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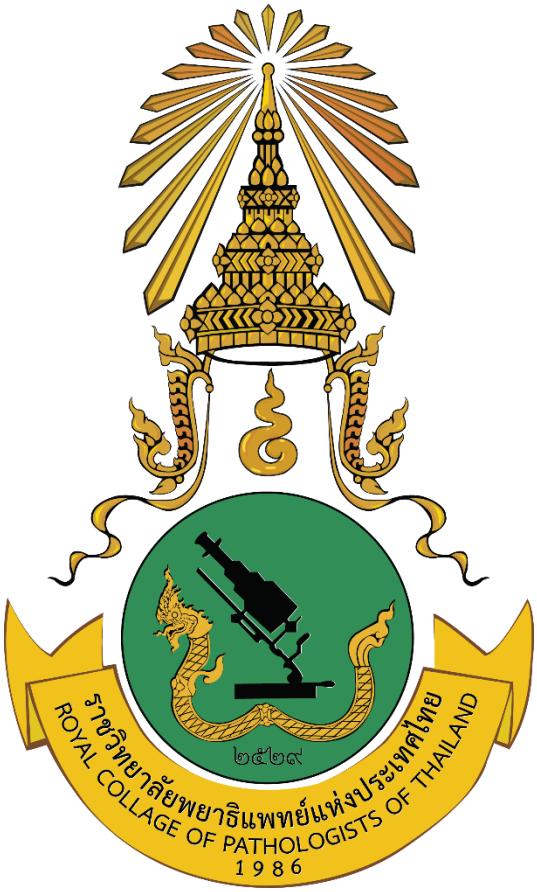

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ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name "วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย" and English name "Journal of the Royal College of Pathologists of Thailand". The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be "Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand", published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

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MANUSCRIPT REVIEWERS

CONTENTS

LETTER TO THE EDITOR

การตรวจคัดกรองมะเร็งเต้านม
(Breast cancer screening)

ณภัทร เพ็ชรศรีกุล

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มะเร็งเต้านมเป็นชนิดของมะเร็งซึ่งพบได้บ่อยที่สุดในเพศหญิงทั้งในประเทศไทยและต่างประเทศ โดยรายงานจากองค์การอนามัยโลกในปี พ.ศ. 2561 (ค.ศ. 2018) พบว่ามีสตรีจำนวน 627,000 คน เสียชีวิตจากมะเร็งเต้านม ซึ่งคิดเป็นร้อยละ 15 ของสาเหตุการเสียชีวิตในสตรีทั่วโลกที่เป็นมะเร็ง นอกจากนี้อุบัติการณ์ของมะเร็งเต้านมในสตรีไทยมีแนวโน้มสูงขึ้นในแต่ละปี[1-9] ซึ่งผู้ที่ตรวจพบมะเร็งเต้านมระยะแรกจะมีอัตราการรอดชีวิตที่สูงกว่า มีคุณภาพชีวิตที่ดีกว่า และเสียค่าใช้จ่ายที่น้อยกว่า[10,11] ทั้งนี้ข้อมูลจากทะเบียนมะเร็งของสถาบันมะเร็งแห่งชาติในประเทศไทยปี พ.ศ. 2560 (ค.ศ. 2017) รายงานว่าผู้ป่วยเพศหญิงรายใหม่ที่ได้รับการวินิจฉัยว่าเป็นมะเร็งเต้านมมีจำนวน 780 คน จากจำนวนสตรีที่ได้รับการวินิจฉัยว่าเป็นมะเร็งทั้งหมด 2,014 คน คิดเป็นร้อยละ 38.73 อนึ่งจากการจำแนกชนิดของมะเร็งเต้านมในผู้ป่วยรายใหม่ดังกล่าวนั้นพบว่า ร้อยละ 78.97 คือ Infiltrating duct carcinoma โดยร้อยละ 86.54 ตรวจพบมะเร็งจากวิธีการตรวจหาเนื้องอกปฐมภูมิ และร้อยละ 6.67 ตรวจพบมะเร็งจากการซักประวัติและตรวจร่างกาย ทั้งนี้ร้อยละของมะเร็งเต้านมที่ถูกตรวจพบนั้น ได้แก่ เป็นมะเร็งระยะที่ 1 ร้อยละ 13.43; เป็นมะเร็งระยะที่ 2 ร้อยละ 33.38; เป็นมะเร็งระยะที่ 3 ร้อยละ 31.07; เป็นมะเร็งระยะที่ 4 ร้อยละ 12.15; และเป็นมะเร็งที่ไม่ทราบระยะ ร้อยละ 9.72[1] แม้กระนั้นก็ตามสัดส่วนของผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นมะเร็งเต้านมระยะที่ 1 ในประเทศไทยยังค่อนข้างน้อยเมื่อเทียบกับประเทศสหรัฐอเมริกาซึ่งสามารถตรวจพบมะเร็งเต้านมในระยะที่ 1 ได้มากที่สุด[12] ดังนั้นประสิทธิภาพการตรวจวินิจฉัยระยะแรกของมะเร็งเต้านมในประเทศไทยจะต้องถูกนำมาพิจารณามากยิ่งขึ้น

ปัจจุบันการใช้ Serum tumour markers ในผู้ป่วยมะเร็งเต้านม ได้แก่ CA 15-3, CA 27-29 และ CEA อย่างไรก็ตาม The National Comprehensive Cancer Network (NCCN) และ American Society of Clinical Oncology (ASCO) ไม่แนะนำการใช้ Serum tumour markers เพื่อการตรวจคัดกรอง การวินิจฉัยและการบอกระยะของโรค รวมถึงเพื่อการติดตามการเกิดมะเร็งซ้ำ (Recurrence) ภายหลังจากได้รับการ

รักษามะเร็งเต้านมครั้งแรก เนื่องจาก Serum tumour markers ดังกล่าวนั้นมีความไวและความจำเพาะต่ำ แต่ควรใช้ Serum tumour markers ในผู้ป่วยมะเร็งเต้านมเพื่อติดตามผลการตอบสนองต่อการรักษาทั้งในผู้ป่วยที่มีและไม่มีการแพร่กระจายของมะเร็งนั้น[13]

