shown recently that this appears to parallel rising levels of obesity (Humphries et al. 2015).

In terms of pathobiological and physiological characteristics, male breast cancer (MBC) has been likened to BC in post-menopausal women (Anderson, et al. 2010). Post-menopausal BC is linked to obesity, due to a combination of peripheral aromatisation of androgens in adipose tissue, leading to increased systemic estrogen levels, and associated metabolic abnormalities (Chen, et al. 2016). Inflammation is also associated with obesity with chronic inflammation observed in a number of different types of cancer, including breast (De Pergola and Silvestris 2013). Furthermore, macrophages, which contribute to the inflammatory state are frequently recruited into adipose tissue (Johnson, et al. 2012). Dead adipocytes are a pathological hallmark of obesity, which is characterised by CD68-positive macrophages surrounding these dead cells, forming so-called crown-like structures (CLS) (Cinti, et al. 2005; Murano, et al. 2008). CLS have been examined in female breast tissue and are significantly more abundant in obese compared to lean women (Mullooly, et al. 2017). In female BC, presence of CLS in adipose tissue from BC resections was associated with worse distant relapse-free survival (Iyengar, et al. 2016), while in women undergoing breast conserving

surgery, a positive association was observed between body composition, size of breast adipocytes, and incidence of CLS (Vaysse, et al. 2017).

As well as their role in CLS-formation, macrophages are frequently the most abundant inflammatory infiltrate in the tumour microenvironment (Balkwill, et al. 2005). When present in high numbers they are associated with poorer disease-free survival in women, particularly in the ER+ setting (Gwak, et al. 2015; Medrek, et al. 2012; Zhao, et al. 2017). Tumour-infiltrating lymphocytes (TILs) are also common in the BC tumour microenvironment (Savas, et al. 2016). Studies on large numbers of patients have indicated that these are typically associated with improved outcome in patients with early stage triple negative and HER2-positive BC (Ali, et al. 2014; Loi, et al. 2013; Yamaguchi, et al. 2012). Despite the infrequency of the HER2-postive phenotype in MBC (Humphries, et al. 2017), higher TIL density has been reported in this subtype in men (Vermeulen, et al. 2017). In this same study, examination of the relationship between TIL density, through review of H&E stained sections, showed that lower TIL densities correlated with reduced overall survival (Vermeulen et al. 2017).

We have previously proposed that not only is obesity a risk factor for male BC, but that increasing obesity trends may contribute to its increased incidence (Humphries et al. 2015). Therefore, the aim of this study was to examine and characterise components of the adipose-inflammatory tumour microenvironment in a contemporary and an archival series of male BCs, the latter diagnosed during 1940s-1970s, at a time when obesity was virtually non-existent in the UK.

Materials and Methods

Cases

Following ethical approval (06/Q1205/156; 15/YH/0025), tissues were obtained from patients diagnosed at Leeds Teaching Hospitals NHS Trust or from tissue donated to the Breast Cancer Now

Tissue Bank (BCNTB). Tissue sections obtained after September 2006 were taken with informed consent from patients prior to surgical resection. Forty-nine formalin-fixed paraffin-embedded (FFPE) contemporary MBC (cMBC) tissue sections were obtained, diagnosed between 1996 and 2008, plus a further 37 archival FFPE MBC tissue sections (aMBC), diagnosed between 1940 and 1978. As we have previously reported in female cases from this archival series (Dowsett, et al. 2014), compared to modern day standards, clinicopathological information recorded at this time is patchy, with details on grade, node status and in some cases, the diagnosis, frequently omitted from clinical reports accompanying aMBC cases. More in depth data such as BMI, is completely lacking. Consequently, digital images were reviewed with a consultant histopathologist (AMS) to identify which could be suitably utilised as comparative MBC cases with the contemporary cohort. Of the 37 archival cases obtained from the BCNTB, 6 were identified as invasive carcinomas and the others were identified as having a benign pathology or were normal male breast tissue. Eighteen cases were identified as gynaecomastia, so were allocated as a control cohort (aGC) for comparison with MBC sections. Gynaecomastia was classified into active, intermediate or late type as previously described (Bannayan and Hajdu 1972). Active was classified as florid immune infiltration and ductal hyperplasia, whereas late type cases were observed to have fewer ducts and frank fibrosis within the stroma. Intermediate type represented cases with features of both. Thirteen archival cases did not have a diagnosis of invasive carcinoma or gynaecomastia, so were excluded from the analysis. A TMA was obtained from Queen Elizabeth Hospital, Birmingham, UK, containing a cohort of 70 patients diagnosed with gynaecomastia. This TMA was constructed with two tissue cores per case taken from formalin-fixed paraffin-embedded material and used as a control for the contemporary MBC sections (cGC) for immunohistochemical analysis of various biomarkers. The baseline characteristics of the cases used are summarised in Table 1.

