

***Cytological criteria distinguishing phyllodes tumour of the
breast from fibroadenoma***

Robert Myles Maritz

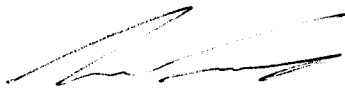
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Johannesburg, 29 July 2015

*A research report submitted to the Faculty of Health Sciences, University of the
Witwatersrand, Johannesburg; in partial fulfilment for the degree of Master of
Medicine (Anatomical Pathology)*

Declaration

I, Robert Myles Maritz, declare that this research report is my own work. It is being submitted in partial fulfillment for the degree of Master of Medicine in Anatomical Pathology. It has not been submitted before for any degree or examination at this or any other university.

A handwritten signature in black ink, appearing to read 'Robert Myles Maritz', with a stylized, sweeping flourish at the end.

Robert Myles Maritz

29th day of July, 2015

Dedication

This research report is dedicated to my wife, Ashleigh, for her endless patience, encouragement and support, and to my children, Luke and Hailey, for giving my life purpose and making me happier than I ever dreamed possible.

Abstract

Cytological criteria distinguishing phyllodes tumour of the breast from fibroadenoma.

Background

Fibroepithelial lesions of the breast include fibroadenomas and phyllodes tumours. Fibroadenomas are benign tumours, whereas phyllodes tumours range from benign, indolent neoplasms to malignant tumours capable of distant metastasis and occasionally resulting in death.

Aim

The aim of this study was to determine whether there are statistically significant differences between fibroadenomas and phyllodes tumours with regard to selected cytological features.

Methods

A ten year retrospective review was performed of patients who had an excision of a fibroadenoma or phyllodes tumour, and on whom a pre-operative fine needle aspirate was performed. The following cytological criteria were assessed: number of stromal and epithelial fragments, stromal to epithelial ratio, stromal cellularity,

stromal borders, stromal atypia and proportion of background wavy spindled cells.

The patient age, tumour laterality and tumour size were recorded.

Results

Fifty fibroadenomas and 17 phyllodes tumours were included. When compared with phyllodes tumours, fibroadenomas had a larger number of epithelial fragments, a smaller number of stromal fragments and a lower stromal to epithelial ratio. The stroma tended to be less cellular and less atypical compared with phyllodes tumours and the background cellular population contained less spindled cells.

Conclusion

Fibroadenomas and phyllodes tumours differ with regard to various cytological features, possibly aiding in their distinction on fine needle aspiration biopsy.

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CHAPTER 1

1.0 Introduction

Fibroepithelial lesions of the breast include fibroadenomas and phyllodes tumours. Both are biphasic neoplasms, comprising a proliferation of glandular breast epithelial elements surrounded by a variably cellular stromal component (Figure 1.1). Fibroadenomas are benign tumours, with no risk of recurrence or metastasis (Rosen, 2008). Phyllodes tumours, on the other hand, represent a spectrum ranging from benign, indolent neoplasms to frankly malignant tumours capable of distant metastasis and occasionally resulting in death (Tan *et al*, 2012). Given the marked variation in clinical behaviour and prognosis associated with these tumours, accurate diagnosis is essential, to prevent both over-treatment of fibroadenomas and under-treatment of phyllodes tumours.

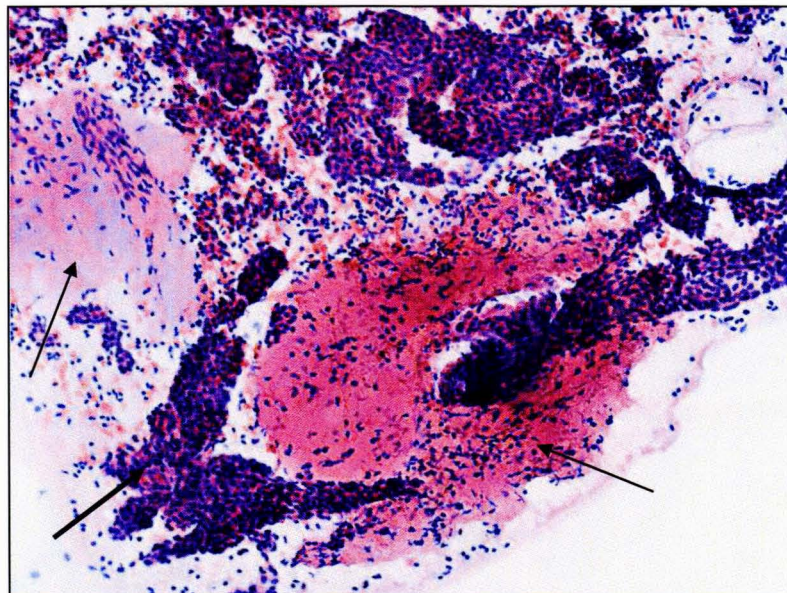


Figure 1.1: Fine needle aspirate of a fibroadenoma showing a biphasic neoplasm comprising epithelial fragments (thick arrow) intimately associated with two large stromal fragments of low cellularity (thin arrows) (Papanicolaou stain, x100)

As fine needle aspiration (FNA) is often performed as part of the initial evaluation of a breast mass, one will encounter both fibroadenoma and phyllodes tumour. Accurately distinguishing between fibroadenoma as well as benign, borderline and malignant phyllodes tumour is important, both from a prognostic point of view as well as for planning possible surgical intervention. Fibroadenomas can be treated conservatively without surgery or can be enucleated, with no risk of recurrence (Rosen, 2008). Phyllodes tumours, on the other hand, require excision with clear surgical margins, as these tumours are prone to local recurrence (Barth *et al*, 1999 and Komenaka *et al*, 2003). Unfortunately, no universally accepted cytological criteria for the distinction of fibroadenoma from phyllodes tumour exist (Foxcroft *et al*, 2007). Several studies comparing the cytological features of fibroepithelial lesions of the breast have been performed (Simi *et al*, 1988; Stanley *et al*, 1989; Dusenbery *et al*, 1992; Rama Rao *et al*, 1992; Shimizu *et al*, 1994; Shabb, 1997; Deen *et al*, 1999; Bhattarai *et al*, 2000; Krishnamurthy *et al*, 2000; Scolyer *et al*, 2001; Veneti *et al*, 2001; Jayaram *et al*, 2002; Shimizu *et al*, 2002; Bandyopadhyay *et al*, 2010 and El Hag *et al*, 2010). Unfortunately, the results of these studies are discordant, with some authors being of the opinion that FNA diagnosis of fibroepithelial lesions is accurate and reproducible, whilst others feel that accurate distinction of fibroadenomas from phyllodes tumours is not possible using cytology. This study will attempt to examine some of the cytological features previously proven helpful in distinguishing between these tumours, with a view to improving the accuracy of preoperative FNA diagnosis in our practice.

CHAPTER 2

2.0 Aims and Objectives

2.1 Aim

To determine whether there are statistically significant differences between fibroadenomas and phyllodes tumours with regard to selected cytological features.

2.2 Objectives

1. To describe the demographics of patients with fibroadenomas and phyllodes tumours in the Johannesburg population, including age, size and laterality.
2. To examine specific cytological features of histologically-proven fibroadenomas and phyllodes tumours.
3. To compare fibroadenomas with phyllodes tumours, fibroadenomas with benign phyllodes tumours and benign, borderline and malignant phyllodes tumours with one another to determine the most helpful cytological features in distinguishing these entities.
4. To determine the intra-observer variability of the various criteria studied.

CHAPTER 3

3.0 Literature review

3.1 Introduction

It is important to distinguish fibroadenoma from phyllodes tumour as the management of the two differs considerably (Komenaka *et al*, 2003 and Jacklin *et al*, 2006). Researchers have attempted various methods to differentiate between these two entities prior to surgery, including epidemiologically, clinically, radiologically, cytomorphologically and with the use of ancillary tests on the cytology specimen.

3.2 Epidemiology

Fibroadenomas are the most common breast tumour in adolescents and young adults (Rosen, 2008) and the most common benign breast tumour overall (Giri, 2009). In contrast, phyllodes tumours are rare, comprising approximately 1% of all breast tumours (Parker *et al*, 2001). Fibroadenomas occur most frequently in patients younger than 30, whereas phyllodes tumours tend to occur in older patients, usually between the ages of 40 and 50 years (Tan *et al*, 2012). However, there is significant overlap between these two groups with regard to age at diagnosis. As phyllodes tumours can occur in young women, the use of age as a

criterion to distinguish between these tumours is not possible, further emphasising the need for a reliable

3.3 Clinical features

Both fibroadenomas and phyllodes tumours present with a well-defined, mobile breast mass which appears clinically benign. Cases with multiple tumours have been described (Rosen, 2008). In general, phyllodes tumours tend to be larger at presentation and show more rapid growth compared with fibroadenomas. However, accurate distinction of these tumours based on clinical grounds is not possible (Rosen, 2008). The widespread use of imaging has led to patients with phyllodes tumours presenting to surgeons at an earlier stage and with smaller lesions, further contributing to the clinical overlap between these tumours (Komenaka *et al*, 2003).

3.4 Imaging

Mammography and ultrasound are the two imaging modalities used most frequently in the assessment of breast masses. Sonographically, fibroadenomas have a more homogeneous internal echo pattern, whereas that of phyllodes tumours tends to be more heterogeneous (Chao *et al*, 2002). According to these authors, both tumour types show lack of microcalcification, are mildly hypoechoic and have smooth margins. In a recent study by Gatta *et al* (2011), fibroadenomas were found to be of lower density than phyllodes tumours on mammogram, with phyllodes tumours

having a greater incidence of calcification. Whilst both ultrasound and mammography have proven invaluable in the assessment of breast carcinomas, their use in distinguishing between fibroadenomas and phyllodes tumours is limited, as significant overlap in the imaging features of these tumours exists (Page *et al*, 1991; Chao *et al*, 2002; Rosen, 2008 and Gatta *et al*, 2011).

In 2009, Awad and Alsaeed attempted to distinguish between these tumours using contrast-enhanced magnetic resonance imaging (MRI). Both tumour types had smooth contours and high signal intensities on T2-weighted images. They concluded that MRI was not useful in their distinction.

3.5 Macroscopy

Macroscopically, fibroadenomas and phyllodes tumours present similar appearances to one another. Both are well-circumscribed, firm, cream/white tumours with a bulging cut surface. Whilst fibroadenomas have small slit-like spaces, a leaf-like architecture is seen in phyllodes tumours (Tan *et al*, 2012). Large phyllodes tumours may have areas of necrosis and haemorrhage.

3.6 Histology

Fibroadenomas are encapsulated, biphasic tumours composed of glandular epithelial elements set in a fibrous stroma. Usual duct hyperplasia of the epithelium may be present, together with areas showing apocrine metaplasia (Tan *et al*, 2012). The

stroma is hypocellular and shows little to no nuclear atypia or mitotic activity. So-called cellular fibroadenoma, which occurs in women younger than 20, may show stromal hypercellularity, leading to histological (and cytological) overlap with phyllodes tumour (Dusenbery *et al*, 1992 and Tan *et al*, 2012).

Phyllodes tumours are also biphasic neoplasms, characterised by hypercellular stroma and a leaf-like growth pattern. They are divided histologically into benign, borderline and malignant categories, based on the degree of stromal hypercellularity, atypia and mitotic activity, stromal overgrowth and the presence or absence of an infiltrative border (Tan *et al*, 2012). Rather than representing three distinct entities, they encompass a spectrum ranging from benign tumours which can be cured with local surgery to frankly malignant tumours capable of metastatic spread (Parker *et al*, 2001).

3.7 Cytology

As alluded to earlier, significant overlap exists between fibroadenoma and phyllodes tumour on a cytological level. This is due in part to the lack of architectural features intrinsic to cytological examination. In addition, phyllodes tumours are heterogeneous, with areas of stromal hypocellularity admixed with areas showing classic features of phyllodes tumour (Jacklin *et al*, 2006). This heterogeneity makes FNA prone to sampling bias, and sometimes leads to an increase in the number of phyllodes tumours misdiagnosed as fibroadenomas.

Several previous studies have compared the cytological features of fibroadenomas and phyllodes tumours, as well as the features of benign, borderline and malignant phyllodes tumour (Simi *et al*, 1988; Stanley *et al*, 1989; Dusenbery *et al*, 1992; Rama Rao *et al*, 1992; Shimizu *et al*, 1994; Shabb, 1997; Deen *et al*, 1999; Bhattarai *et al*, 2000; Krishnamurthy *et al*, 2000; Scolyer *et al*, 2001; Veneti *et al*, 2001; Jayaram *et al*, 2002; Shimizu *et al*, 2002; Bandyopadhyay *et al*, 2010 and El Hag *et al*, 2010). The significant findings of these studies are briefly summarised in Table 3.1.

