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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 baht for Thai authors and 30 United States dollars (USD) for international authors.

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MANUSCRIPT REVIEWERS

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ORIGINAL ARTICLE

The prognostic value of programmed cell death ligand 1 and mismatch repair proteins expression in hereditary non-polyposis colorectal cancer

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Abstract

Background: Hereditary nonpolyposis colorectal cancer (HNPCC) is a genetic cancer that is associated with mismatch repair (MMR) deficiency. Programmed cell death ligand 1 (PD-L1) is associated with immune evasion of cancer cells. The prognostic role of PD-L1 in HNPCC is still unclear.

Objective: To evaluate the association of PD-L1 and MMR protein status, with 3-year overall survival (OS) in HNPCC cases.
Materials and Methods: Tissue microarray was constructed from 38 HNPCC specimens, from Songklanagarind hospital; from January 2004 to December 2015. Diagnostic criteria of HNPCC cases were Amsterdam II or revised Bethesda. All cases had performed immunohistochemical staining to PD-L1 (clone 22C3) and MMR proteins: human mutT homolog 1(MLH1), human mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2).
Results: All cases showed no PD-L1 expression in neoplastic cells. Nine cases (23.7%) were MMR-deficient. In MMR-deficient tumors, the results showed loss of expression in MLH1 (11.0%), MSH6 (11.0%), MSH2 with MSH6 (22.0%), and MLH1 with PMS2 (56.0%). There was a significant association between MMR protein status and three-year OS (p-value = 0.02).
Conclusion: The PD-L1 protein cannot be used to determine the prognosis in HNPCC patients. However, MMR protein status may provide prognostic information.

Keywords: hereditary non-polyposis colorectal cancer, mismatch repair gene, PDL-1

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and in Southern of Thailand [1-2]. Approximately, 30% of all patients with CRC are from an inherited form, with about 5% of them being linked to known genetic alterations [3]. Early detection of this disease, by screening tests, can reduce both morbidity and mortality [4-5].

Hereditary non-polyposis colorectal cancer (HNPCC) is an inherited colorectal cancer, accounting for 2-4% of all colorectal cancers [6]. This tumor has germline mutations of DNA mismatch repair (MMR) genes; including: human mutT homolog 1(MLH1), human mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2). These mutations cause defects of one or more MMR proteins, which can be detected by microsatellite

instability (MSI) molecular testing or via immunohistochemistry (IHC) [7]. MMR defected tumors exhibit a high level of microsatellite instability (MSI-H) [8], and the clinical diagnosis of HNPCC uses Amsterdam II criteria or revised Bethesda guidelines [9-10].

Programmed death receptor-ligand 1 (PD-L1) plays a critical role in immune evasion of tumor cells [11-12]. PD-L1 on tumor cell interacts with programmed death-1 (PD-1) on activated T-cells [13-14]. This interaction inhibits T-cell function, allowing the tumor to evade the immune system [15]. Several studies regarding PD-L1 expression in various tumor cells have shown poor prognosis in patients with PD-L1 expression [16-18].

Recent studies have also found that patients with MMR-deficient CRC have a more favorable stage-adjusted prognosis [19-20]. MMR-deficient tumors tend to have high expression of immune checkpoints, such as PD-L1[21-22]. Therefore, PD-L1 immune checkpoint inhibitors may have an effect and improve survival in MSI-CRC, including HNPCC [23-24]. Some studies have shown that metastatic CRC with MSI-H have responded well to treatment with immune checkpoint inhibitors [25-27]. Although, previous studies have shown that PD-L1 expression is associated with a poor prognosis in CRC patients, studies in HNPCC are still limited [28-29]. Hence, this study endeavored to evaluate the association of expression of PD-L1 and MMR proteins with 3-year overall survival (OS) in HNPCC.

Materials and Methods

Population and Sample

The study groups consisted of 38 cases of HNPCC, who met the Amsterdam II criteria or revised Bethesda guidelines [9-10]. The diagnosis of HNPCC used clinical diagnosis according to our institutional guideline with further molecular study for confirm the diagnosis of HNPCC. All patients were treated by surgical resection of the large intestine, in Songklanagarind Hospital; from January 2004 and December 2015, and had formalin-fixed paraffin embedded tissue. Patients who had previously received chemotherapy, radiation therapy or had second primary cancers were excluded. Clinical data and follow up time data of 3-years were collected, with latest follow up year being 2018. This study was approved by the ethical committee of the faculty of medicine, Prince of Songkhla University (REC.61-081-5-1)

Immunohistochemistry

Tissue microarray (TMA) was performed, by selecting an area of the tumor, using a 2-mm diameter needle. The sections were stained with monoclonal mouse primary antibodies to PD-L1 (clone 22C3, Dako), MLH1 (Cell Marque), MSH2 (Cell Marque), MSH6 (Cell Marque), and PMS2 (Cell Marque). Staining was performed with a Leica BOND-MAX automated immunohistochemical stainer.