Immunohistochemistry

FFPE tissue blocks were sectioned serially at 5 µm and mounted onto Xtra® Slides (Leica, UK). After drying (37^0C overnight), heat-induced antigen retrieval was carried out as previously described (Humphries et al. 2017). Briefly, this was achieved by pressure-cooking in 10% antigen retrieval Access Revelation Solution 10x solution at 125^0C (Menarini Diagnostics, UK). CD68, CD8 and αSMA (all 1:100; Dako, Cambridge, UK) were applied to slides and incubated for 30, 60 and 30 minutes, respectively. Novolink™ Polymer Detection System kit (Leica Biosystems, UK) was used for visualisation of bound antigen following the manufacturer's protocol (27). Subsequently, slides were washed in tris-buffered saline and Polysorbate 20 (Menarini Diagnostics, UK) and placed in Mayer's haematoxylin, dehydrated in graded ethanol, cleared in xylene, and mounted in DPX (Sigma-Aldrich, USA). After drying, slides were scanned (x20; Leica-Aperio AT2 ScanScope™ scanner; Leica Biosystems) and staining quantified by image analysis software (Aperio ImageScope™ Positive Pixel Count Algorithm, version 9). Digital slides were processed by the software, with positively stained areas appearing orange/red and negative areas blue. Digital scores of 3 or above were considered positive for CD8 and CD68, while αSMA was categorised into high and low staining based on the mean value.

Analysis of CLS

CLS were identified in the archival and cMBC sections and aGC sections using anti-CD68 antibody, a pan macrophage marker used routinely by other groups to detect CLS (Iyengar et al. 2016). In order to quantify CLS objectively, image analysis software was used as described above. CLS were defined by the complete encirclement of an adipocyte by orange/red-coloured macrophages, with quantification in Aperio ImageScope™ using the counter tool. The number of CLS in each area was summed to give the total number per case. cGC cases were not evaluated for presence of CLS, given the omission of adipose tissue from most of the TMA cores.

Adipocyte measurement

H&E stained sections from each case were scanned to create digital images (x20; Aperio ScanScope™). Within each case, two random areas of adipose tissue were outlined based on the distance from the invading edge of the tumour. The area closest to the tumour (defined as less than 200 µm) was named the 'close' area, and the adipose area greater than 200 µm was named the 'far' area. In the aGC, areas of adipose were similarly identified as 'close' and 'far' based on their distance from stroma. Randomisation of adipocytes to be selected for measurement was achieved using RandomSpot (Wright, et al. 2015). Twenty square boxes of 100 pixel diameter were overlaid on the outlined 'close' and 'far' areas of adipose. Each box was observed systematically and the adipocyte directly underneath the boxes was measured. Two measurements (horizontal and vertical, regardless of orientation) per adipocyte were taken, giving a total of 40 measurements of diameter per area of adipose. The annotations were exported to Microsoft Excel, then the mean diameter was calculated for each case. To exclude the possibility that fixation may have altered adipocyte size, frozen samples from 3 female BC cases were also evaluated as described.

Statistical Analysis

All statistical analyses were carried out using IBM SPSS Statistics version 24. The mean values for 'close' and 'far' adipose areas were calculated, and a paired t test was conducted to calculate differences in each tissue type. Independent samples t tests were conducted to identify the inter-group differences in mean adipocyte sizes. CLS were analysed using a Mann-Whitney U test. Charité Cutoff Finder was used to calculate the optimal cut off within the 'close' and 'far' group, above which would be considered 'large' adipocytes and below which would be considered 'small' adipocytes for that area(Budczies, et al. 2012). Kaplan-Meier survival analysis was then conducted for the cMBC cases and plotted using GraphPad Prism version 7.03. P values of < 0.05 were considered statistically significance, with cut offs pre-determined before data analysis.