ข้อจำกัดของการตรวจคัดกรองมะเร็งเต้านมในประเทศไทย คือ ไม่มีคำแนะนำสำหรับการตรวจคัดกรองมะเร็งเต้านมในประชากรไทยโดยเฉพาะ และแบบประเมินความเสี่ยงมะเร็งเต้านมในปัจจุบันไม่เหมาะสมกับประชากรไทย[14] นอกจากนี้สตรีไทยมีความหนาแน่นของเนื้อเยื่อเต้านมมากกว่าชาวตะวันตก จึงเป็นอุปสรรคต่อการตรวจวินิจฉัยมะเร็งเต้านมด้วยวิธีการถ่ายภาพรังสีเต้านมหรือแมมโมแกรม (Screening mammography) ในผู้ป่วยบางราย[15,16] เพราะความหนาแน่นของเนื้อเยื่อเต้านมอาจพรางก้อนมะเร็ง (Masking effect) ได้[17] จึงส่งผลให้ความไวในการตรวจพบลดลงจากร้อยละ 80 เหลือเพียงร้อยละ 30[18] ในอีกทางหนึ่งประเทศไทยจะมีการแนะนำให้ทำแมมโมแกรมในเพศหญิงที่มีอายุตั้งแต่ 40 ปีขึ้นไป[19] แต่ข้อมูลจากทะเบียนมะเร็งของสถาบันมะเร็งแห่งชาติในประเทศไทยปี พ.ศ. 2560 (ค.ศ. 2017)[1] แสดงว่าร้อยละ 15 ของมะเร็งเต้านมในสตรีไทยพบได้ในผู้ที่มีอายุต่ำกว่า 40 ปี

ด้วยเหตุนี้จึงมีความจำเป็นอย่างยิ่งที่ต้องพัฒนาการตรวจคัดกรองมะเร็งเต้านมในประเทศไทยให้มีประสิทธิภาพมากขึ้น เพื่อที่จะสามารถตรวจคัดกรองผู้ป่วยมะเร็งเต้านมได้ตั้งแต่ในระยะแรก ๆ อันส่งผลให้ผู้ป่วยมะเร็งเต้านมมีอัตราการรอดชีวิตที่สูงขึ้น มีอัตราการเสียชีวิตที่ลดลง และมีคุณภาพชีวิตที่ดีกว่า

<u>เอกสารอ้างอิง</u>

(1). Information Technology Division, National Cancer Institute. Hospital Based Cancer Registry 2017 [online]. 2017 [cited 2019 June 30]. Available from: http://www.nci.go.th/th/File_download/Nci%20Cancer%20Registry/HOSPITAL-BASED%202016%20Revise%204%20Final.pdf.

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ORIGINAL ARTICLE

Pathological assessment of activity and chronicity indices in lupus nephritis patients

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Abstract

Lupus nephritis (LN) is one of a major complication of systemic lupus erythematosus (SLE). Ten percent of patients with LN will develop end-stage renal disease (ESRD). Renal biopsy is the gold standard for diagnosis of LN and evaluate activity and chronicity of disease to predict renal function outcome. Activity and chronicity indices by National Institutes of Health (NIH) provide prognostic value and treatment guidance, however these indices are poor intra and interobserver reproducibility to be used as therapeutic guides or as prognosticators. The aim of this retrospective study was using individual morphologic variables is easier to understand and apply in clinical practice for predicting renal outcome. This retrospective study was enrolled 38 patients with biopsy proven LN class III and IV seen over 3-year period. The demographic, clinical and laboratory data were obtained at the time of biopsy. Activity and

chronicity indices were calculated and correlation between outcome parameters and the histological findings were investigated. Thirty-eight cases of LN were evaluated, of which 71% had LN class IV. The mean age was 29.63 ± 9.56 years, and 84% were females. The mean scores of activity index (AI) (NIH), chronicity index (CI) (NIH), modified NIH AI were 7.97, 2.79, and 6.47, respectively. Serum creatinine and eGFR correlated significantly with all indices as well as haematuria showed significant correlation with all indices except the chronicity index. Serum creatinine level was the strongest clinical parameter determining outcome. Urine protein to creatinine ratio (UPCR) showed limited correlation with leucocyte infiltration ($r = 0.399$, $p = 0.013$). In activity index, correlations with serum creatinine/estimated glomerular filtration rate (eGFR) were strongest with the interstitial infiltration ($r = 0.557$, $p = 0.001$) and fibrinoid necrosis/cellular crescent ($r = 0.466$, $p = 0.003$). In the chronicity index, correlations with serum creatinine/eGFR were strongest with glomerulosclerosis ($r = 0.587$, $p = 0.001$) and interstitial fibrosis (IF)/tubular atrophy (TA) ($r = 0.448$, $p = 0.005$). The eGFR was significantly decreased (less than 60 mL/min/1.73 m^2), independently with these pathologic lesions, including presence of endocapillary hypercellularity ≥ 50% of total glomeruli, presence of subendothelial hyaline deposits ≥ 25% of total glomeruli, presence of fibrinoid necrosis/cellular crescent ≥ 25% of total glomeruli, presence of glomerulosclerosis ≥ 25% of total glomeruli, presence of fibrous crescent ≥ 5% of total glomeruli, IF/TA ≥ 10% of cortical area, and presence of adhesion to bowman' s capsule ≥ 25% of total glomeruli, respectively. Base of these findings, we suggest the presence of any of the histological features of the AI (endocapillary hypercellularity, cellular/fibrocellular crescent and/or necrosis) reportedly defines patients at risk of developing renal failure. Similarly, the presence of any of the histological features of the CI (glomerulosclerosis, interstitial fibrosis and tubular atrophy) reportedly defines patients at risk of developing renal failure. In conclusion, modified NIH AI showed better correlation with clinical and outcome parameters as compared to the standard AI and CI scores, however these current scoring of AI and CI for LN exhibit poor interpathologist agreement. We suggest it could be improved by using individual morphologic variables that are easier to be performed in routine clinical practice for predicting renal outcome.

Keywords: activity index; chronicity index; lupus nephritis; renal biopsy; renal outcome

Introduction

Lupus nephritis (LN) is one of a major complication of systemic lupus erythematosus (SLE). Ten percent of patients with LN will develop end-stage renal disease (ESRD)[1]. The various clinical presentations are recognised in patients with lupus nephritis, ranging from mild asymptomatic to rapidly progressive glomerulonephritis[2,3] and usually correlating with the histologic type of lesion. The renal biopsies play an important role in the confirm diagnosis, evaluate disease activity, determine prognosis and management of patients with lupus nephritis (LN)[4,5]. Classification of the renal pathology of lupus patients is based on light microscopic changes combined with immunofluorescence microscopy (IF) and electron microscopy[6]. The diagnosis needs criteria in accordance with the 2003 International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification into six different classes based on quantitative assessment of histological lesions[7] (*Table 1*). Parameters of activity and chronicity should be described in accordance with activity and chronicity index by National Institute of Health modified by Austin et al.[8] (*Table 2*).

Table 1 Abbreviated International Society of Nephrology/ Renal Pathology Society (ISN/RPS) classification of lupus nephritis (2003).