Table 3.1: Previous studies comparing the cytological features of fibroadenomas and phyllodes tumours

Author(s)	Number of FA	Number of PT*	Useful cytological features
Bandyopadhyay <i>et al</i> , 2010	25	10 (8/1/1)	Large, hypercellular stromal fragments with well-defined borders in PT; increased numbers of background spindled cells in PT
Bhattarai <i>et al</i> , 2000	0	80(53/18/9)	Hypercellular stromal fragments, numerous bare nuclei, stromal atypia and lack of apocrine cells in PT
Deen <i>et al</i> , 1999	18	19(17/0/2)	Apocrine cells in PT and FA; foamy macrophages in FA; increased spindled stromal cells in PT; >6 leaf-shaped stromal fragments in PT
Dusenbery <i>et al</i> , 1992	0	6 (4/0/2)	Foam cells and oval bare nuclei present in PT; cellular stromal fragments may be absent
El Hag <i>et al</i> , 2010	12	15 (8/6/1)	Increased numbers of stromal fragments in PT; stromal fragments in PT comprise spindled cells, in FA comprise plump cells; atypia present in borderline PT; <10% background spindled cells in FA
Jayaram <i>et al</i> , 2002	0	28 (20/1/7)	Large, hypercellular stromal fragments and dissociated stromal cells in PT; background spindled cells with cytoplasm in PT, bipolar naked nuclei in FA
Krishnamurthy <i>et al</i> , 2000	33	12 (1/8/3)	>30% long, spindled background cells in PT, <10% in FA; hypercellular stromal fragments in PT
Rama Rao <i>et al</i> , 1992	0	17 (10/2/5)	Cellular stromal fragments in PT; mild-to-moderate nuclear atypia in benign/borderline PT; marked atypia with mitoses in malignant PT

Scolyer <i>et al</i> , 2001	13	8 (6/0/2)	Hypercellular stromal fragments with well-defined borders in PT; nuclear atypia in malignant PT
Shabb, 1997	0	8 (5/0/1)**	Cellular stromal fragments present in all cases of PT
Shimizu <i>et al</i> , 1994	19	17 (9/3/5)	Large folded epithelial sheets, hypercellular stromal fragments, atypical background cells and foamy macrophages in PT
Shimizu <i>et al</i> , 2002	25	18 (18/0/0)	Epithelial clusters larger in PT; wavy clusters seen in PT, tubular clusters seen in FA
Simi <i>et al</i> , 1988	20	5 (4/1/0)	Apocrine cells absent in PT but present in FA; cellular stromal fragments in PT; mesenchymal atypia in borderline PT
Stanley <i>et al</i> , 1989	0	23***	Isolated spindle cells present; stromal cellularity variable
Veneti <i>et al</i> , 2001	18	18 (18/0/0)	Large stromal fragments in PT but cellularity and number similar to FA; ratio of stromal to epithelial bare nuclei increased in PT
<i>Abbreviations:</i> FA, fibroadenoma; PT, phyllodes tumour * The numbers in parenthesis indicate the numbers of benign, borderline and malignant phyllodes tumours studied ** Two cases of metastatic PT were included *** Information regarding the subtypes of PT was not included			

The majority of reviewers found the presence of hypercellular stromal fragments to be the most useful cytological feature in distinguishing fibroadenoma from phyllodes tumour. Unfortunately, no strict definition of “hypercellular” exists, with most authors grading cellularity as 1+, 2+ or 3+. This limits the usefulness of comparisons between these studies with regard to cellularity of the stroma. Apart from the cellularity of the stroma, size and number of stromal fragments were of value in some studies, with larger and more numerous fragments present in phyllodes tumours (Veneti *et al*, 2001; Jayaram *et al*, 2002; Bandyopadhyay *et al*, 2010 and El Hag *et al*, 2010).

In the studies by Deen *et al* (1999), Krishnamurthy *et al* (2000), Scolyer *et al* (2001), Bandyopadhyay *et al* (2010) and El Hag *et al* (2010), no significant differences

between fibroadenomas and phyllodes tumours were found with regard to the characteristics of the epithelial clusters. This is in contrast to the two studies performed by Shimizu *et al* (1994 and 2002), in which folded and wavy epithelial sheets were found in phyllodes tumours, compared with tubular clusters seen in fibroadenomas. The study performed by Shimizu *et al* in 2002 also showed the epithelial clusters in phyllodes tumours to be larger than those seen in fibroadenomas.

Besides the epithelial and stromal component, the number and cytomorphology of the background dispersed cells also showed differences between fibroadenomas and phyllodes tumours. This was particularly well studied by Veneti *et al* (2001). This group showed the presence of bland, round-to-oval epithelial bare nuclei in fibroadenomas, whereas both epithelial and stromal bare nuclei were present in phyllodes tumours. The stromal bare nuclei were larger than their epithelial counterparts, and had more vesicular chromatin. Three cases of phyllodes tumour showed epithelial and stromal atypia.

In an interesting article by Krishnamurthy *et al* (2000), long spindled background cells were demonstrated in phyllodes tumours, whereas round-to-oval nuclei were present in fibroadenomas. They felt that in cases with greater than 30% of stromal cells having a spindled morphology, a diagnosis of phyllodes tumour could be made. On the other hand, cases with less than 10% spindled cells should be classified as fibroadenoma. An intermediate zone of 10-30% exists, and cases falling into this category should be classified as indeterminate. In contrast to some of the studies

mentioned above, this study found the presence of hypercellular stromal fragments an unreliable indicator of phyllodes tumour. El Hag and colleagues (2010) recommended using a cut-off of background spindled cells of 10% as opposed to 30% in order to increase the sensitivity of diagnosis of phyllodes tumour, and to prevent misdiagnosing phyllodes tumours as fibroadenomas.

Controversy exists regarding the presence of apocrine metaplasia and foam cells in distinguishing fibroadenomas from phyllodes tumours. These specific features were studied by a number of the authors listed above. Foam cells were identified in the aspirates from both phyllodes tumours and fibroadenomas, proving to be of little value in distinguishing the two lesions. Similarly, apocrine cells, which were originally believed to occur only in fibroadenomas, were demonstrated in phyllodes tumours in a number of studies.

3.8 Ancillary techniques

As reviewed by Jacklin *et al* (2006), few studies have been performed to assess the use of the Ki-67 proliferation index by immunohistochemistry to distinguish between fibroadenoma and phyllodes tumour, with conflicting results. These studies, however, were performed on histology specimens and not on specimens obtained using FNA. Furthermore, these authors state that expression of p53 using immunohistochemistry is associated with malignancy in phyllodes tumour. Unfortunately, no studies have been performed using this antibody to distinguish between the different types of fibroepithelial lesions. Similarly, no studies assessing

differences between fibroadenomas and phyllodes tumours using flow cytometry, fluorescence in-situ hybridisation or polymerase chain reaction have been published. This shows that the distinction between these tumours is based on morphological features alone, with ancillary techniques having little if any role to play.

3.9 Importance of a correct pre-operative diagnosis

Fibroadenomas and phyllodes tumours differ widely in their clinical behaviour, with fibroadenomas behaving in a benign fashion and phyllodes tumours having an inherent risk of local recurrence and metastasis (Rosen, 2008). According to Barth *et al* (1999), the recurrence rate for benign, borderline and malignant phyllodes tumours was 21, 46 and 65% respectively for tumours treated with local excision, decreasing to 8, 29 and 36% following wide local excision. This shows that the higher the grade of phyllodes tumour, the higher the likelihood of local recurrence, necessitating a wider initial excision, especially for malignant tumours. Furthermore, recurrent tumours tend to be of a higher grade (Jayaram *et al*, 2002), with an increased risk of chest wall invasion.

According to El Hag *et al* (2010), an accurate pre-operative diagnosis of phyllodes tumour is essential for planning surgery. Whereas fibroadenomas can be cured by enucleation, excision with clear surgical margins is required for phyllodes tumours (Komenaka *et al*, 2003). As reviewed by Jacklin *et al* (2006), a 1cm surgical margin is required for phyllodes tumours. This can usually be achieved by wide local excision, with mastectomy required for cases in which a 1cm margin is not possible (Parker *et*

al, 2001). According to Khosravi-Shahi (2011), negative surgical margins leads to improved disease-free survival and decreased local recurrence.

Recently, conservative management for fibroadenomas has been advocated (Rosen, 2008). In cases diagnosed as benign fibroadenomas (either by needle core biopsy (NCB) or FNA) and treated conservatively, clinical and mammographic follow-up is recommended, preferably at six-monthly intervals (Foxcroft *et al*, 2007).

The importance of an accurate pre-operative diagnosis is threefold. Firstly, to prevent a false diagnosis of fibroadenoma in a patient with a phyllodes tumour, which could lead either to the lesion being treated conservatively or excised with inadequate surgical margins, necessitating repeat wider excision. Secondly, to prevent misdiagnosis of phyllodes tumour in a patient with a fibroadenoma, leading to more extensive surgery and even mastectomy. Thirdly, to enable patients with phyllodes tumours to undergo surgery at an earlier date, minimising the risk of metastatic spread. As FNA is often used in the pre-operative diagnosis of these tumours, established, reproducible cytological criteria for distinguishing between fibroadenomas and phyllodes tumours are essential.

CHAPTER 4

4.0 Materials and methods

4.1 Study design

This study is a retrospective review of cytology and histology slides from patients with fibroadenomas or phyllodes tumours, identified from the archives of the Department of Anatomical Pathology, University of the Witwatersrand, Johannesburg, over a ten year period between January 2003 and December 2012.

4.2 Ethics

Ethics approval for the study was granted by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Ethics Clearance Certificate Number M131159 – Appendix 1).

4.3 Collection and recording of data

- A search of the laboratory information system database, DisaLab, at the National Health Laboratory Service (NHLS) Anatomical Pathology Department at the Johannesburg Hospital laboratory was undertaken. The search included cases of phyllodes tumour diagnosed between January 2003 and December 2012. The search terms used included “Breast, NOS (not otherwise specified)”, “Left breast”, “Right breast”, “Phyllodes tumor, NOS”, “Phyllodes tumor, benign” and

“Phyllodes tumor, malignant”. No search term for borderline phyllodes tumour was available for the DisaLab database. These cases were, however, identified using the “Phyllodes tumour, NOS” search term.

- A search of the DisaLab database of the Cytology Unit, NHLS in Johannesburg was then performed to search for patients with a histological diagnosis of phyllodes tumour who had undergone pre-operative breast FNA. The search terms as listed above were used.
- The cytology slides of these cases were retrieved from the Cytology Unit archives.
- Any patient with a histological diagnosis of phyllodes tumour for whom pre-operative FNA cytology slides were not available for review, was excluded from the study.
- The histology slides from the relevant cases of phyllodes tumour were then retrieved from the histology archives.
- A similar procedure was then followed for cases of fibroadenoma.
- The DisaLab search terms used to identify these cases included “Breast, NOS”, “Left breast”, “Right breast” and “Fibroadenoma, NOS”.
- There were a very large number of histologically-diagnosed fibroadenoma cases over the period January 2003 to December 2012. On the advice of a Biomedical statistician from the University of the Witwatersrand, only histologically-diagnosed cases of fibroadenoma between January 2012 to December 2012 were included in the study.
- Once the cases were identified, each case was assigned a random case number.

- Where available, the histology slides from each case were reviewed and the diagnosis confirmed using standard histological criteria (Tan *et al*, 2012).
- One suitable Papanicolaou-stained cytology slide was selected from each case. If more than one Papanicolaou-stained slide was available, the one with the most diagnostic material was selected for examination.
- The cases were randomised, ensuring that the diagnosis was not known to either investigator prior to reviewing the cytology slides.
- The cytology slides were reviewed concurrently by the candidate and supervisor using a double-header Olympus microscope and a consensus regarding the cytological features was reached.
- The following cytological features were evaluated for each case:
 - Number of stromal fragments in ten randomly-selected medium-power fields (10x eyepiece, 10x objective).
 - Number of epithelial fragments in ten randomly-selected medium-power fields.
 - Stromal to epithelial ratio.
 - Overall stromal cellularity – 1+, 2+, 3+. If the stromal cells were far apart from one another, with the stromal fragment appearing “pink” at low power, a cellularity of 1+ was applied. If the stromal fragment comprised cells showing nuclear overlap, and the fragment appeared “blue” at low power, a cellularity of 3+ was applied. Stromal cellularity of 2+ was applied to stromal fragments intermediate between these two extremes.
 - Stromal borders – well-defined or poorly-defined. If the stromal fragments had clean, crisp borders, and the separation of one fragment

from another was easy to visualise, the stromal borders were classified as well-defined. If the borders were irregular, and the separation of one fragment from another was not clearly visible, the borders were classified as poorly-defined.

- Stromal atypia – absent, mild, moderate or severe (based on the regularity of nuclear borders, variation in cell size, nuclear hyperchromasia and the presence of nucleoli).
- Proportion of wavy spindled cells in the background cellular population – <10%, 10-30%, >30%. Five high-power fields (10x eyepiece, 40x objective) away from any epithelial or stromal fragments were examined and the proportion of spindled cells estimated.
- The following additional variables from each case were recorded:
 - Age at diagnosis in years
 - Tumour laterality
 - Tumour size in centimetres
- Once each slide was reviewed, the data collection sheet was completed (Appendix 2).
- Once all the cases were reviewed blindly, the histological diagnosis was added to the data collection sheet and the data analysis performed (Section 4.6).
- Every third case (twenty cases in total) was selected for repeat microscopic examination by the candidate and supervisor using the same double-header microscope, in order to assess intra-observer variability.

4.4 Inclusion criteria

Patients with a histological diagnosis of phyllodes tumour between January 2003 and December 2012 and a histological diagnosis of fibroadenoma between January and December 2012 with a pre-operative FNA cytological diagnosis were included in the study.

4.5 Exclusion criteria

- Cases of patients for whom the cytology slides were not available for review.
- Patients for whom the pre-operative FNA was inadequate or not representative of the lesion. This could only be determined by examining the slides under the microscope during the course of the study.
- Patients for whom a definitive diagnosis of either fibroadenoma or phyllodes tumour could not be made histologically on the excision biopsy specimen.

4.6 Statistical analysis

- Statistical advice was sought from postgraduate students in Epidemiology and Biostatistics from the School of Public Health, University of the Witwatersrand prior to embarking on the study, during the study and for the final data analysis.
- The data was captured onto a spreadsheet using Microsoft Excel® 2003. Statistical analysis was performed using STATISTICA 12, StatSoft®, 2013

(www.statsoft.com) and R: A Language and Environment for Statistical Computing, R Core Team, Vienna, Austria, 2014 (www.R-project.org).