Results

Adipocytes are smaller closer to the invading edge of the tumour

Of the 49 cMBC cases, 6 were excluded for adipocyte counting due to the absence of adipose tissue. Therefore, the total number of cases for analysis of adipocyte diameter for cMBC, aMBC and aGC was 43, 6 and 18, respectively. In the cMBC cohort, adipocytes closer to the tumour edge were consistently smaller; the mean far adipocyte diameter was 92.22 $\pm$ 19.62 µm and the mean close 66.74 $\pm$ 15.11 µm, 25.48 µm ± 11.26 µm larger (95% CI 22.01 – 28.94 µm, $p < 0.001$). This trend was similar for aMBC cases with the diameter of far adipocytes 83.94 $\pm$ 11.92 µm and close 57.05 $\pm$ 12.14 µm, 26.89 µm ± 10.00 µm larger (95% CI 16.40 - 37.38 µm, $p < 0.001$). While there was no difference in the size of close (60.17 $\pm$ 21.48 µm) and far (63.90 $\pm$ 16.45 µm) adipocytes in aGC cases ($P = 0.16$), there was a statistically significant difference between the mean diameters of far but not close adipocytes in aGC cases compared to those from archival ($P = 0.012$) and cMBC groups ($P < 0.001$),. The results are depicted in Fig 1. To eliminate the possibility that adipocyte size may have been affected by processing to FFPE, size was also evaluated in frozen female breast tissue with a similar smaller adipocyte diameter closer to the invasive tumour (Supplementary Fig 1).

Crown-like structures are more frequently observed in MBC than gynaecomastia

As assessing CLS can be subjective, the Aperio positive pixel count algorithm was utilised to facilitate counting. An example is shown in Fig 2a. CLS (arrows) were identified by the complete encirclement of an adipocyte by CD68 positive macrophages. CLS were frequently observed in the MBC cases. Of the 49 cMBC cases, 39 (80%) displayed CLS. Additionally, CLS were observed in 5/6 (83%) of aMBC cases. CLS were less frequent in aGC (11/18, 61%). The range varied enormously between cases, with 10/49 (20.4%) cMBC displaying no CLS at all, and up to 500 CLS observed in one aMBC case.

Number of CLS was greater in both MBC cohorts compared to gynaecomastia cases (Table 2). No relationship was found between CLS number and adipocyte diameter (Fig 2b), nor was there a difference when stratified into adipocytes with or without CLS (Fig 2c). There were 2 obvious outliers in each of the contemporary and archival series where 500 and 496 CLS were recorded; when these were removed there was a clear trend of increased numbers of CLS in both MBC compared to gynaecomastia (Fig 2d).

Inflammatory cell infiltrates in the tumour microenvironment influences MBC survival in the contemporary series

Immunohistochemical staining of CD8, CD68 and αSMA was quantified by image analysis (SFig2). T cells were identified using CD8. Kaplan Meier univariate survival analysis showed that higher levels of CD8 in the tumour-stroma had a statistically significant negative effect on survival duration ($p = 0.016$, log rank; Fig 3a). As well as using CD68 to detect CLS, in these same sections we also examined the impact of CD68-stained macrophages within the tumour stroma on survival. This showed that CD68 expression was significantly associated with reduced survival ($p = 0.001$, log rank; Fig 3b). Cancer-associated fibroblasts (CAFs) in the breast tumour microenvironment typically adopt a myofibroblast-like phenotype and express αSMA (Sappino, et al. 1988), hence we took advantage of this to identify these cells in MBC tissue sections. αSMA was expressed in all cases but when categorised into high or low stroma no significant effects were seen on survival ($p = 0.14$, log rank; Fig 3c). These analyses were not possible in the archival series due the lack of any follow up data.

Discussion

The aim of this study was to explore the adipose-inflammatory microenvironment of 4 cohorts of male breast tissue diagnosed in two different time periods, when obesity was rare (archival cohort) and more common (contemporary cohort).