All sections were examined by a pathologist, using a light microscope. The area of PD-L1 positive staining were the cell membranes, and the MSI markers (MLH1, MSH2, MSH6, and PMS2) were the nucleus. Percentages of PD-L1 positive tumor cells and staining intensity were evaluated in each core. Cell membrane staining was divided in 3 groups: no expression (< 1% of tumor cells), low expression (1-49% of tumor cells), and high expression (≥ 50% of tumor cells) [15]. The interpretation of MMR protein was defined as negative (0%) and positive nuclear staining of any tumor cells (>0%) according to Collage of American Pathologist (CAP) protocol.

Statistical Analysis

Clinicopathological characteristic comparisons used Chi-square tests or Fisher's exact tests. The Kaplan-Meier method was used to estimate the overall survival (OS) distributions, and the log-rank test was performed to compare the survival difference within each group. Univariate and multivariate-adjusted Cox regression models were used to evaluate independent prognostic factors. Difference was considered significant when the p-value < 0.05. All statistical analyses were calculated by R program studio 3.3.1.

Results

Demographic data from 38 cases of HNPCC-related CRC are shown in table 1. Most cases were female (60.5%), and the mean age of the study group was 52.1 ± 17.3 years. Most tumors were in stage IV (36.8%) and grade 1 (51.4%). There were 31 cases (81.6%) that were diagnosed as HNPCC, by using the Revised Bethesda criteria. Most tumors were located at left-sided colon (76.3%), with mean size of tumors being 5 cm. mean follow-up duration was 43.6 ± 24 months.

Table 1. Demographic data of patients with HNPCC cases (n=38)

Characteristics	n (%)
Gender	
Female	23 (60.5)
Male	15 (39.5)
Age (years, mean ± S.D.)	52.1 (17.3)
Staging	
I	2 (5.3)
II	12 (31.6)
III	10 (26.3)
IV	14 (36.8)
Criteria diagnosis	
Amsterdam II criteria	7 (18.4)
Revised Bethesda Guidelines	31 (81.6)
Location	
Right side	9 (23.7)
Left side	29 (76.3)
Tumor size (mean (IQR))	5 (4,6.4)
Follow-up time (months, mean ± S.D.)	43.6 (24)

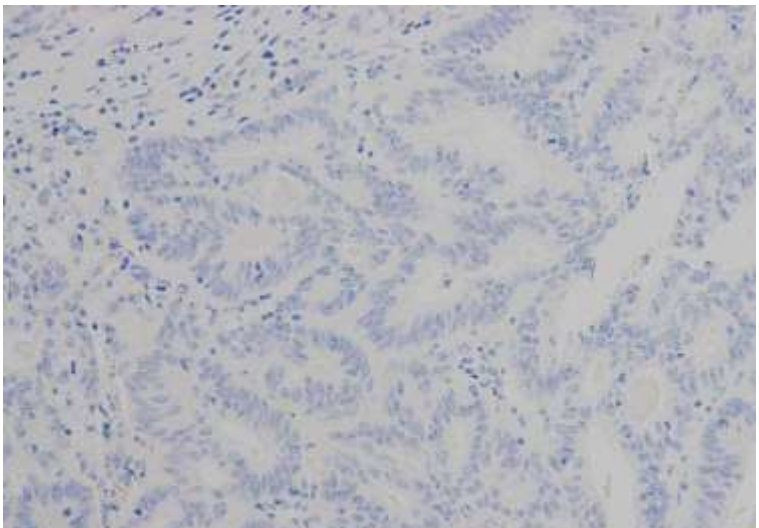

Figure 1. This picture shows the negative immunostaining for PD-L1 in the tumor cells.

Immunohistochemical findings

All 38 cases showed no expression of PD-L1 in neoplastic cells. The example case of negative results of PD-L1 expression are shown in figure 1. IHC staining for MLH1, MSH2, MSH6, and PMS2 were positive in 32 (84.2%), 36 (94.7%), 35 (92.1%), and 33 (86.8%) cases, respectively. MMR-deficient was found in 9 cases (23.7%). In this group, IHC results showed loss of expression in MLH1 (11%), MSH6 (11%), MSH2 combined with MSH6 (22%), and MLH1 combined with PMS2 (56%). The IHC results of MMR proteins are shown in table 2; and the example cases are shown in figure 2.