ISN/RPS classification of lupus nephritis (2003)	
Class I	Minimal mesangial lupus nephritis
Class II	Mesangial proliferative lupus nephritis
Class III	Focal lupus nephritis
Class IV	Diffuse lupus nephritis
Class V	Membranous lupus nephritis
Class VI	Advanced sclerosing lupus nephritis

Activity Index (AI) is useful in assessing activity of LN. It consisted of the following items considered to represent measures of active lupus nephritis (endocapillary hypercellularity, glomerular leucocyte infiltration, subendothelial hyaline deposits, fibrinoid necrosis/karyorrhexis, cellular crescents and interstitial inflammation). These are scored from 0 to 3 depending on severity except fibrinoid necrosis/karyorrhexis, cellular crescents which assigned score was weighted by a factor of two[2] because such lesions were considered to be

disproportionately severe[9]. The maximum score was 24 points for the activity Index. Chronicity Index (Cl) is useful in assessing chronicity of LN. It consisted of the following items considered to represent measures of chronic irreversible lupus nephritis (glomerular sclerosis, fibrous crescents, tubular atrophy and interstitial fibrosis). These were semiquantitatively graded on a scale of 0, 1, 2 or 3. The maximum score was 12 points for the chronicity Index[8,9]. The activity index (AI) represents the degree of inflammatory injury to renal parenchyma and generally comprises lesions that may be response to immunosuppressive therapy. The chronicity index (CI) represents the degree of chronic damage the kidney, and generally comprises lesions that are associated with refractoriness to aggressive therapy[10]. Studies at the NIH correlated both a high activity index (score > 12) and high chronicity index (score > 4) with a poor 10-year renal survival rate. These provides useful information about the efficacy of therapy and the relative degree of reversible versus irreversible lesions[11]. Wernick et al. compared the reproducibility in a setting of four community hospitals and one university medical centre[12]. They found that the activity and chronicity indices were only moderately reproducible in a non-referral setting. Cecile Grootscholten et al. revealed that five specialised nephropathologists scored 126 biopsies from 87 patients with biopsy-proven proliferative LN. They found that there was a wide range of the agreement[13]. The activity index for LN showed good [Intraclass correlation coefficient (ICC) = 0. 716] and the chronicity index showed moderate (ICC = 0. 494) interobserver agreement. Schwartz et al. studied the comparison of the activity (AI) and chronicity indices (CI) in the renal biopsies calculated by different pathologists and concluded that these indices are too subjective to be used as therapeutic guides or as prognosticators[14].

Gary S Hill et al. developed a new morphologic index for the evaluation of renal biopsies in lupus nephritis, comprised four elements: Glomerular Activity Index (GAI), Tubulointerstitial Activity Index (TIAI), Chronic Lesions Index, interstitial fibrosis index (IFI)[15]. The Biopsy Index showed better correlations with clinical and outcome parameters than the standard AI and CI and other similar indices but this schema is very complex and its reproducibility has not been demonstrated. The international nephropathology working group in Leiden, Netherlands, in 2016 re-evaluation of activity and chronicity. In the modified NIH activity index, they link the presence of karyorrhexis to neutrophil infiltration and modified fibrinoid necrosis into a stand-alone. These were semiquantitatively graded on a scale of 0, 1, 2 or 3 (< 25%, 25 – 50% or > 50% of glomeruli, respectively)[16]. However, the semiquantitative system for grading and scoring for each morphologic various lesions for assessing activity and chronicity index of both

NIH and modified NIH scoring system exhibits poor interpathologist agreement[18] and it is subject to interobserver variability. The aim of this retrospective study was using individual morphologic variables is easier to understand and apply in clinical practice for predicting renal outcome.

Table 2 National Institutes of Health (NIH) and modified NIH lupus nephritis activity and chronicity scoring system.

NIH activity index	Modified NIH activity index	Score
1. Endocapillary proliferation	1. Endocapillary hypercellularity	0 – 3
2. Glomerular leucocyte infiltration	2. Neutrophils and/or karyorrhexis	0 – 3
3. Fibrinoid necrosis/karyorrhexis	3. Fibrinoid necrosis	(0 – 3) x 2
4. Hyaline deposits	4. Hyaline deposits	0 – 3
5. Cellular crescent	5. Cellular and/or fibrocellular crescents	(0 – 3) x 2
6. Interstitial inflammation	6. Interstitial inflammation	0 – 3
Total		**0 – 24**
NIH chronicity index	**Modified NIH chronicity index**	**Score**
1. Global sclerosis	1. Total glomerulosclerosis score	0 – 3
2. Fibrous crescents	2. Fibrous crescents	0 – 3
3. Tubular atrophy	3. Tubular atrophy	0 – 3
4. Interstitial fibrosis	4. Interstitial fibrosis	0 – 3
Total		**0 – 12**

Materials and Methods

Selection of patients:

We searched the pathology database to identify native renal biopsies of 38 patients from the archives of Army Institute of Pathology, Bangkok, Thailand from the period of 2016 to 2018 were evaluated. The patients fulfilled the revised American College of Rheumatology (ACR) criteria for SLE[17] as determined by their physicians. Renal biopsy confirmed lupus

nephritis cases were classified as class III and IV according to the 2003 ISN/ RPS LN classification[7].

Clinical and laboratory data:

The following clinical parameters were evaluated at the time of each biopsy, i.e. age, sex, body weight, height, body surface area (BSA), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), underlying diseases, ISN/RPS classification of lupus nephritis, duration of SLE, serum creatinine, estimated glomerular filtration rate (eGFR), haematocrit (Hct), serum albumin, urine protein/creatinine ratio (UPCR), haematuria and previous immunosuppressive treatments at the time of kidney biopsy were obtained from the patient records.

Pathological data:

Standard light microscopy (LM), sectioned with 2 micrometres thickness and the staining included haematoxylin and eosin stain, periodic acid-Schiff stain, and Jones methenamine silver stain was reviewed; the information collects include ISN/ RPS classification of lupus nephritis and presence or absence of these features (endocapillary hypercellularity, subendothelial hyaline deposits, neutrophils infiltration, karyorrhexis, fibrinoid necrosis, cellular/fibrocellular crescents, interstitial infiltration, glomerular sclerosis, fibrous crescent, tubular atrophy, interstitial fibrosis and adhesion to Bowman's capsule). Adequate renal biopsy samples for histological diagnosis, including at least 5 glomeruli. The immunofluorescence (IF) images in computer files were reviewed in all cases; the information collects location of immunofluorescence staining, intensity of each staining (IgG, IgA, IgM, C3 and C1q) with the degree of intensity of 0 (negative), trace, 1, 2 and 3. All renal biopsies were reviewed by a Thai board-certified pathologist blinded to the clinical data. Six outcome parameters were measured, i.e. serum creatinine, estimated glomerular filtration rate (eGFR), Hct, serum albumin, urine protein/creatinine ratio (UPCR) and microscopic haematuria.