We focused first on adipose tissue as adipocytes comprise a major part of the breast tumour stroma. Our data indicated that in both the archival and cMBC series the size of adipocytes at the invading edge of the tumour were significantly smaller compared with those in distant stroma. This was not observed in benign male breast tissue, suggesting it may be a cancer-specific phenomenon. Concerned that this may be an artefact associated with tissue processing, we evaluated this on frozen cases of female BC, with identical results. It was also possible that the size differences in adipocytes may have resulted from the plane in which the sections were cut, however this seems unlikely, as our data is supported by findings in female breast where adipocytes within/immediately adjacent to tumour tissue were also smaller in size compared to those in adjacent far breast tissue (DeFilippis, et al. 2012; Fletcher, et al. 2017). To our knowledge this has not been reported in male breast tissue. The biological consequences are unknown; however it may be related to adipocyte maturity since perilipin, a marker of adipocyte maturity and adiponectin, a well-documented anti-proliferative marker, were both significantly reduced in BC adipocytes (Kang, et al. 2005). Furthermore, co-culturing BC cell lines with mature but not immature adipocytes resulted in increased cell growth (Manabe, et al. 2003). It remains to be elucidated if phenotypic changes observed in adipocytes close to and within breast tumours may result from bi-directional communication with the tumour cells.CLS are considered a reasonable hallmark of pathological obesity (Murano et al. 2008). Their presence and frequency within adipose tissue was examined in 3 of our male breast cohorts and we developed a digital pathology algorithm to facilitate their quantification. This helped remove subjectivity associated with visual assessment of CLS employed by others (Koru-Sengul, et al. 2016; Mullooly et al. 2017). CLS were observed all 3 cohorts, however were seen in a higher proportion of archival and contemporary MBCs and at a greater frequency than in benign breast tissue. The numbers per case varied from none to up to several hundred,

similar to that reported in female breast (Koru-Sengul et al. 2016; Mullooly et al. 2017). Removing 2 outliers from the archival and cMBC series showed the numbers of CLS ranked aMBC > cMBC > gynaecomastia. However, in contrast to reports in female BC (Vaysse et al. 2017), CLS number and adipocyte diameter did not correlate, even when stratified into adipocytes with or without CLS. Our cohort was roughly half of that analysed by Vaysse et al (Vaysse et al. 2017), which might explain the discrepancy, or it could be due to gender-related differences. Further analysis of a larger male cohort, where full face sections are available, is required for confirmation.

Fibroblasts, endothelial cells and immune infiltrates reside within the tumour microenvironment, collectively comprising the stroma, which can influence growth and progression of neighbouring tumour cells. Using the cMBC series only, fibroblasts were identified by αSMA (Sappino et al. 1988), then stratified into high and low αSMA-expression, determined on the basis of expression above and below the mean value obtained from the Aperio algorithm. There was no difference in outcome according to αSMA stratification. However all cases in the contemporary series were ERα+. We have shown previously that a high proportion of stroma in ERα+ male and female BC is associated with better survival (Downey, et al. 2014), so this finding might be anticipated. We also examined the relationship of macrophages and T cells in the cMBC tumour microenvironment with survival. In line with studies in female BC, macrophages were associated with reduced survival (Bense, et al. 2017; Campbell, et al. 2011; Leek, et al. 1996; Zhang, et al. 2013).

We found high expression of CD8 in the MBC tumour microenvironment resulted in reduced survival. In women, the presence of CD8 is typically associated with better outcome in ERα- and to a lesser extent ERα+HER2+ BC (Ali et al. 2014; Liu, et al. 2012), however these subtypes are uncommon in MBC, which is predominantly ERα+ (Humphries et al. 2017). Indeed our cohort was all ERα+. Conversely, it has been demonstrated using tissue microarrays (TMAs) that where the proportion of stroma is high, CD8 expression is low (Gujam, et al. 2014). This is at odds with our study, although we used full face sections, not TMAs. Additionally, we noted CD8 positivity was

heterogeneous; in some cases this was entirely stromal CD8 while in others it was restricted to tumour with some instances of positivity in both tumour and stroma (data not shown). In the BC field, international working groups have established guidelines in favour of assessment of TILs in H&E stained sections (Hendry, et al. 2017; Salgado, et al. 2015). As a result, scientists are gradually moving away from defining specific T cell populations identified by immunohistochemistry. A recent study assessed TIL density in 1,196 MBCs, reporting that a lower density correlated with reduced overall survival (Vermeulen et al. 2017). Collectively these results may indicate it is the repertoire of TILs, rather than specific subtypes, which are important in dictating BC outcome.