Table 2. IHC results of each MMR protein expression in HNPCC cases (n=38)

MMR protein	Positive (n (%)	Negative (n (%)
MLH1	32 (84.2)	6 (15.8)
MSH2	36 (94.7)	2 (5.3)
MSH6	35 (92.1)	3 (7.9)
PMS2	33 (86.8)	5 (13.2)

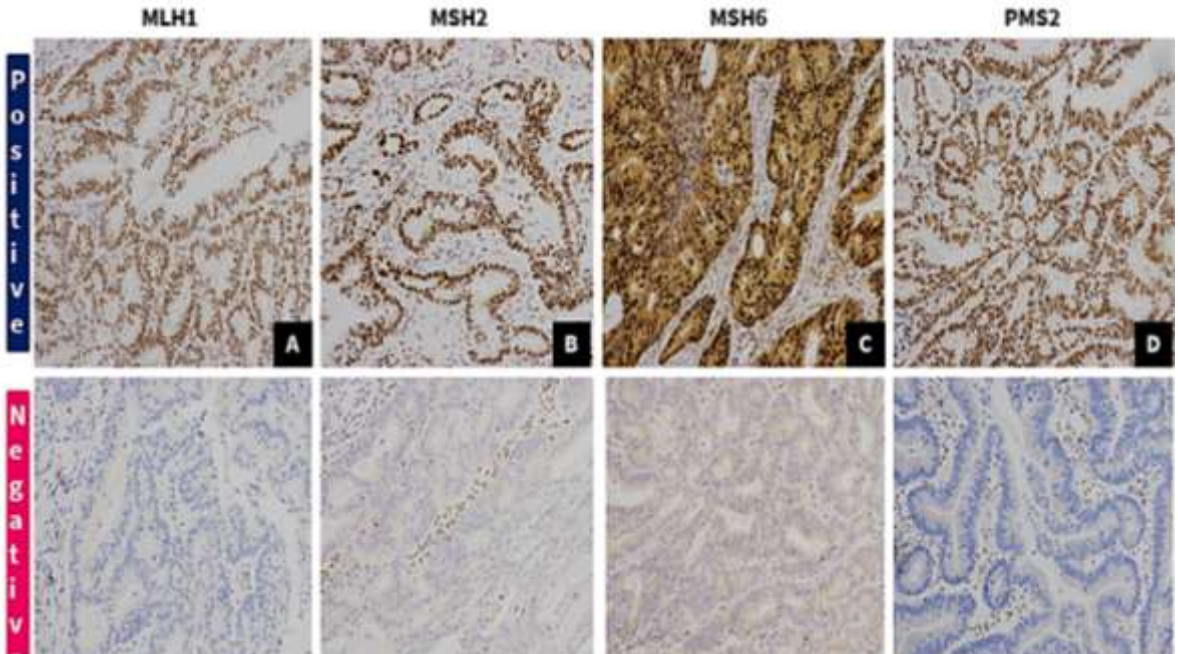

Figure 2. The expression of MMR proteins in HNPCC cases (A-D = positive staining in any nuclear of tumor cells) (E-H = negative nuclear staining)

Survival Analysis

The Kaplan-Meier curve for HNPCC by MMR protein status showed significant effect on 3-year OS by log rank test (p-value = 0.02) (figure 3). Additionally, the Kaplan-Meier curve for HNPCC by TNM staging (AJCC) also showed significant effect on 3-year OS by log rank test (p-value = 0.01) (figure 4). However, Cox regression analysis showed no significance of prognostic factors of TNM staging for 3-year OS. The factors of MMR-protein status could not be evaluated, because not all patients in the MMR deficient group were deceased.

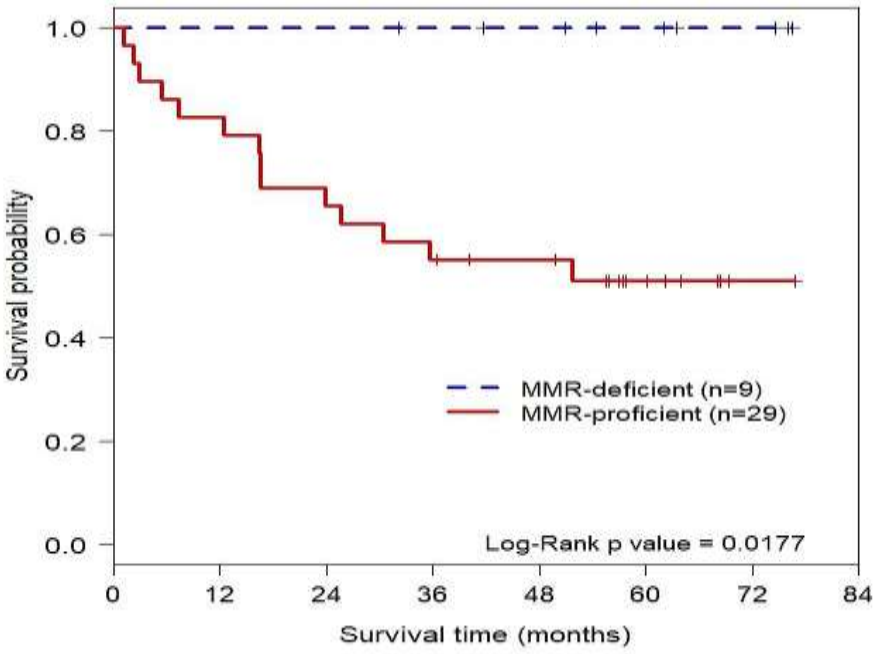

Figure 3. Kaplan-Meier curve of 3-year overall survival, with MMR protein status in HNPCC patients (n=38)