Statistical analysis:

All continuous values were expressed as mean ± standard deviation (SD) and categorical variables were presented as percentage. Pearson's correlation and Chi square tests were used to compare frequency variables and correlation among different variables. Receiver Operating Characteristic (ROC curve) to determine a cutoff value. Data were analyzed by Stata software (Stata Corp. 2011. Stata Statistical Software: Release 12. College Station, TX: Stata Corp LP). The *p*-value of less than 0.05 was assumed to be significant.

Ethical Statement:

This study was reviewed by the Institutional Review Board of Royal Thai Army Medical Department (S003b/62_Exp).

Results

Baseline data of patients with lupus nephritis:

A total of 38 patients were included in this study with age ranged from 16 to 56 years. Thirty-two patients (84.2%) were female. Clinical data at biopsy time is shown in *Table 3*. According to International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification, 11 patients are classified as class III (28.9 %) and 27 (71 %) cases are class IV (*Table 4*).

Comparison with NIH activity and chronicity indices with other indices:

The mean score (± SD) for the activity index was 7.97 ± 5.76, and the mean score for the chronicity index was 2.79 ± 2.3 (*Table 5*). Ranges for the activity and chronicity indices were 0 to 19 and 0 to 7, respectively. For the activity index, 28.9 % were scored in the high-risk range of 12 or greater[9]. For the chronicity index, 36.9% of scores were in the low-risk range of 0 to 1, 34.2% were in the intermediate risk range of 2 to 3, and 28.9% were in the high-risk category of 4 or greater[9]. The mean score (± SD) of the modified NIH activity index was 6.47 ± 4.34 which is lower than the mean score of standard NIH activity index.

Correlation between morphologic lesions and parameter outcome:

Leucocyte infiltration, karyorrhexis/fibrinoid necrosis, cellular crescents, interstitial infiltration, neutrophils infiltrate/karyorrhexis, fibrinoid necrosis/cellular crescent, glomerulosclerosis, tubular atrophy, interstitial fibrosis and adhesion to Bowman's capsule had correlation with both serum creatinine and eGFR (*Table 6*). In the activity index, serum creatinine/eGFR were strongest correlated with the interstitial infiltration variables ($r = 0.557$, $p = 0.001$) and fibrinoid necrosis/cellular crescent variables ($r = 0.466$, $p = 0.003$). In the chronicity index, correlations with serum creatinine/eGFR were strongest with the glomerulosclerosis variables ($r = 0.587$, $p = 0.001$) and interstitial fibrosis/tubular atrophy ($r = 0.448$, $p = 0.005$) (*Table 6*).

Table 3 Clinical and laboratory characteristics at the time of initial biopsy.

Characteristics at the time of initial biopsy	
Number of patients	38
Age (Years old)	29.63 ± 9.56
Gender (Male : Female)	1 : 5.3 (15.8 % : 84.2 %)
Body surface area (BSA)	1.65 ± 0.19
Body mass index (BMI) (kg/m^2)	23.73 ± 4.36
Systolic blood pressure (SBP) (mmHg)	135.84 ± 19.6
Diastolic blood pressure (DBP) (mmHg)	86.16 ± 16.75
Duration of SLE (Years)	5.44 ± 6.18
Serum creatinine (mg/dL)	1.17 ± 0.57
Estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2)	78.61 ± 40.17
Serum albumin (g/dL)	2.8 ± 0.66
Haematocrit (Hct) (%)	32.31 ± 6.89
Urine protein/creatinine ratio (UPCR) (g/g creatinine)	3.58 ± 2.83
Microscopic haematuria (n, %)	18 (47.4 %)
Hypertension (n, %)	21 (56.8 %)
Diabetes mellitus (n, %)	1 (2.7 %)

Note: *n = Number of cases; and SLE = Systemic lupus erythematosus*

Haematuria showed parallel to the correlation of serum creatinine/eGFR with above morphologic lesions, hematuria correlated more closely with karyorrhexis/fibrinoid necrosis lesions (r = 0.581, p = 0.001) in parameters of NIH activity index as well as neutrophil/karyorrhexis lesions (r = 0.601, p = 0.001) in parameters of modified NIH activity index. Haematuria shows correlation with endocapillary hypercellularity (r = 0.505, p = 0.001).

Declined of haematocrit (Hct) significantly correlated with presence of endocapillary hypercellularity, leucocyte infiltration, karyorrhexis/fibrinoid necrosis and

neutrophil/karyorrhexis. Haematocrit (Hct) showed no significant correlation with any morphologic variable in chronicity index.

Urine protein/creatinine ratio (UPCR) showed limited correlation with leucocyte infiltration ($r = 0.399$, $p = 0.013$). No significant correlation of serum albumin was found with any morphologic variable.

Correlations between parameter outcome and pathological indices:

The Pearson's correlations coefficient between renal outcome and pathological indices is shown in *Table 7*. Serum creatinine and eGFR were significantly correlated with all pathological indices. The most significant correlations were between serum creatinine and modified AI index ($r = 0.461$, $p = 0.004$) and between eGFR and modified AI index ($r = -0.483$, $p = 0.002$). Haematocrit (Hct) level and haematuria showed significant correlation with all indices, but no significant correlation was observed with chronicity index. In addition, there were no correlation between serum albumin/UPCR and any pathological indices (*Table 7*).

The eGFR was significantly decreased (less than 60 mL/min/1.73 m^2), independently with these pathologic lesions, including presence of endocapillary hypercellularity ≥ 50% of total glomeruli, presence of subendothelial hyaline deposits ≥ 25% of total glomeruli, presence of fibrinoid necrosis/cellular crescent ≥ 25% of total glomeruli, presence of glomerulosclerosis ≥ 25% of total glomeruli, presence of fibrous crescent ≥ 5% of total glomeruli, interstitial fibrosis/tubular atrophy ≥ 10% of cortical area, and presence of adhesion to Bowman's capsule ≥ 25% of total glomeruli, respectively (*Table 8*).

Table 4 The frequency distribution of different classes of lupus nephritis according to International Society of Nephrology/ Renal Pathology Society (ISN/RPS) 2003 classification.

ISN/RPS classes	Frequency	Percent
Class III	11	28.9
Class IV	27	71.1

Table 5 NIH activity and chronicity indices with other indices.

Score	Mean ± SD	Mean difference[a]	95% CI	*p*-value
NIH activity index	7.97 ± 5.76			
Modified NIH activity index	6.47 ± 4.34	1.50 ± 1.74	0.93 - 2.07	< 0.001
NIH/modified NIH chronicity index	2.79 ± 2.3			

Note:

[a] To compare with modified NIH activity index

CI = Confidence interval; and SD = Standard deviation

Discussions

In our study, no significant correlation was identified between any morphologic variables and level of hematocrit. Austin et al. revealed that haematocrit less than 20% was a strong clinical predictor of poor prognosis[8] while there was no patient with haematocrit values less than 20% in our study that could affect the parameter outcome.