The main purpose of using the CD68 biomarker was to define CLS (Iyengar et al. 2016). While we acknowledge that this is sometimes regarded as marker of total macrophage population (Sousa, et al. 2015), rather than defining specific M1 and M2 macrophage phenotypes, we used this to explore the possible significance of intra-tumoral CD68 expression on outcome. We found high CD68 expression was associated with poorer outcome, which parallels findings in female breast cancer (Medrek et al. 2012; Zhang et al. 2013). As male breast tissue is rare, this precluded a more detailed analysis of other markers of macrophage activity such as CD163.

By analysing contemporary and aMBC, alongside non-cancerous gynaecomastia, which is often used as a MBC control (Alali, et al. 2010), our data suggest that changes in the adipose-inflammatory microenvironment since the 1940s may be a contributing factor to the increase in MBC diagnosis over this time. Access to a unique cohort of archival male breast tissues has permitted this analysis. There have been considerable lifestyle changes during the time period that these samples were collected, one of which is the rising level of obesity. We do, however, acknowledge our study has limitations. The biggest limitation is the lack of recorded BMI data in any of the cohorts studied. Nevertheless, while high BMI has been shown to predict the presence of CLS in adipose tissue adjacent to the tumour in women (Vaysse et al. 2017), BMI does not always correlate with obesity, particularly in athletes e.g. rugby players or American footballers whose large bulk contributes to

high BMI. Additionally, we were only able to identify 6 cases of *bona fide* aMBC from our original cohort of 37. Typically, pathology departments do not store archival material for more than 30 years, making it challenging to obtain larger numbers for this type of work. Indeed two recent studies of cMBC are restricted in numbers, including 42 and 38 patients respectively, (Cui, et al. 2018; Turashvili, et al. 2018), reflecting its rarity. Nevertheless, our study has uncovered novel and thought provoking information which should be considered as hypothesis generating for future work examining potential relationships between obesity and BC.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure legends

Figure 1

Mean difference in adipocyte diameter (μm) between 'close' and 'far' areas for contemporary and archival male breast cancer and gynaecomastia. P values refer to paired sample Student's t-test. * denotes P < 0.001; # denotes P = 0.012.

Figure 2

Example of CLS observed in MBC case with and without the Positive Pixel Count Algorithm applied (right and left images, respectively; a). CLS is illustrated by the arrowhead and identified by the complete encirclement of an adipocyte by macrophages, coloured red by applying the algorithm and identified by the asterisk. Original magnification of scanned image = 20x (Aperio ScanScope™). A scatter plot of adipocyte size and CLS density is shown in (b) and a box plot showing adipocyte size stratified in those with or without CLS is in (b). When 2 obvious outliers were removed, numbers of CLS showed a significant, stepwise increase in number from gynecomastia through aMBC to cMBC (d). *P<0.04 *vs.* gynaecomastia.

Figure 3

Kaplan-Meier survival analysis (log rank test) of the in cMBC cohort showed CD8+ cells were significantly associated with reduced overall survival (p = 0.016; a). The same association was also observed with CD68 positive cells p = 0.001; b). No significant association was seen with the dichotomisation of αSMA (c). HR = Hazard Ratio.

SuppFigure 1

Mean difference in adipocyte diameter (μm) between 'close' and 'far' areas in 3 cases of female breast cancer in which frozen and formalin-fixed paraffin-embedded (FFPE) material was available for each. Smaller adipocyte diameter was observed closer to the invasive tumour in both frozen and FFPE material, eliminating the possibility that adipocyte size may have been affected by processing to FFPE. P values refer to paired sample Student's t-test. * denotes P < 0.001