- Descriptive statistics (median and interquartile range) were used to describe the various groups, and the data represented using tables, histograms and box-and-whisker plots.
- Two groups of statistical analyses were performed. Firstly, fibroadenomas were compared with phyllodes tumours as a group, fibroadenomas were compared with benign phyllodes tumours (a point of particular importance as distinguishing between these two entities is notoriously difficult cytologically (Dusenbery *et al*, 1992; Deen *et al*, 1999; Krishnamurthy *et al*, 2000 and Ibrahim *et al*, 2001) and benign, borderline and malignant phyllodes tumours were compared with one another. Statistical tests used included the Mann-Whitney U test and Kruskal-Wallis analysis of variance (ANOVA) for continuous variables, and Fisher's exact two-tailed test for categorical variables (Dakhale *et al*, 2012). A p value of <0.05 was chosen to represent statistical significance.
- Secondly, the intra-observer variability was described using Bland and Altman plots for continuous variables and Kendall's coefficient of concordance for categorical variables (Petrie & Sabin, 2009).

CHAPTER 5

5.0 Results

5.1 Sample size

Seventy-seven cases were identified initially. Of these, ten (seven phyllodes tumours and three fibroadenomas) were excluded from the study based on the cytology slides. Three cases had no stroma for assessment, one had no epithelium for assessment, one represented granulomatous inflammation and one represented an intra-mammary lymph node. One case comprised free-lying stromal cells and large numbers of neutrophils with no epithelium, and one had scattered atypical cells with no stromal or epithelial fragments. The remaining two cases were excluded due to multiplicity of tumours in the same breast, leading to lack of certainty as to whether the pre-operative FNA was actually from the lesion which was eventually excised.

A total of 67 cases were included in the study. Of these, 50 were fibroadenomas (Figure 5.1) and 17 were phyllodes tumours. The phyllodes tumours included all the cases over the ten year study period (2003 to 2012) which had a pre-operative FNA and subsequent excision, whereas the fibroadenomas were cases from 2012 only. Of the 17 cases of phyllodes tumour, six were diagnosed histologically as benign (Figure 5.2), six as borderline (Figure 5.3) and five as malignant (Figure 5.4). The histology slides for 57 of the 67 cases were available for review. There was agreement with the original diagnosis in all 57 cases.



Figure 5.1: Excision specimen of a fibroadenoma showing a biphasic tumour composed of benign breast epithelial elements set in a hypocellular stroma devoid of atypia (Haematoxylin & Eosin (H&E), x40)



Figure 5.2: Excision specimen of a benign phyllodes tumour displaying the characteristic "leaf-like" architecture and mild stromal hypercellularity (H&E, x40)

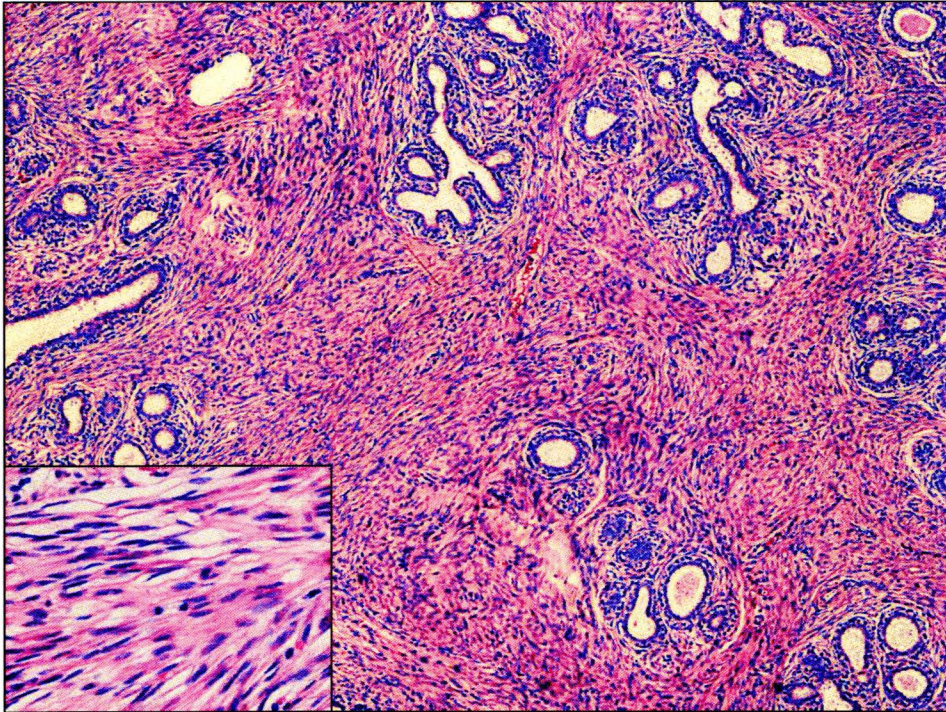


Figure 5.3: Excision specimen of a borderline phyllodes tumour showing breast epithelial elements set in a moderately cellular stroma (H&E, x40). Inset: Moderately atypical stromal cells with a mitotic figure seen centrally (H&E, x400)

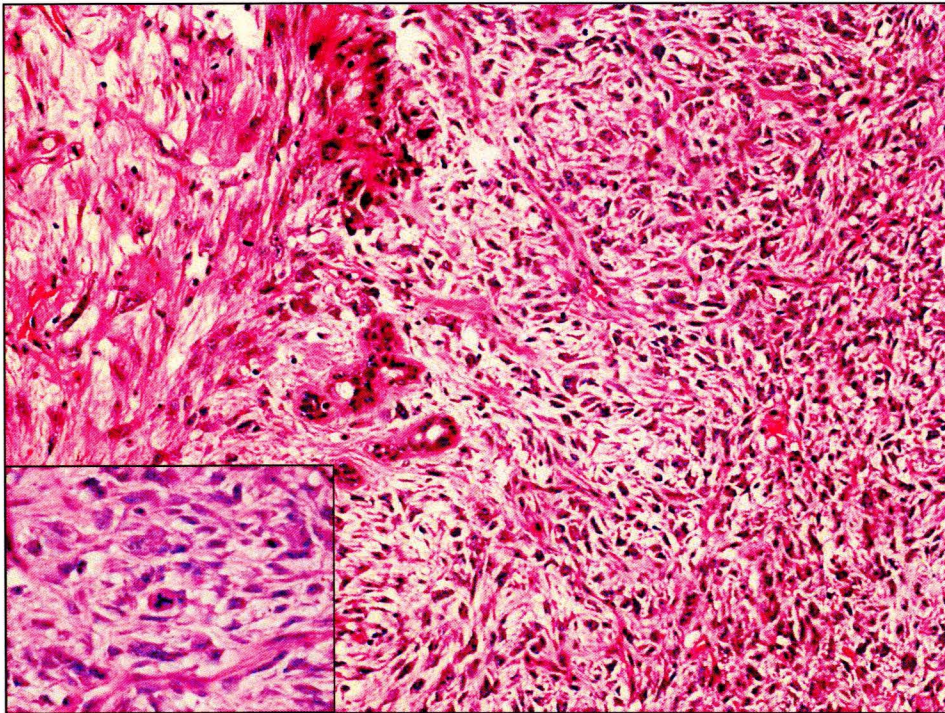


Figure 5.4: Excision specimen of a malignant phyllodes tumour showing benign epithelial tubules surrounded by markedly cellular, atypical stroma (H&E, x100). Inset: Marked stromal pleomorphism with an atypical mitotic figure (H&E, x400)

5.2 Age and laterality

The age of the study population ranged from 14 to 67 years, with a median age of 22 (interquartile range (IQR) = 19-40). The median age of fibroadenomas was 22 years (IQR = 19-33) and phyllodes tumours was 44 years (IQR = 23-55). The difference between these two groups was statistically significant (Mann-Whitney U test, $p=0.001$), with fibroadenomas occurring in a younger age group. There was no statistically significant difference between fibroadenomas and benign phyllodes tumours (Mann-Whitney U test, $p=0.399$) or between benign, borderline and malignant phyllodes tumours (Kruskal-Wallis ANOVA, $p=0.059$) with regard to age at diagnosis (Figure 5.5; Table 5.1). The histogram presented in Figure 5.5 does, however, indicate a trend towards older age in malignant phyllodes tumours.

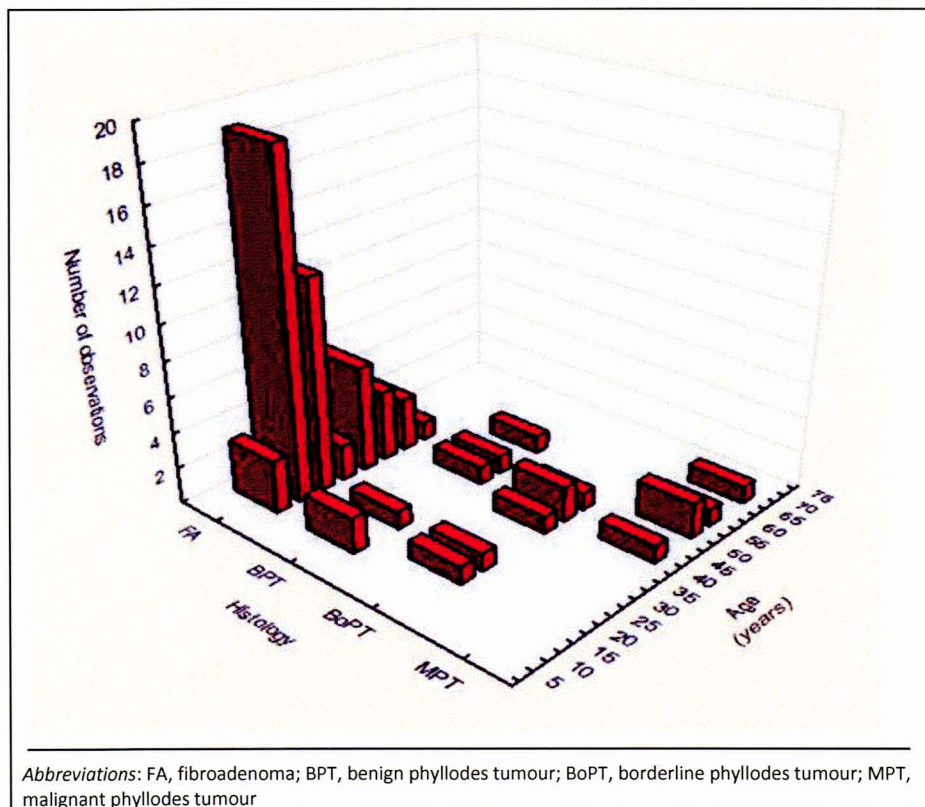


Figure 5.5: Histogram of age (in years) versus histological subtype

There were 25 left-sided and 25 right-sided fibroadenomas. Of the phyllodes tumours, eight were right-sided and nine were left-sided. Two benign phyllodes tumours were right-sided and four were left-sided, four borderline phyllodes tumours were right-sided and two were left-sided and two malignant phyllodes tumours were right-sided and three were left-sided.

5.3 Tumour size

Information regarding the size of the tumour (as measured macroscopically during routine processing) was available for 62 of the 67 cases. Fibroadenomas were significantly smaller than phyllodes tumours (Mann-Whitney U test, $p=0.011$). Fibroadenomas had a mean size of 4.0cm, whereas benign, borderline and malignant phyllodes tumours had a mean size of 4.5cm, 4.8cm and 10.5cm respectively. There was no statistically significant difference between the different groups of phyllodes tumours (Kruskal-Wallis ANOVA, $p=0.128$) or between fibroadenomas and benign phyllodes tumours (Mann-Whitney U test, $p=0.569$) with regard to tumour size. A summary of the demographic data, tumour size and laterality is provided in Figure 5.6 and Table 5.1.

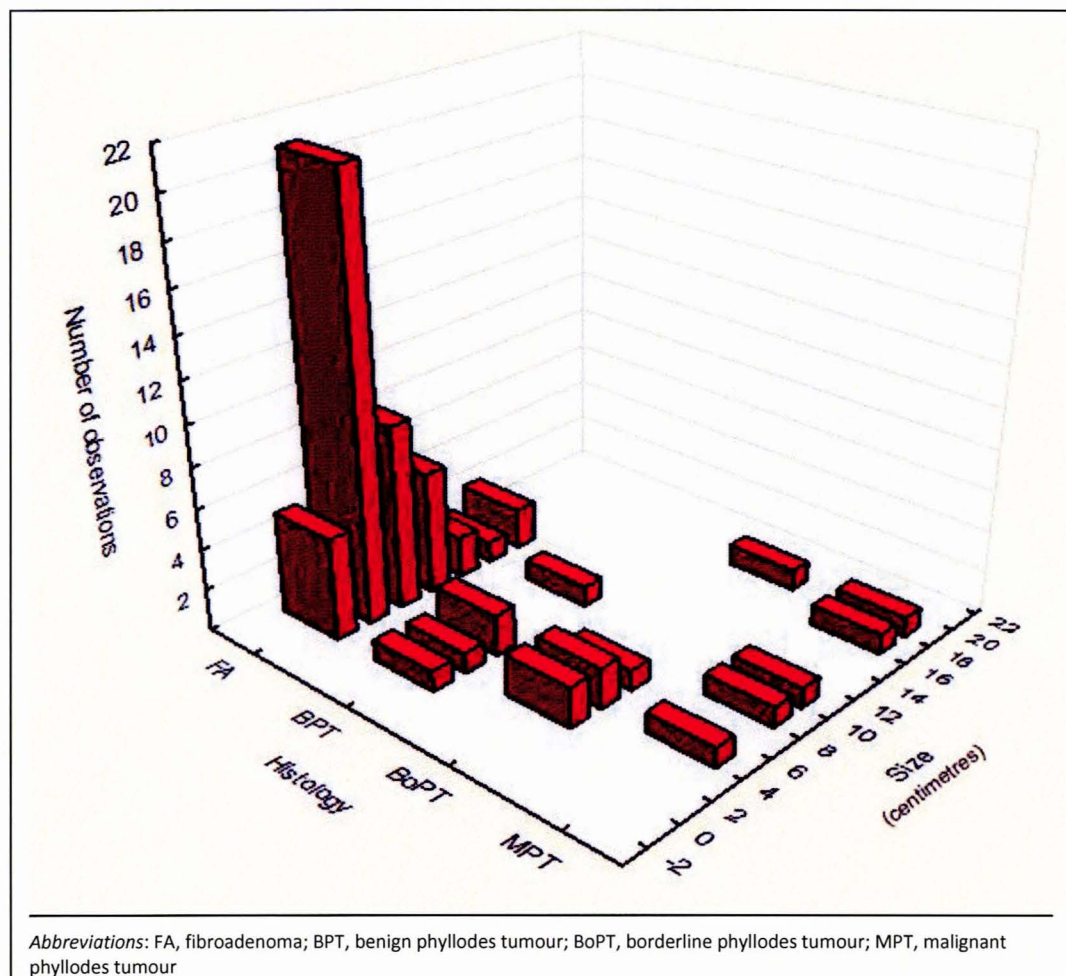


Figure 5.6: Histogram of size (in centimetres) versus histological subtype

Table 5.1: Summary of the patient demographics, tumour laterality and size

	All Cases (n=67)	FA (n=50)	PT (n=17)	BPT (n=6)	BoPT (n=6)	MPT (n=5)
Median age in years (IQR)	22 (19-40)	22 (19-33)	44 (23-55)	32.5 (15-48)	42 (22-45)	55 (55-57)
Median Size in cm (IQR)	4.5 (3.0-7.0)	4.0 (3.0-5.5)	5.5 (4.3-10.5)	4.5 (4.0-6.0)	4.8 (4.0-6.3)	10.5 (9.0-17.0)
Laterality	R=33 L=34	R=25 L=25	R=8 L=9	R=2 L=4	R=4 L=2	R=2 L=3
Abbreviations: FA, fibroadenoma; BPT, benign phyllodes tumour; BoPT, borderline phyllodes tumour; MPT, malignant phyllodes tumour						