Fibrous adhesion of glomerular tuft to Bowman's capsule favours scarring from a previously active lesion rather than usual type segmental sclerosis[19]. However, no attempts have been made to include the presence of adhesion to Bowman's capsule in the chronicity indices of lupus nephritis. We found that presence of adhesion to Bowman's capsule on renal biopsy was significantly correlated with high serum creatinine and decrease in eGFR, so it may provide useful prognostic information on renal survival in patients with lupus nephritis.

Our results revealed that the modified NIH indices show better correlations with clinical and outcome parameters than the standard NIH indices. We also observe significance difference in the mean of the NIH activity index scores and modified activity index scores ($p < 0.001$). In our study, the mean score (± SD) of modified NIH activity index was 6.47 ± 4.34 that lower than the mean score of standard NIH activity index. In our study, fibrinoid necrosis was present in 4 patients (10.5%) compare with 22 patients (57.9%) had fibrinoid necrosis and/or karyorrhexis that could affect these index scores.

Table 6 Correlations between serum creatinine and various morphologic variables: Pearson product-moment correlations.

Morphologic variable	Pearson correlation (r)			
	Serum creatinine	eGFR	Hct	Haematuria
Endocapillary hypercellularity	0.317	-0.231	-0.360[a]	0.505[a]
Subendothelial hyaline deposits	0.231	-0.250	-0.078	0.121
Leukocyte infiltration	0.432[a]	-0.464[a]	-0.368[a]	0.566[a]
Karyorrhexis/Fibrinoid necrosis[+]	0.439[a]	-0.416[a]	-0.422[a]	0.581[a]
Cellular crescents	0.455[a]	-0.349[a]	-0.295	0.346[a]
Interstitial infiltration	0.557[a]	-0.462[a]	-0.319	0.375[a]
Fibrinoid necrosis	0.161	-0.209	-0.282	0.106
Neutrophil/Karyorrhexis[$]	0.439[a]	-0.434[a]	-0.402[a]	0.601[a]
Fibrinoid necrosis/Cellular crescent	0.466[a]	-0.364[a]	-0.316	0.352[a]
Glomerulosclerosis	0.587[a]	-0.494[a]	-0.014	-0.199
Fibrous crescent	-0.075	0.011	-0.099	0.018
Tubular atrophy	0.448[a]	-0.333[a]	0.049	-0.131
Interstitial fibrosis	0.448[a]	-0.333[a]	0.049	-0.131
Interstitial fibrosis/Tubular atrophy	0.448[a]	-0.333[a]	0.049	-0.131
Adhesion to Bowman's capsule	0.430[a]	-0.338[a]	-0.014	-0.057

Note:

[a] Significant at p < 0.05

[+] National Institutes of Health (NIH)

[$] Modified NIH

eGFR = Estimated glomerular filtration rate; and Hct = Haematocrit

For the activity index, we observed the presence of cellular/ fibrocellular crescents and/or fibrinoid necrosis on renal biopsy was significantly correlated with several laboratory abnormalities, including high serum creatinine, decrease in eGFR, and increase microscopic haematuria. We found the presence of endocapillary hypercellularity was also associated with increase microscopic haematuria while there was no correlation between the presence of

subendothelial deposits on renal biopsy with any laboratory outcome data. Similarly, the chronicity index had also shown correlation with the renal function. The presence of glomerulosclerosis, interstitial fibrosis and tubular atrophy, and presence of adhesion to Bowman's capsule on renal biopsy were significantly correlated with high serum creatinine and decrease in eGFR. There was no correlation between the presence of fibrous crescent on renal biopsy with any laboratory outcome data.

Furthermore, although modified NIH indices and standard NIH indices have been associated with renal outcome in LN, the interobserver reproducibility for the standard NIH activity and chronicity indices is relatively poor[18]. Base of these findings, we suggest the presence of any of the histological features of the AI (endocapillary hypercellularity, cellular/ fibrocellular crescent and/ or necrosis) reportedly defines the patients at risk of developing renal failure. Similarly, the presence of any of the histological features of the CI (glomerulosclerosis, interstitial fibrosis and tubular atrophy) reportedly defines the patients at risk of developing renal failure.

Table 7 Pearson's correlations coefficient of various clinical outcome and pathologic parameters.

	AI	MAI	CI	AI/IF	MAI/IF
Serum creatinine	0.442*	0.461*	0.408*	0.470*	0.491*
eGFR	-0.473*	-0.483*	-0.347*	-0.456*	-0.459*
Serum albumin	-0.391	0.344	0.179	-0.406	-0.367*
Hct	-0.235*	-0.293*	0.103	-0.287*	-0.354*
UPCR	0.106	0.121	0.005	0.228*	0.280
Haematuria	0.606*	0.588*	-0.269	0.647*	0.602*

Note:

***** *p-value < 0.05*

AI = National Institutes of Health activity index; AI/IF = NIH activity index with presence of subendothelial hyaline deposit in IF; CI = National Institutes of Health chronicity index; eGFR = Estimated glomerular filtration rate; Hct = Haematocrit; IF = Interstitial fibrosis; MAI = Modified National Institutes of Health activity index; MAI/IF = Modified NIH activity index with presence of subendothelial hyaline deposit in IF; and UPCR = Urine protein/creatinine ratio

Table 8 Receiver operating characteristic curve for determining specific cut-off for percent of glomerular involvement in each parameter for estimated patient with GFR less than 60 mL/min/1.73 m^2.

Morphologic variable	Cut-point	Sensitivity (%)	Specificity (%)	95% CI of sensitivity	95% CI of specificity
Active lesions					
Endocapillary hypercellularity	≥ 50	42.86	58.33	17.7 – 71.1	53.3 – 90.2
Subendothelial hyaline deposits	≥ 25	57.14	79.17	23.0 – 77.0	57.8 – 92.9
Fibrinoid necrosis/ Cellular crescent	≥ 25	42.86	91.67	17.7 – 71.1	73.0 – 99.0
Chronic lesions					
Glomerulosclerosis	≥ 25	64.29	79.17	35.1 – 87.2	57.8 – 92.9
Fibrous crescent	≥ 5	7.14	95.83	0.18 – 33.9	78.9 – 99.9
Interstitial fibrosis/ Tubular atrophy	≥ 10	35.71	79.17	12.8 – 64.9	57.8 – 92.9
Adhesion to Bowman's capsule	≥ 25	21.43	95.83	4.66 – 50.8	78.9 – 99.9

Note: *CI = Confidence interval; and GFR = Glomerular filtration rate*

The limitation of this study was the small sample size and this is a single centre study, further validation of these indices needs to be studied in larger center studies for their reproducibility.

Conclusion

We suggest that the standard activity and chronicity indices in lupus nephritis could be improved by using individual morphologic variables that are easier to be performed in clinical practice for predicting renal outcome.