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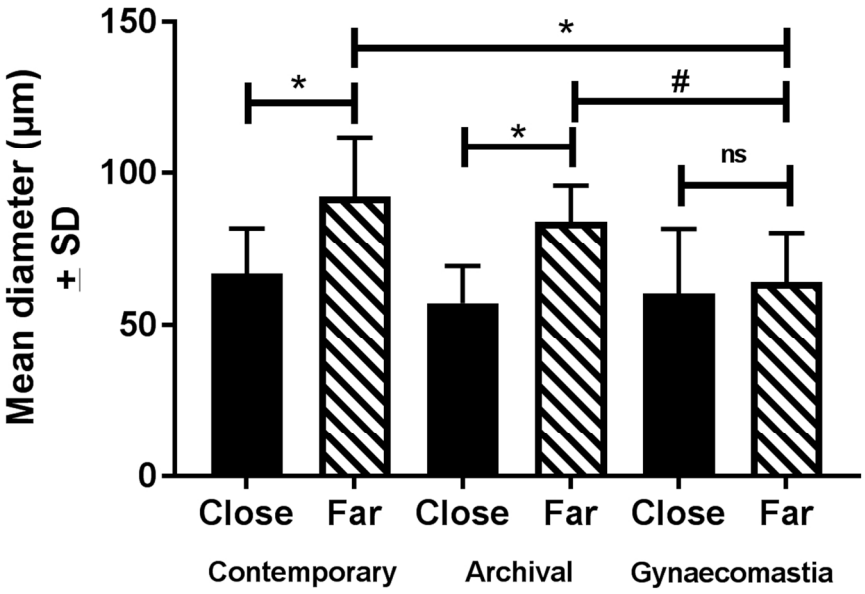

Fig 1

111x74mm (300 x 300 DPI)

Figure 3

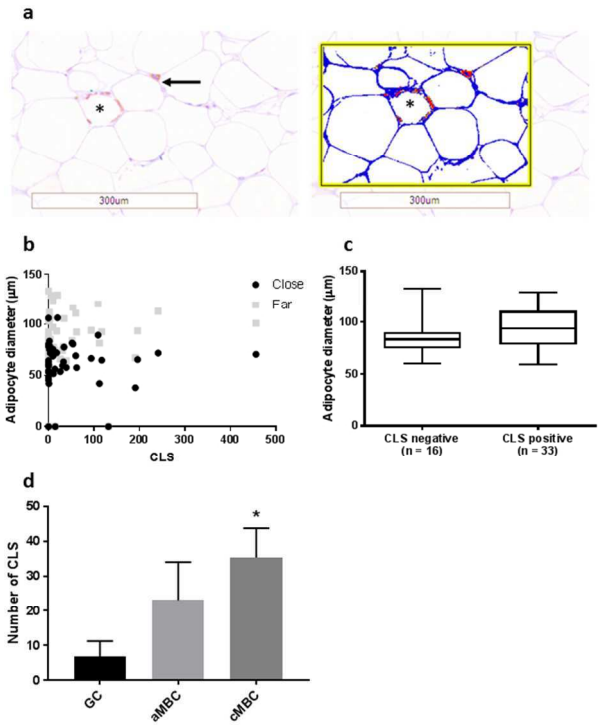

Fig 2

190x338mm (96 x 96 DPI)