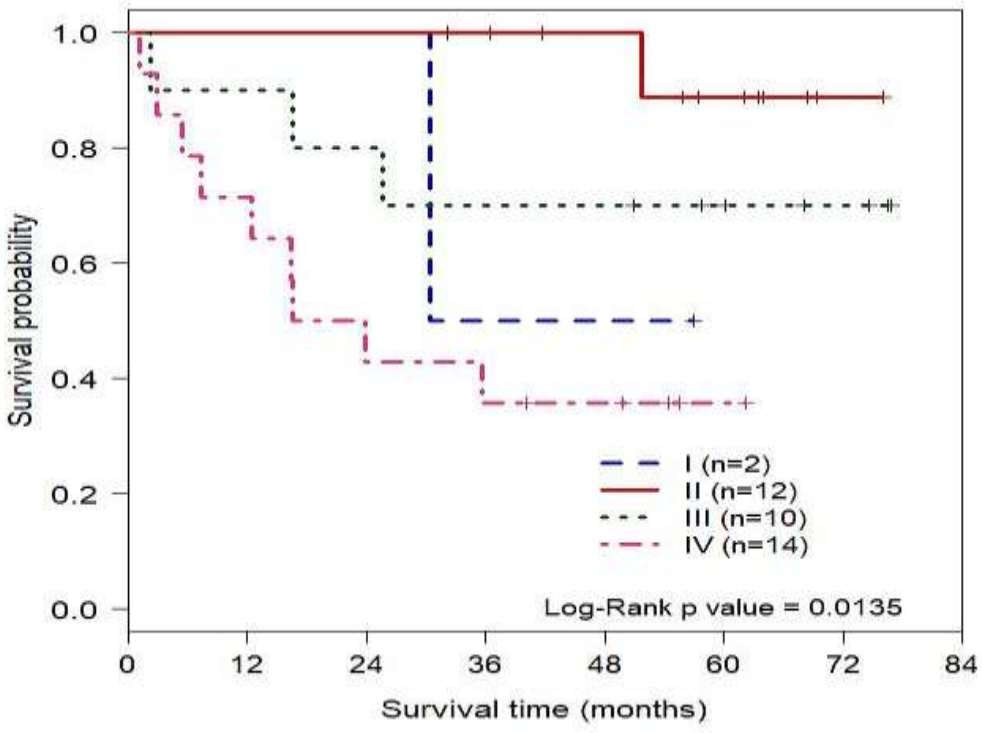

Figure 4. Kaplan-Meier curve of 3-year overall survival, with TNM staging (AJCC) (n=38)

Discussion

This study used clinical criteria for diagnosis of HNPCC cases; including, Amsterdam II criteria (sensitivity 22%, specificity 98%) and the Revised Bethesda guidelines (sensitivity 82%, specificity 77%) [30]. However, these two criteria did not include MSI/MMR protein testing for diagnosis. This study revealed that most CRC patients (76.3%) were MMR-proficient. This result was concordant with Kawakami H, et al. [31]

PD-L1 plays an important role in host immune evasion of tumor cells. In our study, we used IHC for PD-L1 (clone 22C3), and found that it was negative in neoplastic cells of all our 38 HNPCC cases. This result was discordant with previous studies [32]. This conflicting result may be due to a small sample size and different clones of antibodies. The frequency of PD-L1 expression in CRC patients was variable, depending on the clone of antibody, and ranged from 19% (clone SP142) to 45% (clone 28-8) [28,32]. The previous study showed higher PD-L1 expression in MMR-deficient tumors than MMR-proficient tumors [33]. In our study, we used PD-L1 (clone 22C3), because it is a diagnostic IHC approved by the FDA, and is used to select NSCLC patients for anti-PD-L1 therapy [34]. The negative staining of PD-L1 in this study may be from differences in patient groups, low sensitivity of antibodies or low incidence of PD-L1 expression in HNPCC patients.

In addition, this study also found that patients with MMR-deficient tumors had a significantly better 3-year OS (p=0.017). This result was the same in many previous retrospective and population-based studies [35-36]. The pathogenesis of MMR-deficient tumor differs from MMR-proficient tumor that has defect of adenomatous polyposis coli (APC) gene. The defect of APC gene causes loss of tumor suppressor gene function that promote tumor cell growth and metastasis. Therefore, patients with MMR-deficient tumors were found to rarely have any metastatic disease [37]. Moreover, lymphocytic reaction by tumor-infiltrating lymphocytes (TILs) was increased in MMR-deficient CRC, and it was associated with longer survival [38].

Our study had limitations, including: a small number of patients, due to this being a rare disease, and using clinical diagnosis of HNPCC without MSI/MMR protein testing. We suggest a larger numbers of cases with known MSI/MMR protein status and longer periods of follow-up times for the further study.

Conclusion

PD-L1 protein cannot be used to determine the prognosis in HNPCC patients. However, MMR protein status may provide valuable prognostic information for these patients.

References

(1). Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 93 - 7.

(2). Virani S, Bilheem S, Chansaard W, et al. National and Subnational Population-Based Incidence of Cancer in Thailand: Assessing Cancers with the Highest Burdens. Cancers (Basel). 2017; 9: 1-27.

(3). Patel SG, Ahnen DJ. Familial colon cancer syndromes: an update of a rapidly evolving field. Curr Gastroenterol Rep 2012; 14: 428-38.

(4). Ballester V, Boardman L. Next generation multigene panel testing: the next step for identification of hereditary colorectal cancer syndromes? Gastroenterology 2015; 149: 526-8.

(5). Møller P, Seppälä T, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut 2017; 66: 464-72.

(6). Jasperson KW, Tuohy TM, Neklason DW, et al. Hereditary and familial colon cancer. Gastroenterology 2010; 138: 2044 - 58.

(7). Steinke V, Engel C, Büttner R, et al. Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome. Dtsch Arztebl Int 2013; 110: 32-8.

(8). Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58: 5248 - 57.

(9). Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. A National Cancer Institute Workshop on hereditary nonpolyposis colorectal cancer syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997; 89: 1758 - 62.

(10). Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004; 96: 261-8.

(11). Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646 - 74.

(12). Swaika A, Hammond WA, Joseph RW. Current state of anti-PD-L1 and anti-PD-1 agents in cancer therapy. Mol Immunol 2015; 67: 4-17.

(13). Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. Genome Biol 2005; 6: 223. Available from https://doi.org/10.1186/gb-2005-6-6-223

(14). Wang S, Chen L. T lymphocyte co-signaling pathways of the B7-CD28 family. Cell Mol Immunol 2004; 1: 37-42.

(15). Bryan LJ, Gordon LI. Releasing the brake on the immune system: the PD-1 strategy for hematologic malignancies. Oncology (Williston Park) 2015; 29: 431-9.
(16). Ghebeh H, Mohammed S, Al-Omair A, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia 2006; 8: 190-8.
(17). Hamanishi J, Mandai M, Iwasaki M, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A 2007; 104: 3360 - 5.
(18). Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res 2006; 66: 3381-5.
(19). Mlecnik B, Bindea G, Angell HK, et al. Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. Immunity 2016; 44: 698-711.
(20). Kim JH, Kang GH. Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. World J Gastroenterol 2014; 20: 4230-43.
(21). Bupathi M, Wu C. Biomarkers for immune therapy in colorectal cancer: mismatch-repair deficiency and others. J Gastrointest Oncol 2016; 7: 713-20.
(22). Bever KM, Le DT. An expanding role for immunotherapy in colorectal cancer. J Natl Compr Canc Netw 2017; 15: 401-10.
(23). Gatalica Z, Snyder C, Maney T, et al. Programmed Cell Death 1 (PD-1) and Its Ligand (PD-L1) in Common Cancers and Their Correlation with Molecular Cancer Type. Cancer Epidemiology Biomarkers & Prevention. 2014 Nov 12; 23(12): 2965–70.
(24). Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015; 5: 43-51.
(25). Dudley JC, Lin MT, Le DT, et al. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res 2016; 22: 813-20.
(26). Kim JH, Park HE, Cho NY, et al. Characterisation of PD-L1-positive subsets of microsatellite-unstable colorectal cancers. Br J Cancer 2016; 115: 490-6.
(27). Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017; 357: 409-13.
(28). Shi SJ, Wang LJ, Wang GD, et al. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. PLoS One 2013; 8: e 76012-23.
(29). Rosenbaum MW, Bledsoe JR, Morales-Oyarvide V, et al. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes. Mod Pathol 2016; 29: 1104-12.

(30). Giardiello FM, Allen JI, Axilbund JE, et al. Guidelines on genetic evaluation and management of Lynch Syndrome: A consensus statement by the US Multi-Society Task Force on colorectal cancer. Am J Gastroenterol 2014; 147: 502–26.
(31). Kawakami H, Zaanan A, Sinicrope FA. MSI testing and its role in the management of colorectal cancer. Curr Treat Options Oncol 2015; 16: 30-44.
(32). Kim ST, Klempner SJ, Park SH, et al. Correlating programmed death ligand 1 (PD-L1) expression, mismatch repair deficiency, and outcomes across tumor types: implications for immunotherapy. Oncotarget 2017; 8: 77415-23.
(33). Lee LH, Cavalcanti MS, Segal NH, et al. Patterns and prognostic relevance of PD-1 and PD-L1 expression in colorectal carcinoma. Modern Pathology 2016; 29: 1433-42.
(34). Sul J, Blumenthal GM, Jiang X, et al. FDA Approval Summary: Pembrolizumab for the Treatment of Patients with Metastatic Non-Small Cell Lung Cancer Whose Tumors ExpressProgrammed Death-Ligand 1. Oncologist 2016; 21: 643-50.
(35). Sinicrope FA, Rego RL, Halling KC, et al. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. Gastroenterology 2006; 131: 729-37.
(36). Lanza G, Gafà R, Santini A, et al. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. J Clin Oncol 2006; 24: 2359-67.
(37). Sinicrope FA. DNA mismatch repair and adjuvant chemotherapy in sporadic colon cancer. Nat Rev Clin Oncol 2010; 7: 174-7.
(38). Srdjan M, Jadranka A, Ivan D, et al. Microsatellite instability & survival in patients with stage II/III colorectal carcinoma. Indian J Med Res 2016; 143: 104-11.