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ORIGINAL ARTICLE

A comparison study of molecular classification of primary invasive breast carcinoma and corresponding metastatic carcinoma in axillary lymph nodes by immunohistochemistry

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Abstract

Breast cancer is a heterogeneous disease. There is a high degree of diversity between and within tumours as well as among cancer-bearing individuals, and all of these factors together determine the risk of disease progression and therapeutic resistance. The significant difference between primary tumour and paired lymph node metastases has been reported to be awareness about tumour heterogeneity to decrease treatment failure rate. The objective of this study was to compare molecular subtypes of primary invasive breast carcinoma and corresponding metastatic carcinoma in axillary lymph nodes by immunohistochemistry. The study subjects consisted of formalin-fixed, paraffin-embedded tissue blocks from 59 patients who was diagnosed primary breast tumour with axillary lymph node metastases. They were assessed for ER, PR, HER2, Ki67, CK5/6, EGFR and p53 immunoexpression. Differences in expression for each of the immunohistochemical markers and molecular phenotypes were analysed. The immunohistochemical markers showed significant concordance in expression of ER, PR, HER2, Ki67, EGFR and p53 ($p < 0.05$) except for CK5/6 between primary tumour and paired lymph node (s). There was significant concordance in molecular phenotype from the primary tumour compared with the paired lymph node (s) in any subtypes ($p < 0.05$). In conclusion, the immunohistochemical expression in metastatic lymph node (s) may refer to the similar molecular phenotypes for adjuvant therapy.

Keywords: axillary lymph node metastasis; breast cancer; immunohistochemistry; molecular subtypes; tumour heterogeneity

Introduction

Breast cancer is the most common cancer in Thai women with the incidence of 22.8% of new female cancer cases in 2018[1]. In addition to pathological grade and stage, breast cancer is routinely assessed for hormone receptor status (oestrogen and progesterone receptors, ER and PR) by immunohistochemistry (IHC) and human epidermal growth factor receptor 2 (HER2) expression by either IHC or in situ hybridisation (ISH). According to DNA microarrays and Gene Expression Profiling (GEP), breast cancer is classified into 5 molecular subtypes, i.e. luminal A, luminal B, HER2-enriched, basal-like and normal breast-like[2]. The therapeutic purposes are additionally based on the recognition of intrinsic biological subtypes within the breast cancer spectrum. Thus, 'Luminal A' disease generally requires only endocrine therapy, which also part of the treatment of the 'Luminal B' subtype. Chemotherapy is considered for most patients with 'Luminal B', 'HER2 positive', and 'Triple negative (ductal)' diseases, with the addition of trastuzumab in 'HER2 positive' cancer[3]. However, 60% of patients have no benefit from endocrine therapies, and only 30 – 40% of patients receiving trastuzumab get benefit[4,5]. The basal-like subtype is most commonly revealed as triple negativity for immunoexpression of myoepithelial markers such as CK 5/6 and also overexpression of epidermal growth factor receptor (EGFR) which has no targeted therapy in the present time[6]. In addition, breast cancer with p53 expression has been reported to be associated with poor prognosis[7]. Therapeutic decision is based on the molecular pathology of the core biopsy or resection specimen of the primary tumour. The immunoexpression of the primary cancer tissue is assumed to be identical with the corresponding metastatic lymph node (s). If immunoexpression in metastatic lymph nodes differs from the primary tumor, this might be an important reason of treatment failure. As the hypothesis, breast cancer is a heterogeneous disease. There is a high degree of diversity between and within tumours as well as among cancer-bearing individuals, and all of these factors together determine the risk of disease progression and therapeutic resistance[8]. Since the immunohistochemical expression in the primary tumour is significantly different from its paired lymph node metastases[9-12], the aim of this study was to compare the molecular subtypes of primary invasive breast carcinoma with metastatic carcinoma in the corresponding axillary lymph nodes by immunohistochemistry.

Materials and Methods

Study design and population:

The formalin-fixed, paraffin-embedded (FFPE) tissue blocks from 59 patients with primary breast cancer stored in the Army Institute of Pathology, Bangkok, Thailand were recruited in this study. The breast cancer tissues were diagnosed between 2009 and 2018 and already had their ER, PR, HER2 and Ki67 immunostainings. The selective criteria included infiltrating ductal carcinoma (IDC) of no special type (NST) of the breast with metastasis to axillary lymph node (s). The characteristics of 59 breast cancer samples are shown in *Table 1*.

Table 1 The characteristics of 59 breast cancer samples.

Characteristic	
Age (Years old)	
• ***Range (Average)***	***29 – 85 (53.9)***
≤ 50	25/59 cases (42.37%)
> 50	34/59 cases (57.63%)
Tumour size (cm)	
• ***Range (Average)***	***1.0 – 9.5 (3.5)***
≤ 2.0	48/59 cases (81.36%)
> 2.0	11/59 cases (18.64%)
Tumour grade (differentiation)	
• ***Low grade [Grades I (Well) and II (Moderate)]***	***33/59 cases (55.93%)***
Grade I (Well)	3/59 cases (5.08%)
Grade II (Moderate)	30/59 cases (50.85%)
• ***High grade [Grade III (Poor)]***	***26/59 cases (44.07%)***

Immunohistochemistry (IHC):

The tissue sections of 3 μm FFPE samples were stained with CONFIRM™ Anti-Estrogen Receptor (SP1) Rabbit Monoclonal Antibody, CONFIRM™ Anti-Progesterone Receptor (1E2) Rabbit Monoclonal Antibody, PATHWAY® Anti- HER-2/neu (4B5) Rabbit Monoclonal Antibody, CONFIRM™ Cytokeratin 5/6 Clone D5/16 B4 Mouse Monoclonal, CONFIRM™ EGFR (5B7) Rabbit Monoclonal Antibody and CONFIRM™ p53 Clone BP53-11 Mouse Monoclonal.

Evaluation of tumour grade and IHC:

An anatomical pathology resident and three Thai board-certified pathologists assessed tumour grade in primary breast tumour and immunohistochemical expression of ER, PR, HER2, Ki67, CK5/6, EGFR and p53 in cancer cells of both primary breast tumour and corresponding metastatic axillary lymph node tissues. The Nottingham combined histologic grade (NCHG) system (Elston-Ellis modification of Scarff-Bloom-Richardson grading system) was used for evaluating tumour grade. Expressions of ER and PR were determined by Allred (quick) scoring[13]. HER2 expression was scored as the followings: 0/1+ is negative; 2+ is equivocal; and 3+ is positive[14]. Immunoexpression of Ki67 was categorised as low (< 20%) and high (≥ 20%)[15]. Expressions of CK5/6[16,17] and EGFR[18] were positive if there was cytoplasmic membrane staining greater than 10% of the cancer cells. For p53 expression, the positive result was nuclear immunostaining at least 10% of the cancer cells[19].