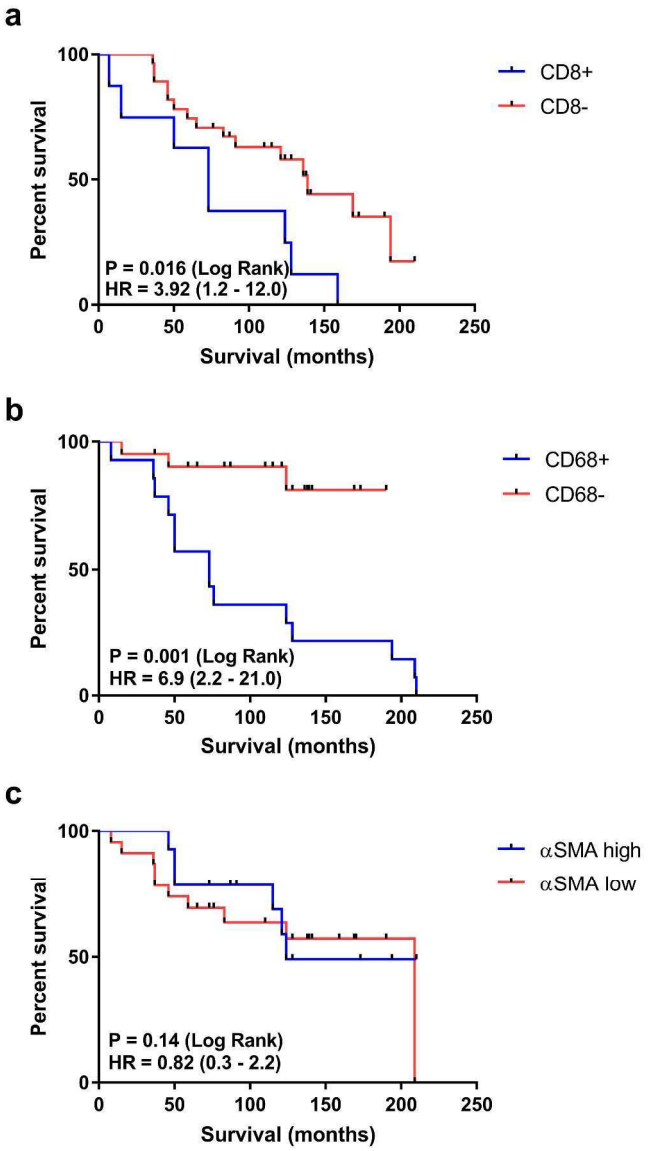

Fig 3

264x461mm (300 x 300 DPI)

Table 1

Clinicopathological details of the cases analysed

Characteristic	Male breast cancer Number (%)		Gynaecomastia Number (%)	
	Contemporary[1]	Archival[2]	Contemporary	Archival
Age				
13-30 years	0 (0)	0 (0)	49 (70)	4 (22.2)
30-39 years	0 (0)	0 (0)	8 (11.4)	0 (0)
40-49 years	3 (6.1)	0 (0)	7 (10)	1 (5.6)
50-59 years	6 (12.2)	2 (33.3)	2 (2.9)	0 (0)
60-69 years	10 (20.4)	0 (0)	2 (2.9)	1 (5.6)
70-79 years	15 (30.6)	1 (16.7)	2 (2.9)	1 (5.6)
80-89 years	15 (30.6)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	3 (50)	0 (0)	11 (61.1)
Diagnosis (year)				
1940-1949	N/A	2 (33.3)	N/A	1 (5.6)
1950-1959	N/A	2 (33.3)	N/A	1 (5.6)
1970-1979	N/A	2 (33.3	N/A	16 (88.9)
1996 – 2000	18 (36.7)	N/A	3 (3.5)	N/A
2001 – 2005	16 (32.7)	N/A	13 (15.1)	N/A
2006 - 2008	12 (24.5)	N/A	51 (59.3)	N/A
2009-2011	0 (0)	N/A	3 (4.3)	N/A
Unknown	3 (6.1)	0 (0)	(0)	0 (0)
Histology				
Ductal	43 (87.8)	5 (83.3)	N/A	N/A
Papillary	4 (8.2)	1 (16.7)		
Lobular	1 (2)	0 (0)		
Unknown	1 (2)	0 (0)		

Grade				
1	7 (14.3)	0 (0)	N/A	N/A
2	33 (67.3)	4 (66.7)		
3	8 (16.3)	2 (33.3)		
Unknown	1 (2)	0 (0)		
Lymph nodes				
+	15 (30.6)	2 (33.3)	N/A	N/A
-	32 (65.3)	3 (50)		
Unknown	2 (4.1)	1 (16.7)		
Size (mm)				
≤20mm	31 (63.3)	2 (33.3)	N/A	N/A
>20mm	14 (28.6)	1 (16.7)		
Unknown	4 (8.2)	3 (5)		
Gynaecomastia type				
Active	N/A	N/A	6 (33.3)	Information not available
Intermediate			6 (33.3)	
Late			6 (33.3)	

[1]All ERα positive, [2]ERα status not defined, N/A = not available

Table 2

Crown-like structures are increased in male breast cancer compared to gynaecomastia

Pathology	Median number CLS (IQR)	CLS range	Cases with CLS (%)
cMBC	18 (59.5)	0 - 496	80
aMBC	26 (161)	0 - 500	83
Gynaecomastia	1 (2.5)	0 - 81	61