REVIEW ARTICLE

ตัวบ่งชี้ทางชีวภาพสำหรับมะเร็ง (Cancer Biomarkers)

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บทนำ (Introduction)

ในปัจจุบันโรคมะเร็งนับเป็นสาเหตุของการเสียชีวิตอันดับที่ 1 ของประชากรในประเทศไทย[1-2] โดย 5 อันดับของโรคมะเร็งที่พบบ่อยในเพศชาย ได้แก่ มะเร็งตับ มะเร็งลำไส้ใหญ่ มะเร็งปอด มะเร็งทวารหนัก และ มะเร็งช่องปาก ส่วนที่พบในเพศหญิง ได้แก่ มะเร็งเต้านม มะเร็งลำไส้ใหญ่ มะเร็งปากมดลูก มะเร็งอวัยวะสืบพันธุ์ และ มะเร็งตับ[3]

โรคมะเร็งเกิดจากเซลล์ต้นกำเนิดมะเร็ง (cancer stem cells) ซึ่งอาศัยความได้เปรียบจากการที่มีความผิดปกติของสารพันธุกรรม (genetic mutations) หรือความผิดปกติที่ระดับเหนือพันธุกรรม (epigenetics) อยู่ภายในเซลล์ ซึ่งส่งผลให้เซลล์เหล่านั้นสามารถแบ่งตัวได้มากผิดปกติ จนกระทั่งเบียดบังการเจริญเติบโตของเซลล์ปกติ[4-5] โดยทั่วไปโรคมะเร็งสามารถแบ่งออกได้เป็น 4 ระยะ ได้แก่ ระยะที่ 1 มะเร็งมีลักษณะเป็นก้อนเนื้อ แผลขนาดเล็กยังไม่ลุกลาม ระยะที่ 2 ก้อนมะเร็งมีลักษณะเป็นแผลขนาดใหญ่ขึ้น เริ่มลุกลามภายในเนื้อเยื่อ ระยะที่ 3 มะเร็งมีลักษณะเป็นก้อนใหญ่ขึ้น เริ่มลุกลามเข้าเนื้อเยื่ออวัยวะข้างเคียง และ ระยะที่ 4 ก้อนมะเร็งมีขนาดใหญ่มาก และลุกลามไปยังเนื้อเยื่ออวัยวะข้างเคียงจนทะลุ และ แพร่กระจายเข้าสู่กระแสโลหิตและอวัยวะที่อยู่ไกลออกไป[6]

สารบ่งชี้มะเร็ง (tumor marker) เป็นสารชีวโมเลกุลที่ถูกสร้างและหลั่งออกมาจากเซลล์มะเร็งเอง หรือผลิตจากเซลล์ร่างกายที่ตอบสนองต่อมะเร็ง ดังนั้นจึงตรวจพบได้ในกระแสเลือดหรือสารคัดหลั่งอื่น ๆ ของร่างกาย โดยสามารถตรวจสารบ่งชี้มะเร็งได้จากเลือด ปัสสาวะ และ เนื้อเยื่อ[7-8] อย่างไรก็ดีสารบ่งชี้มะเร็งมีความจำเพาะเจาะจงต่ำ นั่นคืออาจตรวจพบการเพิ่มขึ้นของสารบ่งชี้มะเร็งตัวเดียวกันได้ในมะเร็งหลายชนิด หรือ อาจตรวจพบการเพิ่มขึ้นของสารบ่งชี้มะเร็งในบางภาวะซึ่งไม่ใช่โรคมะเร็งก็ได้ และปัญหาที่พบอีกประการหนึ่งคือ สารบ่งชี้มะเร็งอาจมีความไวต่ำ ทำให้ไม่สามารถพบการเปลี่ยนแปลงของสารบ่งชี้มะเร็งในผู้ป่วยโรคมะเร็งในบางราย

คุณสมบัติของสารบ่งชี้มะเร็งในอุดมคติ[9-10]

1. มีความจำเพาะสูงเฉพาะโรคมะเร็งนั้น ๆ (Highly specific)
2. มีความไวสูงสามารถนำมาใช้ตรวจหาโรคมะเร็งได้ตั้งแต่ระยะต้น ๆ (Highly sensitive)
3. มีการเปลี่ยนแปลงที่สามารถนำมาใช้ตรวจติดตามผลการรักษาได้ (Levels correlate with tumor)
4. มีวิธีการตรวจที่ทำได้ง่าย ค่าใช้จ่ายต่ำ (Simple and cheap test)
5. ตัวอย่างที่หาได้ง่าย (Easily obtainable specimens)
6. ครึ่งชีวิตสั้น (Shot half-life)
7. ระยะเวลาการรอคอยที่นาน (Long lead-time)
8. ภาระโรค (burden)