Evaluation of tumour grade and IHC:

The molecular phenotypes were classified into 5 categories, i.e. luminal A, luminal B, Her2-enriched, basal-like and normal breast –like (*Table 2*)[20].

Statistical analysis:

The immunohistochemical expressions of ER, PR, HER2, KI67, CK5/6, EGFR and p53 and the molecular subtypes were analysed in the primary breast cancer tissues and their corresponding metastatic axillary lymph nodes at the 95% confidence interval (CI).

Table 2 Putative molecular subtypes of breast cancer based on immunohistochemical expression and histological grade[20].

Putative molecular subtype	Histological grade	Immunohistochemical expression						
		ER	PR	HER2	Ki67	CK5/6	EGFR	p53
Luminal A	I & II	+	+	-	Low	-	-	+
Luminal B	III	+	-	-	High	-	-	+
HER2-enriched	III	-	-	+	High	-	-	+
Basal-like	III	-	-	-	High	+	+	+
Normal breast-like	I & II	+	-	-	Low	-	-	+

Note: *- = Negative; + = Positive; EGFR = Epidermal growth factor receptor; ER = Oestrogen receptor; and PR = Progesterone receptor*

Results

Fifty-nine patients were included within this study. The patient's age ranged from 29 to 85. An average age was about 54 years old. The tumor ranged in size from 1 cm to 9.5 cm. There was an average tumour size of 3.5 cm. The majority of histologic tumour grades were grade II breast cancer obtaining from 30 patients (50.85%). The immunohistochemical expressions of ER, PR, HER2, Ki67, EGFR and p53 of primary tumour cells were significantly concordant with these immunoexpressions of metastatic cancer cells in paired axillary lymph node ($p < 0.05$). The expression of CK5/6 of primary tumour cells does not correspond with its expression of metastatic cancer cells ($p = 0.219$) (*Table 3*). The molecular subtypes of primary breast cancer were in agreement with that of metastatic cancers ($p < 0.05$) (*Table 4*).

Discussions

It is well established that there is heterogeneous expression of molecular phenotypes in breast cancer patients. According to the hypothesis that metastatic heterogeneity described about cells with different metastatic properties have been isolated from the same parent tumour by the role of clonal selection during the process of metastasis supported by studies in which individual cells were tagged by unique markers allowing them to be tracked[8,21]. However, our results were not support this hypothesis but revealed significant concordance

of ER, PR, HER2, Ki67, EGFR and p53 immunoexpression between primary tumours and corresponding nodal metastases. This was in agreement with the previous studies[22-25].

Since we found immunoexpressions of ER, PR, HER2, Ki67, EGFR and p53 were able to evaluate in paired axillary lymph node (s), the assessment receptor expression may reflect or predict response in corresponding lymph node(s)[10]. However, changes in CK 5/6 expression was detected in minority cases of paired lymph node (s). Therefore, a decision about adjuvant therapy was possibly impacted by the difference in immunoreactivity-based molecular subtypes between primary invasive breast cancer and metastatic carcinoma in corresponding axillary lymph node.

Table 3 The concordance between immunoexpression of ER, PR, HER2, Ki67, CK5/6, EGFR and p53 of primary breast cancer and paired axillary lymph node metastasis.

Number of cases yielding immunohistochemical expression (%)																	
Primary breast cancer		Paired axillary lymph node metastasis															
		ER		PR		HER2			Ki67		CK5/6		EGFR		p53		
		+	-	+	-	+	E	-	+	-	+	-	+	-	+	-	
ER	+	36 (61.0)	2 (3.4)														
	-	4 (6.8)	17 (28.8)														
		Kappa = 0.773 *p < 0.001*[$]															
PR	+			23 (39.0)	5 (8.5)												
	-			7 (11.9)	24 (40.7)												
				Kappa = 0.594 *p < 0.001*[$]													

Note:

[$] Cohen's kappa test

- = Negative; + = Positive; E = Equivocal; EGFR = Epidermal growth factor receptor; ER = Oestrogen receptor; and PR = Progesterone receptor

Table 3 (Continued) The concordance between immunoexpression of ER, PR, HER2, Ki67, CK5/6, EGFR and p53 of primary breast cancer and paired axillary lymph node metastasis.

Number of cases yielding immunohistochemical expression (%)																	
Primary breast cancer		Paired axillary lymph node metastasis															
		ER		PR		HER2			Ki67		CK5/6		EGFR		p53		
		+	-	+	-	+	E	-	+	-	+	-	+	-	+	-	
HER2	+					11 (18.6)	2 (3.4)	0 (0.0)									
	E					3 (5.1)	2 (3.4)	8 (13.6)									
	-					1 (1.7)	4 (6.8)	28 (47.5)									
						Kappa = 0.547 *p < 0.001*[$]											
Ki67	+								28 (47.5)	6 (10.2)							
	-								8 (13.6)	17 (28.8)							
									Kappa = 0.509 *p < 0.001*[$]								

Note:

[$] Cohen's kappa test

- = Negative; + = Positive; E = Equivocal; EGFR = Epidermal growth factor receptor; ER = Oestrogen receptor; and PR = Progesterone receptor

Table 3 (Continued) The concordance between immunoexpression of ER, PR, HER2, Ki67, CK5/6, EGFR and p53 of primary breast cancer and paired axillary lymph node metastasis.

Number of cases yielding immunohistochemical expression (%)																			
Primary breast cancer		Paired axillary lymph node metastasis																	
		ER		PR		HER2			Ki67		CK5/6		EGFR		p53				
		+	-	+	-	+	E	-	+	-	+	-	+	-	+	-			
CK5/6	+										1 (1.7)	4 (6.8)							
	-										3 (5.1)	51 (86.4)							
											Kappa = 0.159 *p = 0.219*[$]								
EGFR	+												3 (5.1)	3 (5.1)					
	-												2 (3.4)	51 (86.4)					
													Kappa = 0.499 *p < 0.001*[$]						
p53	+														18 (30.5)	1 (1.7)			
	-														3 (5.1)	37 (62.7)			
															Kappa = 0.849 *p < 0.001*[$]				

Note:

[$] *Cohen's kappa test*

- = Negative; + = Positive; E = Equivocal; EGFR = Epidermal growth factor receptor; ER = Oestrogen receptor; and PR = Progesterone receptor

Table 4 The concordance between molecular subtypes of primary breast cancer and metastatic cancer in paired axillary lymph nodes.