5.4 Epithelial fragments, stromal fragments and the stromal to epithelial ratio

Fibroadenomas had significantly more epithelial fragments (Figure 5.7) and less stromal fragments (Figure 5.8) compared with phyllodes tumours (Mann-Whitney U

test, $p=0.000$ and $p=0.038$ respectively). The stromal to epithelial ratio of fibroadenomas was significantly less than phyllodes tumours (Mann-Whitney U test, $p=0.000$). A summary of the data collected is presented in Table 5.2 and Figures 5.9 to 5.11.

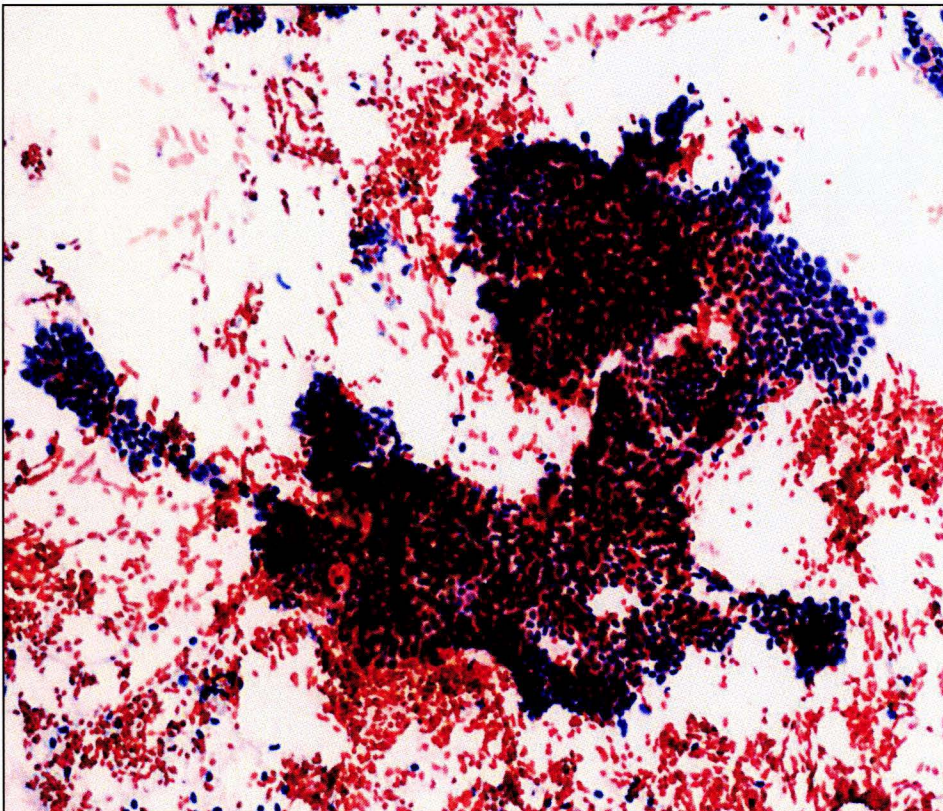


Figure 5.7: Fine needle aspirate of a fibroadenoma demonstrating epithelial fragments (Papanicolaou stain, x100)

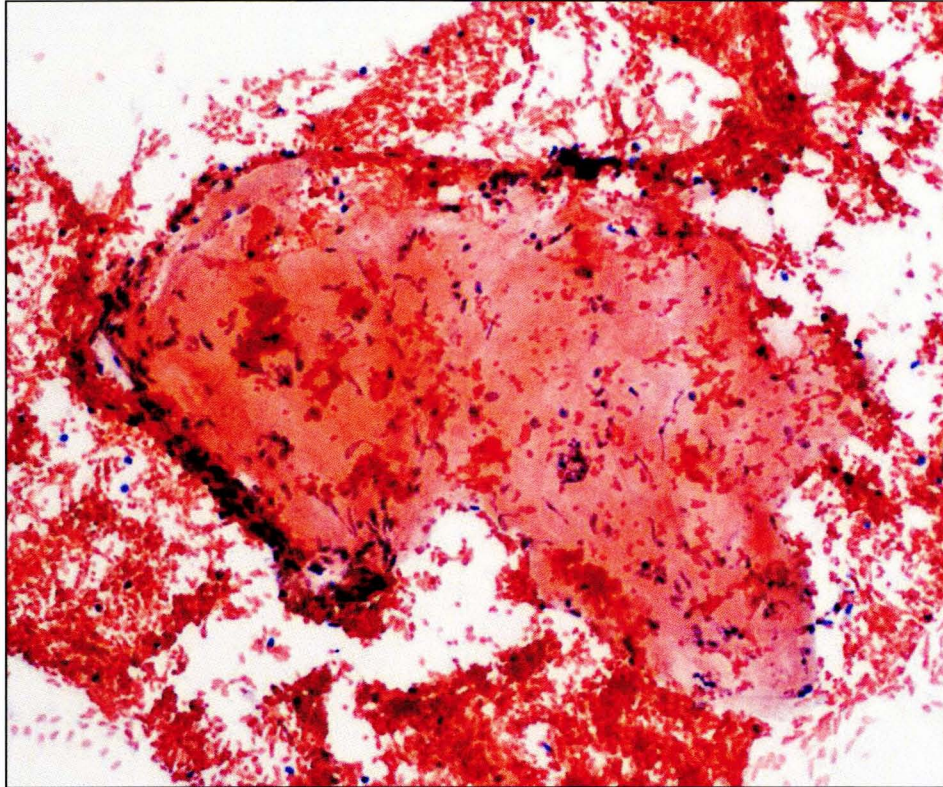


Figure 5.8: Fine needle aspirate of a stromal fragment in a fibroadenoma, showing low (1+) cellularity, absence of nuclear atypia and a well-defined border (Papanicolaou stain, x100)

Table 5.2: Summary of epithelial fragments, stromal fragments and stromal to epithelial ratio (per ten medium power fields)

	FA (n=50)	PT (n=17)	BPT (n=6)	BoPT (n=6)	MPT (n=5)
Median number of EF (IQR)	94.5 (52.5-125.2)	23.0 (18.0-53.0)	27.0 (18.5-63.3)	28.5 (23.3-62.3)	15.0 (9.0-19.0)
Median number of SF (IQR)	4.5(2.0-8.8)	7.0 (5.0-15.0)	8.5 (4.0-55.8)	5.5 (5.0-20.3)	9.0 (7.0-14.0)
Median SER (IQR)	0.048 (0.027-0.093)	0.250 (0.113-0.933)	0.119 (0.093-4.285)	0.234 (0.168-0.323)	0.778 (0.474-0.933)
<i>Abbreviations:</i> FA, fibroadenoma; PT, phyllodes tumour, BPT, benign phyllodes tumour; BoPT, borderline phyllodes tumour; MPT, malignant phyllodes tumour; EF, epithelial fragments; SF, stromal fragments; SER, stromal to epithelial ratio; IQR, interquartile range					

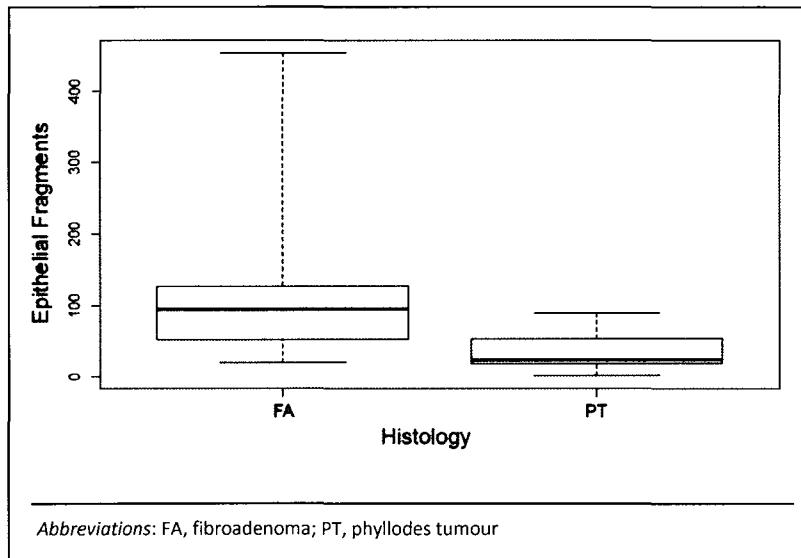


Figure 5.9: Box-and-whisker plot of number of epithelial fragments per ten medium-power fields (fibroadenoma versus phyllodes tumour)

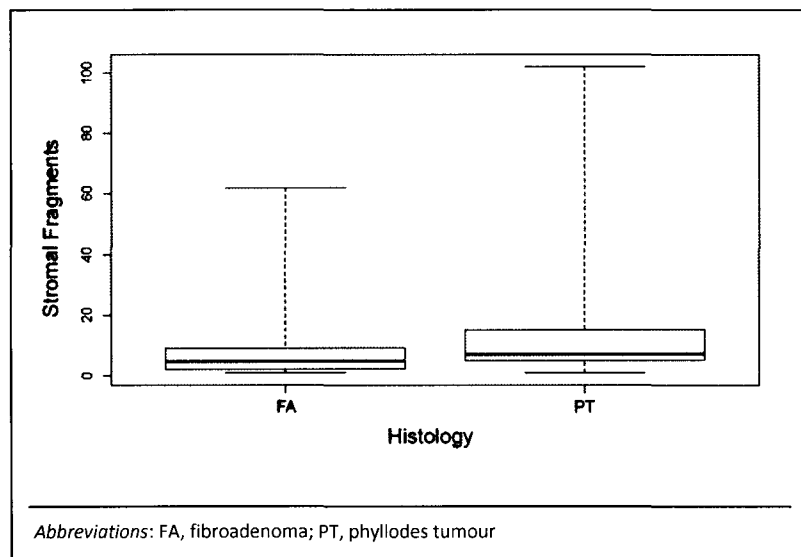


Figure 5.10: Box-and-whisker plot of number of stromal fragments per ten medium-power fields (fibroadenoma versus phyllodes tumour)

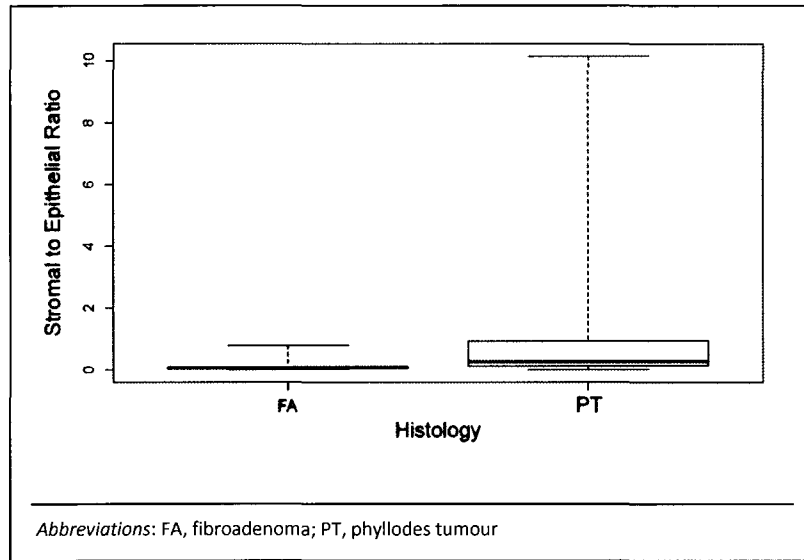


Figure 5.11: Box-and-whisker plot of stromal to epithelial ratio per ten medium-power fields (fibroadenoma versus phyllodes tumour)

When fibroadenomas were compared with benign phyllodes tumours, the former were found to have significantly more epithelial fragments and a lower stromal to epithelial ratio (Mann-Whitney U test, $p=0.003$ and $p=0.004$ respectively). The number of stromal fragments, however, was not significantly different (Mann-Whitney U test, $p=0.186$).

There were no statistically significant different differences between benign, borderline and malignant phyllodes tumours with regard to the number of epithelial fragments, stromal fragments or the stromal to epithelial ratio (Kruskal-Wallis ANOVA, $p=0.131$, $p=0.687$ and $p=0.446$ respectively).

5.5 Stromal cellularity

Stromal cellularity was graded as 1+ (Figure 5.8), 2+ (Figure 5.12) or 3+ (Figure 5.13). Fibroadenomas had significantly less cellular stroma when compared with both benign phyllodes tumours as well as phyllodes tumours as a group (Fisher's exact two-tailed test, $p=0.031$ and $p=0.001$ respectively). Phyllodes tumours, when compared with one another, showed no significant differences in the degree of stromal cellularity (Fisher's exact two-tailed test, $p=0.762$). This data is represented in Table 5.3.

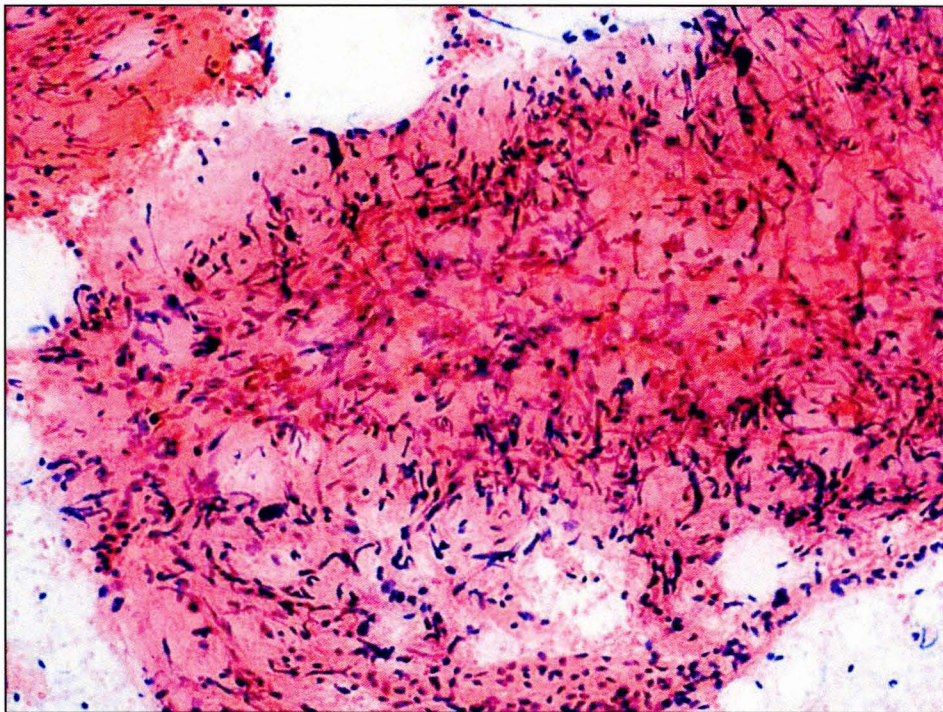


Figure 5.12: Fine needle aspirate of a borderline phyllodes tumour showing moderate (2+) stromal cellularity and mild nuclear atypia (Papanicolaou stain, x100)

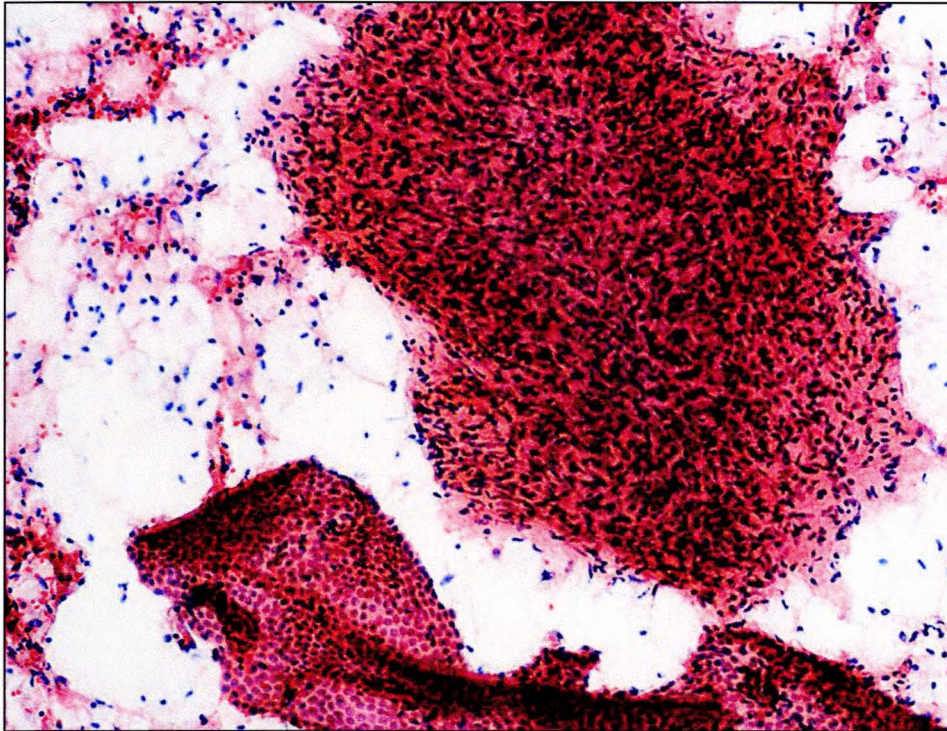


Figure 5.13: Fine needle aspirate from a benign phyllodes tumour showing marked (3+) stromal cellularity (Papanicolaou stain, x100)

Table 5.3: Number of cases grouped according to stromal cellularity

Stromal Cellularity	FA (n=50)	PT (n=17)	BPT (n=6)	BoPT (n=6)	MPT (n=5)
1+	30	3	2	0	1
2+	19	9	2	4	3
3+	1	5	2	2	1
<i>Abbreviations:</i> FA, fibroadenoma; BPT, benign phyllodes tumour; BoPT, borderline phyllodes tumour; MPT, malignant phyllodes tumour					

5.6 Stromal borders

Stromal borders were classified as well-defined (Figure 5.8) or poorly-defined (Figure 5.14). Using Fisher's exact two-tailed test, there were no significant differences between any of the groups with regard to the stromal borders (FA versus PT: $p=0.164$, FA versus BPT: $p=0.244$ and BPT versus BoPT versus MPT: $p=0.818$).

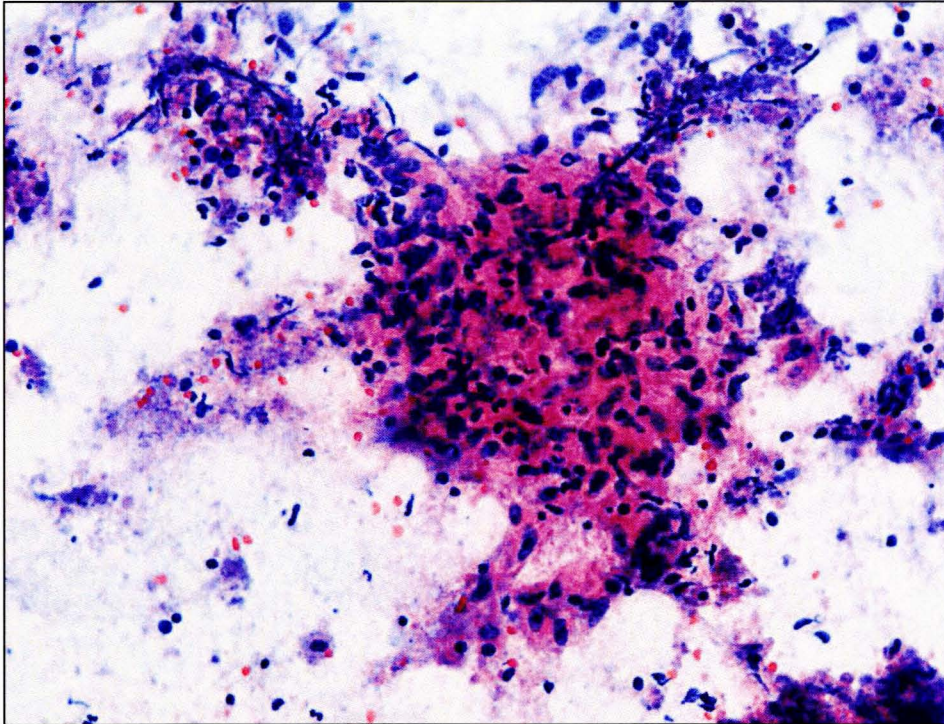


Figure 5.14: Fine needle aspirate of a benign phyllodes tumour demonstrating a poorly-defined stromal fragment with moderate (2+) cellularity and mild nuclear atypia (Papanicolaou stain, x200)

5.7 Stromal atypia

A number of criteria were assessed in order to classify the degree of atypia as absent (Figure 5.8), mild (Figure 5.14), moderate or severe (Figure 5.15), including regularity of nuclear borders, variation in cell size, nuclear hyperchromasia and the presence of nucleoli. The only statistically significant result was obtained when fibroadenomas were compared with phyllodes tumours, with the former having less atypia than the latter (Fisher's exact two-tailed test, $p=0.016$). There was no statistically significant difference between the different groups of phyllodes tumours or between fibroadenomas and benign phyllodes tumours with regard to the degree of atypia (Fisher's exact two-tailed test, $p=0.112$ and $p=0.127$ respectively). This data is represented in Table 5.4.

Table 5.4: Number of cases grouped according to the degree of atypia

Degree of Atypia	FA (n=50)	PT (n=17)	BPT (n=6)	BoPT (n=6)	MPT (n=5)
Absent	43	10	5	4	1
Mild	7	5	0	2	3
Moderate	0	0	0	0	0
Severe	0	2	1	0	1
<i>Abbreviations: FA, fibroadenoma; BPT, benign phyllodes tumour; BoPT, borderline phyllodes tumour; MPT, malignant phyllodes tumour</i>					

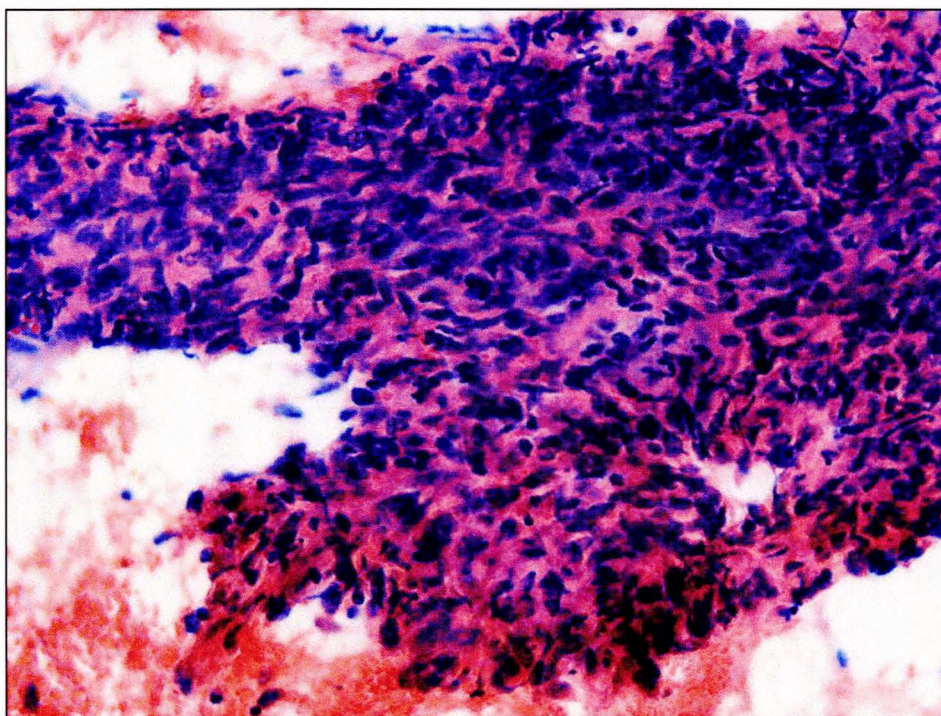


Figure 5.15: Fine needle aspirate of a malignant phyllodes tumour showing marked (3+) stromal cellularity and severe nuclear atypia. The nuclei are hyperchromatic and show nuclear pleomorphism, irregularity of the nuclear borders and visible nucleoli (Papanicolaou stain, x200)

5.8 Proportion of background spindled cells

The proportion of wavy spindled free-lying stromal cells in the background cellular population was categorised as follows: less than 10% (Figure 5.16), 10 to 30% (Figure 5.17) and greater than 30% (Figure 5.18). Phyllodes tumours had a statistically

greater number of these background cells than fibroadenomas (Fisher's exact two-tailed test, $p=0.049$). Furthermore, significant differences were obtained between the three groups of phyllodes tumour, with the number of background spindled cells being directly proportional to the grade of the tumour (Fisher's exact two-tailed test, $p=0.015$). Interestingly, this was the only statistically significant result obtained amongst all the variables studied when the three groups of phyllodes tumours were compared with one another. There was no significant difference between fibroadenomas and benign phyllodes tumours with regard to the background spindle cell population (Fisher's exact two-tailed test, $p=0.620$). This data is represented in Table 5.5.

Table 5.5: Proportion of background spindled cells by histological subtype

Proportion of background spindled cells	FA (n=50)	PT (n=17)	BPT (n=6)	BoPT (n=6)	MPT (n=5)
< 10%	40	9	6	2	1
10-30%	9	6	0	4	2
> 30%	1	2	0	0	2
<i>Abbreviations:</i> FA, fibroadenoma; BPT, benign phyllodes tumour; BoPT, borderline phyllodes tumour; MPT, malignant phyllodes tumour					

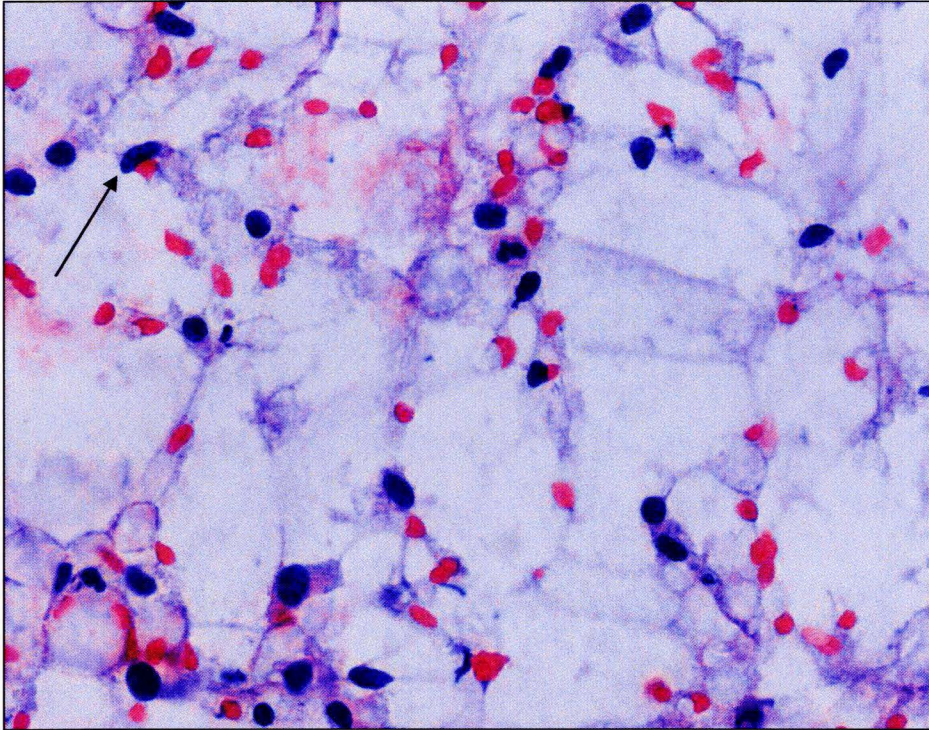


Figure 5.16: Fine needle aspirate of a fibroadenoma showing a background cellular population comprising less than 10% spindled cells (arrow) (Papanicolaou stain, x400)

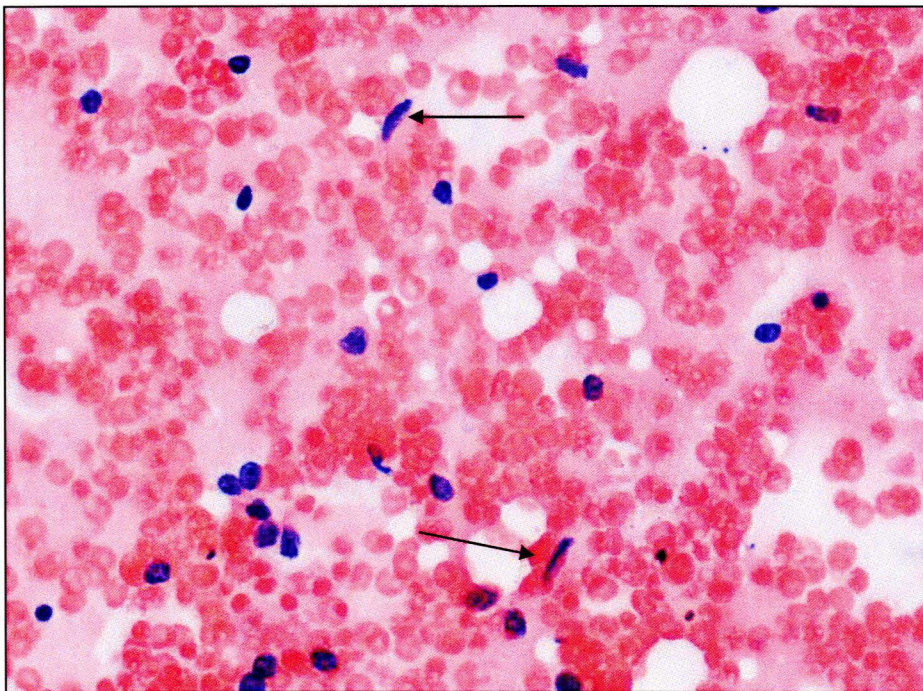


Figure 5.17: Fine needle aspirate of a fibroadenoma showing a background cellular population comprising 10-30% spindled cells (arrows) (Papanicolaou stain, x400)

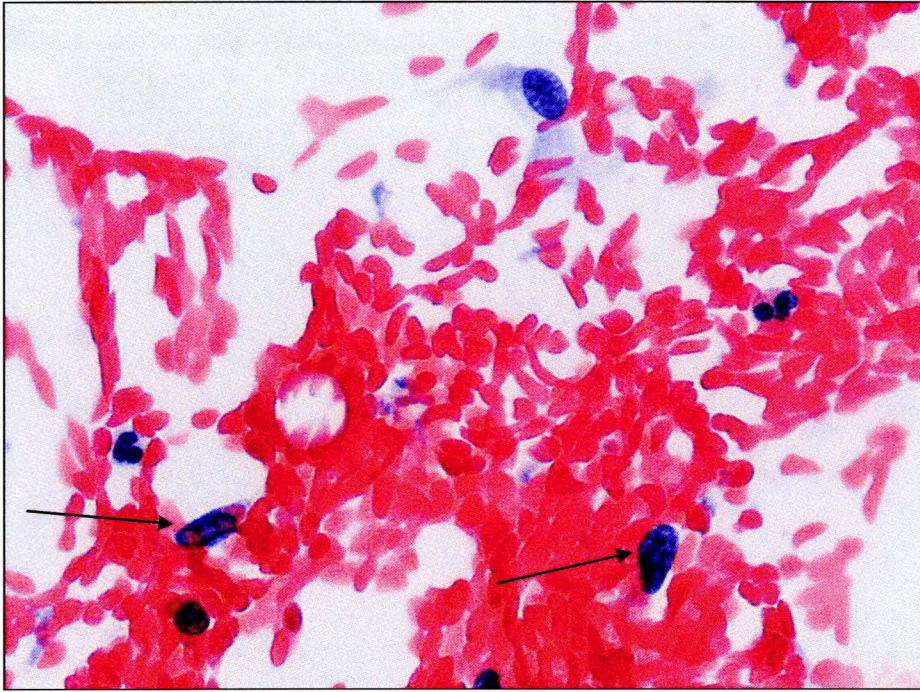


Figure 5.18: Fine needle aspirate of a malignant phyllodes tumour showing a background cellular population comprising greater than 30% spindled cells (arrows). Scattered neutrophils are present (Papanicolaou stain, x400)

5.9 Intra-observer variability

Of the 67 cases examined initially, 20 were randomly selected for repeat examination by the candidate and supervisor. The histological diagnosis of these cases was blinded to both observers. The intra-observer variability for the continuous variables is illustrated below (Figures 5.19 to 5.21) using Bland and Altman plots. The closer the data points are to one another, the better the concordance. Similarly, the fewer the number of data points which fall outside the 95% confidence limits (red horizontal lines on the figures), the better the concordance (Petrie & Sabin, 2009).

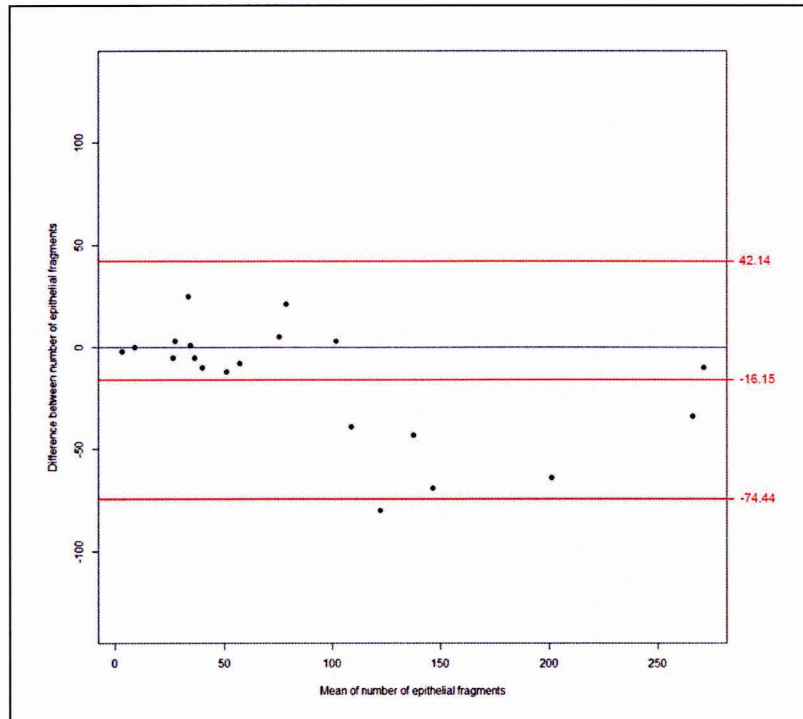


Figure 5.19: Bland and Altman plot – intra-observer variability of epithelial fragments

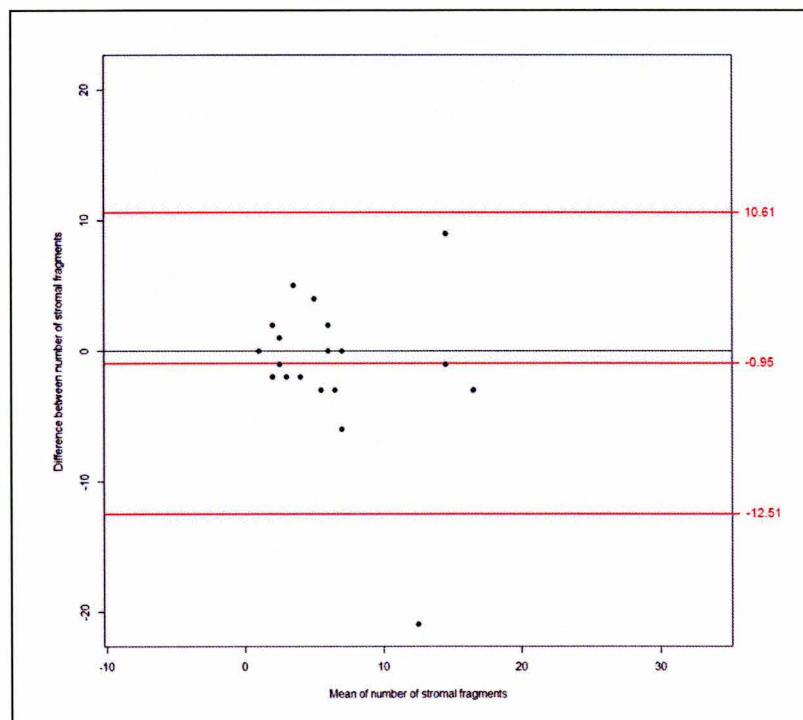


Figure 5.20: Bland and Altman plot – intra-observer variability of stromal fragments

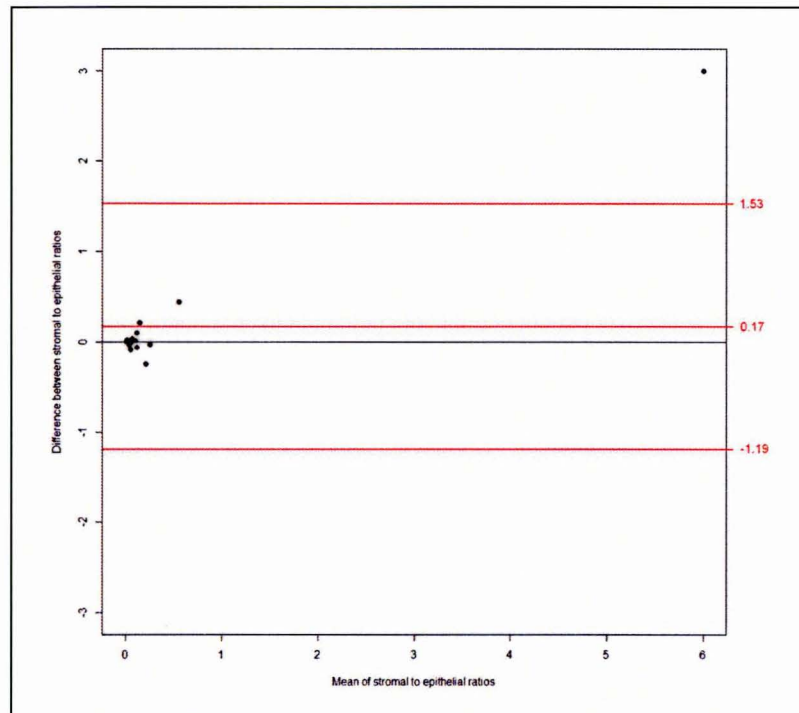


Figure 5.21: Bland and Altman plot – intra-observer variability of stromal to epithelial ratio

For categorical variables, Kendall’s coefficient of concordance was used to assess the intra-observer variability. The closer the number is to one, the better the concordance. These results are displayed in Table 5.6.

Table 5.6: Kendall’s coefficient of variance for categorical variables

Variable	Kendall’s coefficient of concordance
Stromal borders	0.140
Stromal cellularity	0.782
Stromal atypia	0.762
Proportion of background spindled cells	0.709

CHAPTER 6

6.0 Discussion

6.1 Introduction

Fibroadenomas and phyllodes tumours are both fibroepithelial breast lesions. Whereas fibroadenomas are benign, phyllodes tumours range from benign to malignant, with a borderline category falling between these two extremes (Rosen, 2008). Fibroadenomas can be treated conservatively or can be enucleated, with no risk of recurrence or metastasis (Rosen, 2008). Phyllodes tumours, on the other hand, require excision with clear surgical margins, as these tumours are prone to local recurrence and even distant metastasis (Barth *et al*, 1999 and Komenaka *et al*, 2003). FNA is often used as the initial diagnostic investigation in the evaluation of these lesions. Given the different prognosis and surgical management of these tumours, accurate pre-operative diagnosis using FNA is of the utmost importance.

The first part of the discussion considers the findings of the current study while the next part of the chapter is devoted to the comparison of the results of this study to other reports regarding the cytological discrimination of fibroadenomas and phyllodes tumours. The limitations of the current study and suggestions for further research end the discussion.

6.2 Major findings

Several articles have looked at various criteria to distinguish fibroadenoma from phyllodes tumour. In the current study, only the most useful criteria gleaned from the literature were studied, namely number of epithelial and stromal fragments, stromal to epithelial ratio, degree of atypia, regularity of the stromal borders, stromal cellularity and proportion of background spindled cells.

The current study has shown that some statistically significant differences exist between fibroadenomas and phyllodes tumours as a whole (without differentiating benign, borderline or malignant phyllodes), both from a demographic and cytological point of view. Fibroadenomas tended to occur in a younger age group and were smaller at excision than phyllodes tumours. Overlap between these tumours with regards to age and tumour size exists, however, as demonstrated by the current study. The largest fibroadenoma measured 13.5cm, and the smallest phyllodes tumour measured 2.0cm. Furthermore, the oldest patient with a fibroadenoma was 46, and the youngest patient with a phyllodes tumour was 15. This indicates that patient age and tumour size cannot be heavily relied upon when trying to distinguish between fibroadenomas and phyllodes tumours. When compared with phyllodes tumours, fibroadenomas had a larger number of epithelial fragments, a smaller number of stromal fragments and a lower stromal to epithelial ratio. The stroma tended to be less cellular and less atypical compared with phyllodes tumours and the background cellular population contained less spindled cells.

A fewer number of significant differences were demonstrated between fibroadenomas and benign phyllodes tumours. Fibroadenomas had more epithelial fragments and a lower stromal to epithelial ratio; however, the number of stromal fragments was not significantly different. The stromal cellularity of benign phyllodes tumour was greater than fibroadenoma. There were no significant differences between the groups with regard to age, size, stromal atypia or the proportion of background spindled cells.

When the three groups of phyllodes tumours (benign, borderline and malignant) were compared with one another, the only difference which reached statistical significance was the proportion of background spindled cells, which was directly proportional to the grade of the tumour. Six benign phyllodes tumours had <10% background spindled cells, with no tumours having a proportion of background spindled cells greater than 10%. Of the borderline phyllodes tumours, two had less than 10% background spindled cells, four had 10-30% and none had greater than 30%. Of the malignant phyllodes tumours, one tumour had less than 10% background spindled cells, two had 10-30% and two had greater than 30%. These results suggest that, in a phyllodes tumour, the finding of less than 10% spindled cells points to a diagnosis of a benign phyllodes tumour. Similarly, the finding of greater than 30% background spindled cells suggests a diagnosis of a malignant phyllodes tumour. Making this distinction is important from a practical point of view as, whilst all grades of phyllodes tumour have the potential for local recurrence, only the malignant tumours have the ability to metastasise (Tan *et al*, 2012).

The variable which proved to be the least useful when comparing the different tumour groups was that of stromal borders (well-defined or poorly-defined), with no statistically significant difference obtained in any of the comparisons performed, as well as poor intra-observer variability.

6.3 Comparison with previous studies

6.3.1 Epithelial fragments

A number of previous studies compared fibroadenomas and phyllodes tumours with regard to the features of their epithelial fragments (Deen *et al*, 1999; Krishnamurthy *et al*, 2000; Scolyer *et al*, 2001; Bandyopadhyay *et al*, 2010 and El Hag *et al*, 2010). The features studied included epithelial fragment architecture, hyperplasia, atypia, apocrine and squamous metaplasia, presence of overlapping nuclei and myoepithelial cells, degree of epithelial dissociation and mitotic activity. These authors found that the features of the epithelial fragments were unhelpful in distinguishing between these tumours. This is unsurprising, given that the histological features used in this distinction focus on the stromal rather than the epithelial component (Tan *et al*, 2012). It is for this reason that evaluation of the epithelium was not performed in the current study, but rather features relating to the stroma and background cellular population were assessed.

6.3.2 Stromal cellularity

As stated in Chapter 3, the presence of hypercellular stroma was one of the most useful distinguishing features between fibroadenomas and phyllodes tumours in many of the studies on the topic (Rama Rao *et al*, 1992; Shimizu *et al*, 1994; Shabb, 1997; Simi *et al*, 1988; Bhattarai *et al*, 2000; Krishnamurthy *et al*, 2000; Scolyer *et al*, 2001; Jayaram *et al*, 2002 and Bandyopadhyay *et al*, 2010). The results from the current study are consistent with these findings, with benign phyllodes tumour and phyllodes tumours as a group having more cellular stroma than fibroadenomas. This is in contrast to the findings of Veneti *et al* (2001), a study in which benign phyllodes tumours were compared with fibroadenomas. This group found that stromal fragments in phyllodes tumours were larger, but the cellularity and number were not significantly different between the two groups. Differences between this study and the current study are that, in the former, no borderline or malignant phyllodes tumours were included, and the number of fibroadenomas studied was significantly less (18 versus 50). Importantly, in the study by Krishnamurthy *et al* (2000), up to 30% of fibroadenomas showed the presence of hypercellular stromal fragments, indicating that this features cannot be used unequivocally as evidence that one is dealing with a phyllodes tumour. In the current study, 19 of 50 cases of fibroadenoma (38%) showed stromal cellularity of 2+ and a single case (2%) showed 3+ stromal cellularity.

6.3.3 Number of stromal and epithelial fragments

In the studies by Veneti *et al* (2001), Jayaram *et al* (2002), Bandyopadhyay *et al* (2010) and El Hag *et al* (2010), phyllodes tumours were found to have an increased number of stromal fragments compared with fibroadenomas. In addition, these fragments were noted to be larger in the former. Whilst the size of the stromal fragments was not measured in the current study, it was found that phyllodes tumours had relatively more stromal fragments than fibroadenomas. In addition, phyllodes tumours as a group as well as benign phyllodes tumours in isolation had significantly less epithelial fragments and a higher stromal to epithelial ratio than fibroadenomas. Other studies which examined stromal to epithelial ratios include those by Rama Rao *et al* (1992), Bhattarai *et al* (2000) and Bandyopadhyay *et al* (2010). In the third of these studies, this ratio was found to be a useful discriminating factor between fibroadenomas and phyllodes tumours, and in the second it proved useful in distinguishing between the three different grades of phyllodes tumour. In the study by Rama Rao *et al* (1992), on the other hand, the use of the epithelial to stromal ratio did not prove useful in distinguishing between benign and borderline phyllodes tumours, both of which had ratios of greater than one. A weakness of this study is that only two borderline phyllodes tumours were included.

A further two studies (Krishnamurthy *et al*, 2000 and El Hag *et al*, 2010) looked at the number of epithelial and stromal fragments per slide, both classifying the number as greater than or less than five. Neither group found these measurements to be a useful discriminating feature between the two tumours. In the current study, a more accurate approach was used, counting the number of epithelial and stromal

fragments in ten medium-power fields (10x eyepiece, 10x objective) and working out the ratio between the two. A combination of ten fields with the use of a medium-power objective was chosen for practical reasons, as stromal and epithelial fragments were easily visible at this power, enabling their accurate enumeration. No other previous study has used this method of calculation, which, whilst tedious, could realistically be applied to routine cytopathological practice.

6.3.4 Proportion of background spindled cells

A number of studies (Stanley *et al*, 1989; Deen *et al*, 1999; Krishnamurthy *et al*, 2000; Jayaram *et al*, 2002; Bandyopadhyay *et al*, 2010 and El Hag *et al*, 2010) examined the background cellular population for the presence of spindled cells, which were found to be increased in cases of phyllodes tumour. In the study by Krishnamurthy *et al* in 2000, the number of spindled cells was divided into three groups: <10%, 10-30% and >30%, with >30% spindled cells seen only in cases of phyllodes tumour. In the current study, >30% spindled cells was seen in a single case of fibroadenoma (2%) as well as two phyllodes tumours (12%), both of these being malignant. Krishnamurthy and colleagues state that, if the number of spindled cells is <10%, a diagnosis of fibroadenoma is likely and that a diagnosis of phyllodes tumour should be made if the number is >30%, with 10-30% representing an intermediate zone. El Hag *et al* (2010) argue that using a cut-off of background spindled cells of 10% as opposed to 30% would increase the sensitivity of diagnosis of phyllodes tumour and decrease the possibility of misdiagnosing phyllodes tumours as fibroadenomas. In the current study, ten (20%) fibroadenomas showed background

spindled cells of 10% or greater, meaning that these cases would be misdiagnosed as phyllodes tumours if a cut-off of 10% as proposed by El Hag was to be used. It therefore seems that the categories proposed by Krishnamurthy and colleagues are a better discriminator of these tumours, and that the 10% cut-off proposed by El Hag appears to be too low. Further studies clarifying this point are warranted.

A difficulty encountered during the current study was that of accurately determining the proportion of background spindled cells. Personal correspondence with one of the authors of the study by Krishnamurthy *et al* (2000) unfortunately never resolved the issue. In the current study, five high-power fields (10x eyepiece, 40x objective) away from any epithelial or stromal fragments were examined and the proportion of spindled cells estimated. This was a relatively easy method which could be applied to everyday practice. The use of automated cell counting would provide the most accurate result, but this method is not readily available outside the research setting. Again, further studies in this regard are needed.

6.3.5 Stromal atypia

In this study, the assessment of stromal atypia only showed statistically significant differences between fibroadenomas and phyllodes tumours as a group, with the latter having greater degrees of atypia than the former. Stromal atypia was found to be a useful feature in the studies by Rama Rao *et al* (1992); Bhattarai *et al* (2000); Scolyer *et al* (2001) and El Hag *et al* (2010). A problem common to all these studies was that no strict criteria were provided to assist with the subclassification of atypia.

In the current study, none of the cases showed moderate atypia, possibly highlighting the difficulties associated with a three-tiered grading system. In retrospect, it might have been easier and more reproducible to group atypia into absent, low grade and high grade. This is a practice which is not unique to fibroepithelial lesions, and has been used successfully in many other areas of cytopathology, the assessment of cervical smears being one of the most common examples.

According to Dusenbery *et al* (1992), the diagnosis of malignant phyllodes tumour is relatively straightforward in the presence of benign epithelial fragments together with clearly malignant stromal fragments showing marked atypia and mitotic activity. In the current study, of the five cases of malignant phyllodes tumour, only one (20%) showed severe atypia of the stroma. Instead, it appears that the *lack* of atypia was significant, with none of the fibroadenomas showing moderate or severe atypia and only seven (14%) showing mild atypia.

6.3.6 Stromal borders

Both Scolyer *et al* (2001) and Bandyopadhyay *et al* (2010) showed that stromal fragments with well-defined borders were present in phyllodes tumours. Unfortunately, neither of these authors defined exactly what is meant by well-defined and poorly-defined. In the current study, there was no statistically significant correlation between tumour type and the border of the stromal fragments. Furthermore, this feature was associated with poor intra-observer variability

(Kendall's coefficient of concordance = 0.140), possibly resulting from a lack of defined criteria to classify a stromal fragment as having a poorly-defined or well-defined border.

6.4 Intra-observer variability

For the continuous variables (number of epithelial fragments, number of stromal fragments and stromal to epithelial ratio), intra-observer variability was illustrated using Bland and Altman plots (see Figures 5.6 to 5.8). These variables show good intra-observer variability, with only a single outlier falling outside of the 95% confidence limits in each case. For the categorical variables (stromal borders, stromal atypia, stromal cellularity and proportion of background spindled cells), the Kendall's coefficient of concordance (W) was calculated. The closer this number is to one, the better the intra-observer variability. As stated above, the intra-observer variability of the stromal borders was poor, with a W number of 0.140. The other three variables, however, showed good intra-observer variability, with the W numbers ranging from 0.709 to 0.782.

6.5 Study weaknesses

The major weakness of this study, as well as many of the studies quoted, is the low number of phyllodes tumours. Bhattarai *et al* (2000), Jayaram *et al* (2000) and Stanley *et al* (1989) had 80, 28 and 23 phyllodes tumours in their studies respectively. They did, however, not include any fibroadenomas. Of the remaining

studies, eight had between ten and 20 phyllodes tumours, and four had less than ten of these tumours. The reason for these low numbers is that phyllodes tumours as a group are rare, comprising only 1% of all breast tumours (Parker *et al*, 2001). In the current study, there were 17 phyllodes tumours, a number comparable to many of the other similar studies.

A second weakness is the lack of well-defined criteria and methods used to assess some cytological features, including the number of stromal and epithelial fragments, the proportion of background stromal cells, stromal cellularity and atypia. This makes comparison between the various studies difficult, and negatively impacts the intra-observer variability.

Due to the design of the study, the candidate and supervisor assessed the cytology slides together using a double-header microscope. Whilst this allowed for a consensus to be reached concerning the various elements studied, possibly decreasing bias, this also meant that calculation of inter-observer variability was not possible.

A further weakness of the study is that phyllodes tumours from a ten year period (2003 to 2012) were examined, whereas only fibroadenomas from a single year (2012) were included. This was done for practical reasons as the number of fibroadenomas diagnosed during the full ten year period was very large. Randomly selecting fibroadenomas from this period would potentially have been possible. However, given the need for each patient to have had both a fine needle aspirate

and subsequent tumour excision, this selection process would have been extremely time-consuming. It is possible that an element of bias could have been introduced by only including fibroadenomas from a single year, especially if there has been a gradual change in presentation of women with these tumours over the past decade.

Finally, this study has attempted to identify differences between benign, borderline and malignant phyllodes tumours. A problem inherent to these tumours is that they are not separate disease entities, but rather represent a histological (and cytological) spectrum (Parker *et al*, 2001). Histology is the current gold standard for the diagnosis of these lesions. Even though potential histological overlap between these lesions exists, the final histological assessment of the excision specimen has to be relied upon for the purposes of comparative studies.

6.6 Suggestions for future research

The aim of this study was to see if any statistically significant differences exist with regard to the cytological features of fibroadenomas and phyllodes tumours. The next step is to apply these criteria to randomised cases (either retrospectively or prospectively) to assess whether they aid in making an accurate diagnosis. Furthermore, each of these cytomorphological criteria needs to be strictly defined to allow for uniformity across the literature.

As stated above, a major disadvantage in this area of study is the low number of phyllodes tumours available. One way to overcome this problem is to perform large

collaborative studies, pooling the diagnostic material and resources of multiple academic centres, leading to more robust results.

Lastly, studies using automated cell counting techniques should be performed, as these, if incorporated into routine practice, could increase the speed and accuracy of the relevant determinations and, in doing so, increase the diagnostic accuracy when dealing with fibroepithelial lesions.

CHAPTER 7

7.0 Conclusion

FNA remains one of the first-line investigations in patients with palpable and non-palpable breast lesions (Krishnamurthy *et al*, 2000, Rosa *et al*, 2012 and Smith *et al*, 2012). Compared with needle core biopsy (NCB), FNA is cheaper to perform, less invasive, associated with fewer complications and samples more of the lesion (Nassar, 2011; Simsir *et al*, 2012 and Nagar *et al*, 2012). According to a recent article by Smith *et al* in 2012, FNA is a vital component of the so-called triple test, which refers to the combination of clinical findings, radiology (ultrasound or mammogram) and biopsy, either in the form of an FNA or NCB. In a resource-limited setting, such as exists in the state sector of South Africa, FNA is an invaluable tool and the accuracy thereof has great management implications for these patients.

As fibroadenomas and phyllodes tumours cannot be distinguished from one another with certainty on clinical or radiological grounds (Page *et al*, 1991; Chao *et al*, 2002 and Rosen, 2008), biopsy (either for histology or cytology) assumes a very important role. The current study has focused on a variety of cytological features, with a view to determine whether there are any statistically significant differences between the various groups of fibroepithelial lesions of the breast. The best criteria from previous studies were chosen for assessment. The features which have shown the greatest differences include the number of epithelial and stromal fragments, the stromal to epithelial ratio, stromal cellularity, stromal atypia and the proportion of background

spindled cells. In addition, the data obtained is in agreement with the long-held view that phyllodes tumours tend to occur in older women and are larger at presentation than fibroadenomas (Rosen, 2008). It will be exciting to apply these cytological criteria prospectively to help differentiate fibroadenoma from phyllodes tumour, thereby enhancing patient management.

References

- Awad, F.M. & Alsaeed, M. 2009. Can Breast MRI Differentiate Phyllodes Breast Tumors from Fibroadenomas? *Med J Cairo Univ* 77(1):231-35.
- Bandyopadhyay, R., Nag, D., Mondal, S.K., Mukhopadhyay, S., Roy, S. & Sinha, S.K. 2010. Distinction of phyllodes tumour from fibroadenoma: Cytologists' perspective. *J Cytol* 27(2):59-62.
- Barth, R.J. 1999. Histologic features predict local recurrence after breast conserving therapy of phyllodes tumors. *Breast Cancer Res Tr* 57(3):291-95.
- Bhattarai, S., Kapila, K. & Verma, K. 2000. Phyllodes tumor of the breast: A cytohistologic study of 80 cases. *Acta Cytol* 44(5):790-6.
- Chao, T.C., Lo Y.F., Chen, S.C. & Chen, M.F. 2002. Sonographic features of phyllodes tumors of the breast. *Ultrasound Obstet Gynecol* 20(1):64-71.
- Dakhale, G.N., Hiware, S.K., Shinde, A.T. & Mahatme, M.S. 2012. Basic biostatistics for post-graduate students. *Indian J Pharmacol* 44(4):435-42.
- Deen, S.A., McKee, G.T. & Kissin, M.W. 1999. Differential Cytologic Features of Fibroepithelial Lesions of the Breast. *Diagn Cytopathol* 20(2):53-56.
- Dusenbery, D. & Frable, W.J. 1992. Fine needle aspiration cytology of phyllodes tumor: Potential diagnostic pitfalls. *Acta Cytol* 36(2):215-21.
- El Hag, I.A., Aodah, A., Kollur, S.M., Attallah, A., Mohamed, A.A.E. & Al-Hussaini, H. 2010. Cytological Clues in the Distinction Between Phyllodes Tumor and Fibroadenoma. *Cancer Cytopathol* 118(1):33-40.
- Foxcroft L.M., Evans E.B. & Porter A.J. 2007. Difficulties in the pre-operative diagnosis of phyllodes tumours of the breast: A study of 84 cases. *Breast* 16(1):27-37.
- Gatta, G., Iaselli, F., Parlato, V., Di Grezia, G., Grassi, R. & Rotondo, A. 2011. Differential diagnosis between fibroadenoma, giant fibroadenoma and phyllodes tumour: sonographic features and core needle biopsy. *Radiol Med* 116(6):905-18.
- Giri, D. 2009. Recurrent Challenges in the Evaluation of Fibroepithelial Lesions. *Arch Pathol Lab Med* 133(5):713-21.
- Ibrahim, A.E.K., Bateman, A.C., Theaker, J.M., Low, J.L., Addis, B., Tidbury, P., Rubin, C., Briley, M. & Royle, G.T. 2001. The role and histological classification of needle core biopsy in comparison with fine needle aspiration cytology in the preoperative assessment of impalpable breast lesions. *J Clin Pathol* 54(2):121-5.

Jacklin, R.K., Ridgway, P.F., Ziprin, P., Healy, V., Hadjiminis, D. & Darzi, A. 2006. Optimising preoperative diagnosis in phyllodes tumour of the breast. *J Clin Pathol* 59(5):454-9.

Jayaram, G. & Sthaneshwar, P. 2002. Fine-Needle Aspiration Cytology of Phyllodes Tumors. *Diagn Cytopathol* 26(4):222-7.

Khosravi-Shahi, P. 2011. Management of non metastatic phyllodes tumors of the breast: Review of the literature. *Surg Oncol* 20(4):e143-8. Available <http://www.ncbi.nlm.nih.gov/pubmed/21609854> [Accessed 31/07/2013].

Komenaka I.K., El-Tamer, M., Pile-Spellman, E. & Hibshoosh, H. 2003. Core Needle Biopsy as a Diagnostic Tool to Differentiate Phyllodes Tumor from Fibroadenoma. *Arch Surg* 138(9):987-90.

Krishnamurthy, S., Ashfaq, R., Shin, H.J.C. & Sneige, N. 2000. Distinction of Phyllodes Tumor from Fibroadenoma: A Reappraisal of an Old Problem. *Cancer* 90(6):342-9.

Nagar, S., Iacco, A., Riggs, T., Kestenberg, W. & Keidan, R. 2012. An analysis of fine needle aspiration versus core needle biopsy in clinically palpable breast lesions: a report on the predictive values and a cost comparison. *Am J Surg* 204(2):193-8.

Nassar, A. 2011. Core Needle Biopsy Versus Fine Needle Aspiration Biopsy in Breast - A Historical Perspective and Opportunities in the Modern Era. *Diagn Cytopathol* 39(5):380-8.

Page J.E. & Williams J.E. 1991. The Radiological Features of Phylloides Tumour of the Breast With Clinico-Pathological Correlation. *Clin Radiol* 44(1):8-12.

Parker S.J. & Harries, S.A. 2001. Phyllodes tumours. *Postgrad Med J* 77(909):428-35.

Petrie, A. & Sabin, C. 2009. Assessing agreement. In *Medical Statistics at a Glance*, 3rd edition, Blackwell Publishing, 118-20.

Rama Rao, C., Narasimhamurthy, N.K., Jaganathan, K., Mukherjee, G. & Hazarika, D. 1992. Cystosarcoma Phyllodes: Diagnosis by Fine Needle Aspiration Cytology. *Acta Cytol* 36(2):203-7.

Rosa, M., Mohammadi, A. & Masood, S. 2012. The Value of Fine Needle Aspiration Biopsy in the Diagnosis and Prognostic Assessment of Palpable Breast Lesions. *Diagn Cytopathol* 40(1):26-34.

Rosen, P.P. 2008. Fibroepithelial Neoplasms. In *Rosen's Breast Pathology*, 3rd edition, Philadelphia: Lippincott Williams & Wilkins, 188-226.

Scolyer, R.A., McKenzie, P.R., Achmed, D. & Lee, C.S. 2001. Can Phyllodes Tumours of the Breast be Distinguished from Fibroadenomas using Fine Needle Aspiration Cytology? *Pathology* 33(4):437-43.

Shabb, N.S. 1997. Phyllodes Tumour: Fine Needle Aspiration Cytology of Eight Cases. *Acta Cytol* 41(2):321-6.

Shimizu, K., Masawa, N., Yamada, T., Okamoto, K., & Kanda, K. 1994. Cytologic Evaluation of Phyllodes Tumors as Compared to Fibroadenomas of the Breast. *Acta Cytol* 38(6):891-7.

Shimizu, K. & Korematsu, M. 2002. Phyllodes Tumor of the Breast: A Cytomorphologic Approach Based on Evaluation of Epithelial Cluster Architecture. *Acta Cytol* 46(2):332-6.

Simi, U., Moretti, D., Iacconi, P., Arganini, M., Roncella, M., Miccoli, P. & Giacomini, G. 1988. Fine Needle Aspiration Cytopathology of Phyllodes Tumor: Differential Diagnosis with Fibroadenoma. *Acta Cytol* 32(1):63-6.

Simsir, A. & Cangiarella, J. 2012. Challenging Breast Lesions: Pitfalls and Limitations of Fine-Needle Aspiration and the Role of Core Biopsy in Specific Lesions. *Diagn Cytopathol* 40(3):262-72.

Smith, M.J., Heffron, C.C., Rothwell, J.R., Loftus, B.M., Jeffers, M. & Geraghty, J.G. 2012. Fine Needle Aspiration Cytology in Symptomatic Breast Lesions: Still an Important Diagnostic Modality? *Breast J* 18(2):103-10.

Stanley, M.W., Tani, E.M., Rutqvist, L.E. & Skoog, L. 1989. Cystosarcoma Phyllodes of the Breast: A Cytologic and Clinicopathologic Study of 23 Cases. *Diagn Cytopathol* 5(1):29-34.

Tan, P.H., Tse, G., Lee, A., Simpson, J.F. & Hanby, A.M. 2012. Fibroepithelial tumours. In Lakhani, S.R., Ellis, I.A., Schnitt, S.J., Tan, P.H. & van de Vijver, M. (ed.) *WHO Classification of Tumours of the Breast*, Lyon: International Agency for Research on Cancer (IARC), 141-147.

Veneti, S. & Manek, S. 2001. Benign phyllodes tumour vs fibroadenoma: FNA cytological differentiation. *Cytopathology* 12(5):321-8.

Appendix 1 – Ethics Clearance Certificate



HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131159

NAME: Dr Robert Myles Maritz
(Principal Investigator)

DEPARTMENT: Division of Anatomical Pathology
National Health Laboratory Services

PROJECT TITLE: Cytological Criteria Distinguishing Phyllodes
Tumour of the Breast from Fibroadenoma

DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr Pamela Michelow

APPROVED BY: 
Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 15/11/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report.


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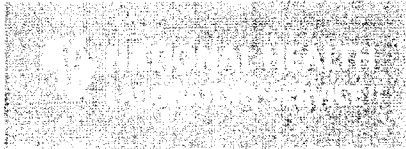
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Appendix 2 – Data Collection Sheet

Case #	Histo ref #	Cyto ref #	Age	Lx	TS	SF	EF	SER	Stromal cellularity (tick)			Stromal borders (tick)			Stromal atypia (tick)			PSC (tick)			Photography (tick)			Histology (tick)		
									1+	2+	3+	WD	PD	Absent	Mild	Mod	Severe	<10%	10-30%	>30%	Histo	Cyto	Both	FA	BPT	BoPT
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Abbreviations: Lx - tumour laterality, TS - tumour size in mm (maximal dimension), SF - number of stromal fragments per slide, EF - number of epithelial fragments per slide, SER - stromal to epithelial ratio, WD - well-defined, PD - poorly-defined, PSC - proportion of wavy spindle cells in the background cellular population, FA - fibrosarcoma, BPT - benign phyllodes tumour, BoPT - borderline phyllodes tumour, MPT - malignant phyllodes tumour

Appendix 3 – Turnitin Plagiarism Report



5 March 2015

Dear Sir/Madam

Plagiarism report for Dr Robert Maritz's MMed: Cytological criteria distinguishing phyllodes tumour of the breast from fibroadenoma.

This letter serves to confirm that this MMed is Dr Maritz's original work. The data was collected by Dr Maritz and I by reviewing glass slides under a double-header microscope.

In the Introduction, Literature Review and Discussion of the above mentioned MMed report, Dr Maritz acknowledged sources and cited them fully and correctly.

I personally submitted Dr Maritz's MMed to Turnitin, the plagiarism check recommended by the University of the Witwatersrand, which determined an 8% similarity index.

Yours sincerely

A handwritten signature in black ink, appearing to read "P. Michelow".

Dr Pamela Michelow

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1.0 Introduction

CHAPTER 1

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breast include fibroadenomas and phyllodes tumours. Both comprising a proliferation of glandular breast epithelial variably cellular stromal component (Figure 1.1). tumours, with no risk of recurrence or metastasis (Rosen, on the other hand, represent a spectrum ranging from frankly malignant tumours capable of distant metastasis death (Tan *et al*, 2012). Given the marked variation in sis associated with these tumours, accurate diagnosis is er-treatment of fibroadenomas and under-treatment of