หน้าที่ของ tumor marker ในทางคลินิก สามารถแบ่งได้ดังนี้

1. Screening คัดกรองหาระยะมะเร็งเริ่มแรกตั้งแต่ยังไม่มีอาการ ปัจจุบันมี tumor marker ที่ได้รับการยอมรับว่าสามารถใช้ในการตรวจกรองหามะเร็งได้ คือ Prostate specific antigen (PSA) เพื่อตรวจกรองภาวะมะเร็งต่อมลูกหมาก [11-12]

2. Diagnosis วินิจฉัยผู้ป่วยที่สงสัยว่าเป็นมะเร็งตั้งแต่ระยะต้น ต้องพิจารณาร่วมกับการตรวจร่างกาย การตรวจทางห้องปฏิบัติการ และ ตรวจทางการแพทย์อื่น ๆ เช่น X-ray, Ultrasound เป็นต้น [11-12]

3. Prognosis การพยากรณ์โรค เป็นการบอกความรุนแรงของโรค เนื่องจากระดับของ tumor marker จะแปรผันไปตามระยะของมะเร็ง ดังนั้นเมื่อตรวจพบ tumor marker ที่มีระดับสูงมากจะแสดงว่ามะเร็งมีขนาดใหญ่ขึ้นหรือมีการแพร่กระจายแล้ว [11-12]

4. Monitoring treatment ใช้ในการติดตามผลการรักษาโรค ค่าของ tumor marker ที่ลดลงหลังการรักษาจะบ่งบอกถึงการตอบสนองต่อการรักษาหรือการหายของโรค [11,13]

5. Detection of relapse ติดตามโอกาสที่โรคจะเป็นซ้ำหรือมีการกระจายของโรค หาก tumor marker ที่เคยลดลงเพิ่มสูงขึ้นหลังการรักษา แสดงว่ามีโอกาสที่จะกลับมาเป็นใหม่ของโรคมะเร็ง [11-12]

การแบ่งกลุ่มการใช้งานของ tumor marker ในแง่ clinical application ในระยะต่างๆ ได้แก่

1. ระยะ screening ได้แก่

- Prostate specific antigen (PSA) เป็นโปรตีนที่สร้างจากต่อมลูกหมาก[14,15] ผู้ที่มีอายุ 45-75 ปี จะมีค่า PSA <1 ng/ml หรือ PSA 1-3 ng/ml แต่ถ้าหาก PSA >3 ng/ml แสดงว่ามีความเสี่ยงที่จะเป็นมะเร็งต่อมลูกหมาก ผู้ที่มีอายุมากกว่า 75 ปี จะมีค่า PSA <4 ng/ml แต่ถ้าหาก PSA >4 ng/ml มีความเสี่ยงที่จะเป็น[16] ซึ่ง PSA มีความสัมพันธ์กับมะเร็งระยะลุกลามเฉพาะที่ และมะเร็งระยะสุดท้าย เมื่อ PSA >4-10 ng/ml พบว่ากลุ่มนี้ 50% มะเร็งมีการลุกลามออกนอกต่อมลูกหมาก ในกรณีที่ผู้ป่วยมีการกระจายของมะเร็งและกำลังรับการรักษาสามารถใช้ PSA เป็นตัวติดตามการรักษาได้อย่างดีเพราะผู้ป่วยเหล่านี้มักมีภาวะมะเร็งเข้ากระดูกซึ่งประเมินได้ยาก[11]

2. ระยะ diagnosis ได้แก่

- Prostatic Acid Phosphatase (PAP) ใช้ในการวินิจฉัยมะเร็งต่อมลูกหมาก มะเร็งเม็ดเลือดขาว มะเร็งต่อมน้ำเหลือง ชนิดนอนฮอดจ์กิน (non-Hodgkin's lymphoma) [17]

- Neuron-specific enolase (NSE) เป็นตัวชี้วัดที่ดีที่สุดในการวินิจฉัยโรคมะเร็งปอดชนิด small cell lung cancer และ neuroblastoma[18]

- Calcitonin หากพบในเลือดเยอะ เป็นสัญญาณบ่งบอกถึงการเป็นมะเร็งชนิด medullary thyroid cancer [19-20] ค่าปกติของ ผู้ชาย <8.5 pg/ml และ ผู้หญิง <5.0 pg/ml [22]