Number of cases for molecular subtypes of breast cancer (%)						Kappa	*p*-value[$]
Primary breast cancer	Metastatic cancer in paired axillary lymph nodes						
	Luminal A	Luminal B	Her2-enriched	Basal-like	Normal breast–like		
Luminal A	8 (72.7)	3 (27.3)	—	—	—	0.417	0.001
Luminal B	9 (32.1)	19 (67.9)	—	—	—	0.521	< 0.001
Her2-enriched	—	1 (12.5)	6 (75.0)	—	1 (12.5)	0.771	< 0.001
Basal-like	—	—	—	3 (75.0)	1 (25.0)	0.732	< 0.001
Normal breast –like	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	4 (50.0)	0.515	< 0.001

Note:

[$] *Cohen's kappa test*

Conclusion

Based on the immunohistochemical expressions of ER, PR, HER2, Ki67, EGFR and P53, the molecular subtypes of primary breast cancer are in agreement with the molecular classification of metastatic carcinoma in corresponding axillary lymph node (s).

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APPENDIX 1
INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

Authors should take care to exclude overlap and duplication in papers dealing with related materials. See also paragraph on Redundant or Duplicate Publication in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/index.html.

The submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript's review. The editor's decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal's general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. Letters to the Editor

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figure or Table: Maximum of 1 (if needed)*

2. Original Articles

The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see ***Organisation of Manuscripts***): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Structured Abstract (see **Organisation of Manuscripts**): 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 6*

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 4*

4. Case Reports

AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

- *Word Count: 1,200 – 2,000 words (excluding abstract, references, and figure or table legends)*
- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 20*
- *Figures or Tables: Maximum of 4*

5. Case Illustrations

Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

- *Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figures: Maximum of 2*
- *Tables: Maximum of 5*

6. Technical Notes

The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

- *Word Count: Maximum of 1,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 5*
- *Figures or Tables: Maximum of 2*

Organisation of Manuscripts

1. General Format

The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

Footnotes are not used in the manuscripts, but parenthetical statements within text are applied instead and sparingly. Abbreviations should be defined at first mention and thereafter used consistently throughout the article. The standard abbreviations for units of measure must be used in conjunction with numbers.

All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

2. Title Page

The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
- The full names, institutional addresses, and email addresses for all authors (If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the address list.)
- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
- Conflict of interest statement (If there are no conflicts of interest for any author, the following statement should be inserted: "The authors declare that they have no conflicts of interest with the contents of this article.")

3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- *Background: The main context of the study*
- *Objective: The main purpose of the study*
- *Materials and Methods: How the study was performed*
- *Results: The main findings*
- *Conclusions: Brief summary and potential implications*

- *<u>Keywords</u>: 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article*

4. Introduction

The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. Conclusions

The Conclusions section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. Acknowledgements

The Acknowledgements section should be any brief notes of thanks to the following:

- *Funding sources*
- *A person who provided purely technical help or writing assistance*
- *A department chair who provided only general support*
- *Sources of material (e.g. novel drugs) not available commercially*

Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write "Not applicable" in this section.

10. References

The Vancouver system of referencing should be used in the manuscripts. References should be cited numerically in the order they appear in the text. The authors should identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts. Please give names of all authors and editors. The references should be numbered and listed in order of appearance in the text. The names of all authors are cited when there are six or fewer. When there are seven or more, only the first three followed by "et al." should be given. The names of journals should be abbreviated in the style used in Index Medicus (see examples below). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. A Smith, unpubl. Data, 2000).

- *Journal article*

  1. Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia – eclampsia. Am J Obstet Gynecol 1990; 162: 1141 – 5.

- *Books*

  2. Remington JS, Swartz MN. Current Topics in Infectious Diseases, Vol 21. Boston: Blackwell Science Publication, 2001.

- *Chapter in a book*

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11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

12. Figure Legends

The legends should be self-explanatory and typed on a separate page titled “Figure Legends”. They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes,

illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

13. Figures

All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are submitted electronically along with the manuscripts. These figures should be referred to specifically in the text of the papers but should not be embedded within the text. The following information must be stated to each microscopic image: staining method, magnification (especially for electron micrograph), and numerical aperture of the objective lens. The authors are encouraged to use digital images (at least 300 d.p.i.) in .jpg or .tif formats. The use of three-dimensional histograms is strongly discouraged when the addition of these histograms gives no extra information.

14. Components

14.1. Letters to the Editor

The Letter to the Editor manuscripts consist of the following order:

- *Title Page*
- *Main Text*
- *References*
- *Table (if needed)*
- *Figure Legend (if needed)*
- *Figure (if needed)*

14.2. Original Articles

The Original Article manuscripts consist of the following order:

- *Title Page*
- *Structured Abstract*
- *Introduction*
- *Materials and Methods*
- *Results*
- *Discussion*
- *Conclusions*

- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.3. Review Articles

The Review Article manuscripts consist of the following order:

- *Title Page*
- *Unstructured Abstract*
- *Introduction*
- *Main Text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.4. Case Reports

The Case Report manuscripts consist of the following order:

- *Title Page*
- *Unstructured Abstract*
- *Introduction*
- *Case Description*
- *Discussion*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.5. Case Illustrations

The Case Illustration manuscripts consist of the following order:

- *Title Page*
- *Clinical Presentation or Description*
- *Laboratory Investigations*
- *Discussion*
- *Final Diagnosis*
- *Multiple Choice Questions (MCQs)*
- *Take-Home Messages (Learning Points)*
- *Acknowledgements*
- *References*
- *Correct Answers to MCQs*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

14.6. Technical Notes

The Technical Note manuscripts consist of the following order:

- *Title Page*
- *Introduction*
- *Main text*
- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

Proofreading

The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

Revised Manuscripts

In many cases, the authors will be invited to make revisions to their manuscripts. The revised manuscripts must generally be received by the Editorial Board within 3 months of the date on the decision letter or they will be considered a new submission. An extension can sometimes be negotiated with the Editorial Board.

APPENDIX 2
BENEFITS OF PUBLISHING WITH ASIAN ARCHIVES OF PATHOLOGY

Asian Archives of Pathology (AAP) is an open access journal. Open Access makes your works freely available to everyone in the world. It provides a significant boost to the readership of your articles, and has been shown to have an increase in positive influence on citations and reuse. Hence, open-access leads to more recognition for our esteemed authors.

The journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

Since AAP is also a peer-reviewed journal, the submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. The decision on these manuscripts is processed very fast without any delay and in shortest possible time. The processing period is 1 – 2 weeks. These decisions of the reviewers are unbiased and the decision (reject, invite revision, and accept) letter coming from the Editorial Board is always conveyed to the authors.

APPENDIX 3
SUBMISSION OF THE MANUSCRIPTS

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Dr Chetana Ruangpratheep

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Editor-in-Chief of Asian Archives of Pathology

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Announcements of academic meetings and conferences that are of interest to the readers of Asian Archives of Pathology (AAP) should be sent to the Editor-in-Chief at least 3 months before the first day of the month of issue. The contact information is shown below.

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WHAT IS INSIDE THIS ISSUE?

Letter to the Editor:

Original Articles: