(PCs) regardless of their original phenotype may acquire stochastic genetic and epigenetic hits that lead to the activation of separate histogenetic pathways for LGBC and HGBC, and that these events determine the phenotype of the pre-invasive and invasive lesions. Furthermore, these hits may be an early event in the progression of 'luminal A' tumours and once committed to this 'molecular pathway', progression to a 'high grade' (basal-like or HER2+) phenotype would be an unlikely.

O-40 An automated breast cancer grading demonstrator – Pathscore

M. Varga, P. Ducksbury, A. Green, K. Copsey*, E. Warner, I.O. Ellis, A.R. Green, R. Hanka. *QinetiQ, Malvern, UK, University of Nottingham, and Cambridge University, UK*

Pathology analysis of tissue entails visual interpretation of complex microscopic images; this is liable to inter- and intra-observer variation. National External Quality Assessment Service (NEQAS) work has improved the concordance of grading from 0.3 Kappa to 0.6; however, the performance still varies. There is a need for a new approach.
The PathScore project has developed automated computer analysis for grading breast cancer using the Elston and Ellis grading scheme. The evaluation dataset consisted of 47 samples from the NEQAS assessed by 733 pathologists. Diagnostic features; gland (acinus) formation, nuclear atypia/pleomorphism, mitotic frequency and overall grade were scored automatically by PathScore. The majority view of all pathologists was used for this evaluation. The grade allocated by pathologists was usually not unanimously agreed, the level of agreement varied widely. Evaluation showed that PathScore's performance was similar to that of the human pathologists. Overall grade agreement between the Pathologists and PathScore was good, although there was some tendency for PathScore to overestimate the severity of Nuclear Pleomorphism. The observed agreement (68.09) is twice as high as could have been observed by chance. The weighted kappa of 0.591 is statistically very highly significant and there is no evidence of any significant disagreement over any category or its asymmetry. By coincidence the level of agreement on the final grade amongst the pathologists yielded an identical kappa value (kappa = 0.59).
PathScore provides the potential for enhanced objectivity and reproducibility offering a standardized reliable method for histological grading of breast cancer on routine clinical samples.

O-41 Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences

R.A.A. Mohammed*, S.G. Martin, M.S. Gill, A.R. Green, E.C. Paish, I.O. Ellis. *University of Nottingham, City Hospital and Queen's Medical Centre Nottingham, UK*

The presence of vascular invasion (VI); encompassing both lymphovascular invasion (LVI) and blood vascular invasion (BVI), in breast cancer has been found to be a poor prognostic factor. It is not clear; however, which type of VI plays the major role in metastasis. The aims of this study were to use an endothelial subtype specific immunohistochemical approach to distinguish between LVI and BVI by comparing the differential expression of blood vascular (CD34 and CD31) and lymphatic markers (podoplanin/D2–40) to determine their prognostic role in a well characterized group of breast cancer patients with known long term follow up.

Sections from177 consecutive paraffin-embedded archival specimens of primary invasive breast cancer were stained for expression of podoplanin, D2–40, CD31 and CD34. BVI and LVI were identified and results were correlated with clinicopathological criteria and patient survival.
VI was detected in 56/177 specimens (31.6%); 54 (96.5%) were LVI and 2 (3.5%) were BVI. The presence of LVI was significantly associated with the presence of LN metastasis, larger tumour size, development of distant metastasis, regional recurrence and shorter disease free interval (DFI) and overall survival (OS). In multivariate analysis, LVI was an independent prognostic factor for poor survival.
In conclusion, VI in breast cancer is predominantly of lymph vessels and is a powerful independent prognostic factor which is associated with risk of recurrence and death from the disease. The use of immunohistochemical staining with a lymphendothelial specific marker such as podoplanin/ D2–40 increases the accuracy of identification of patients with tumour associated LVI.

O-42 OSNA® for rapid molecular analysis of breast cancer lymph nodes: the Guildford experience

K.L. Snook, G.T. Layer, P. Jackson, S. Al-Hashimi, J. Woodland, C.S. de Vries, M.W. Kissin*. *Royal Surrey County Hospital Breast and Histopathology Units, Guildford, The University of Surrey, Guildford*

Introduction: The OSNA® (One Step Nucleic Acid Amplification) system (Sysmex Corporation) has been developed to rapidly amplify CK19 mRNA from tissue lysates of lymph nodes (LNs), detecting metastatic tumour (>0.2 mm) in under 30 minutes. We are conducting a multicentre prospective study to determine concordance of OSNA analysis with multisection intensive haemotoxylin & eosin (H&E) and immunohistochemical (IHC) examination in LNs of breast cancer (BC) patients. This abstract reports results of the initial technical phase from the Guildford Breast Unit.
Methods: Lymph nodes were removed using standard surgical techniques, defatted and cut into 4×1 or 2 mm slices. Alternate slices were snap frozen at –80° for subsequent OSNA analysis; remaining slices underwent H&E and IHC (CK19 and AE1/AE3) examination (0.25 mm multistep sections ×5 levels). Tissue lysates were prepared for OSNA according to standard procedure. OSNA was performed and results correlated with histopathology findings. Ethics approval was obtained prior to commencement of study.
Results: 45 axillary LNs (sentinel and non-sentinel) from 19 patients with BC were investigated by both OSNA and histopathological examination (figure 1).

N = 45		HISTOPATHOLOGICAL EXAMINATION (H&E AND IHC)				
		Positive		**Negative**		
		Macro Metastasis >2 mm	Micro Metastasis 0.2–2 mm	Isolated Tumour Cells* <0.2 mm	No Metastasis	Total
OSNA	**Positive**	**8**	**3**	2	1	14
	Negative	0	0	**4**	**27**	31
	Total	11		34		

Fig. 1. Results of LN analysis by Histology and OSNA ($p \leqslant 0.001$ chi squared); *considered 'histologically negative' (UICC TNM staging).

Overall concordance was 93.3%; sensitivity 100% and specificity 91.2%. Tissue allocation bias may explain some discordant cases; further investigation of these specimens is underway.