3. ระยะ Prognosis ได้แก่

- Carcinoembryonic antigen (CEA) พบได้บ่อยที่สุดในมะเร็งลำไส้ใหญ่ มะเร็งอื่น ๆ ที่พบ คือ มะเร็งตับอ่อน มะเร็งเต้านม หากผู้ป่วยมะเร็งลำไส้ระยะที่สอง ที่มีค่า CEA >5.2ng/ml จะมีโอกาสเกิดการเป็นซ้ำของโรคสูงกว่าผู้ป่วยในระยะเดียวกันที่มีค่า CEA ปกติถึง 1.6-3.2 เท่า [11] หลังจากการผ่าตัดแล้วค่า CEA ควรลดลงใน 6-8 สัปดาห์ หากไม่ลดลงจะบ่งบอกถึงการผ่าตัดออกไม่หมด หรืออาจเพราะเกิดการกระจายตัวของมะเร็งไปตั้งแต่ต้นแล้ว [11,13]

- ระดับของ beta HCG เป็นฮอร์โมนชนิดหนึ่งจะมีค่าขึ้นสูงในหญิงตั้งครรภ์ แต่จะสูงมากในผู้ป่วยมะเร็งรังไข่ มะเร็งเยื่อบุโพรงมดลูก ค่าปกติผู้หญิงไม่ตั้งครรภ์ <3.0 mIU/ml ภายหลังวัยทอง <6.0 mIU/ml และผู้ชาย <2.0 mIU/ml และ AFP สามารถบอกพยากรณ์โรคได้[18,23]

- Beta-2-Microglobulin (B2M) ใช้บอกถึงความรุนแรงของ มะเร็งชนิด multiple myeloma, มะเร็งเม็ดเลือดขาวชนิดเรื้อรัง ค่า B2M ปกติ <2.5 mg/L ถ้าในน้ำไขสันหลังมีระดับค่า B2M สูง อาจบอกถึงว่ามะเร็งได้แพร่กระจายไปยังสมองหรือไขสันหลัง [24]

- Lactate Dehydrogenase (LDH) ไม่จำเพาะเจาะจงในมะเร็งชนิดใดชนิดหนึ่ง [15]

4. ระยะ Monitoring treatment ได้แก่

- Alpha-fetoprotein (AFP) เป็น glycoprotein สร้างจาก yolk sac ตับ และทางเดินอาหารของทารกในครรภ์ โดยค่าจะเพิ่มขึ้นสูงและลดลงเมื่อทารกอายุได้ 1 ปี ค่าปกติในผู้ชายและผู้หญิงที่ไม่ได้ตั้งครรภ์จะอยู่ที่ 0-20 ng/ml แต่ในกลุ่มหญิงตั้งครรภ์ค่าจะสูงกว่าปกติ 2-3 เท่า ประเภทของมะเร็งที่สามารถตรวจพบ คือ เนื้องอกในเซลล์ต้นกำเนิด โดยเฉพาะเซลล์ต้นกำเนิดบางชนิดของรังไข่และอัณฑะ และยังพบมากในมะเร็งตับ [18,21,25-26]

- CA 19-9 เป็น tumor marker ที่ดีที่สุดในการวินิจฉัยและติดตามผลการรักษาของมะเร็งตับอ่อนมะเร็งท่อน้ำดี มะเร็งลำไส้ใหญ่ มะเร็งลำไส้ตรง และ มะเร็งกระเพาะอาหาร การอักเสบของตับอ่อนและท่อน้ำดีค่าจะ <1,000 U/ml ในกรณีที่ค่า CA 19-9 ที่มีระดับสูงมาก >100-120 ng/ml จะช่วยทำให้การวินิจฉัยโรคมีความแม่นยำมากขึ้น [11] และค่า CA19-9 ที่ >1,000 ng/ml นั้นมักบ่งบอกถึงโรคที่ไม่สามารถทำการผ่าตัดได้และมักมีการกระจายตัวของมะเร็งแล้ว หากหลังการผ่าตัดค่า CA19-9 ไม่กลับสู่ปกติผู้ป่วยจะมีอัตราการอยู่รอดต่ำ [11,13]

- CA 15-3 ใช้ในการติดตามผลการรักษาของผู้ป่วยหลังจากผ่าตัดเต้านม [11]

- CA 27-29 ใช้ในการติดตามการรักษาของมะเร็งเต้านมระยะลุกลาม [27] ค่า CA 27-29 ≤38 U/ml [28]

- หากใช้ Beta-human chorionic gonadotropin (beta HCG) ร่วมกับ AFP จะติดตามผลการรักษาและใช้ติดตามผู้ป่วยในกลุ่มที่เป็นมะเร็งต่อมลูกหมากได้[11]

- Prostate specific antigen (PSA) เป็นตัวติดตามการรักษามะเร็งต่อมลูกหมากได้อย่างดี [11]

- Neuron-specific enolase (NSE) เป็นตัวชี้วัดที่ดีที่สุดในการติดตามผลการรักษามะเร็งปอด [15,18]

- Lactate Dehydrogenase (LDH) ระดับค่าของ LDH ใช้ในการติดตามผลการรักษาของมะเร็งได้ [29]

5. Detection of relapse ได้แก่

- prostate specific antigen (PSA) เป็นตัวติดตามการเป็นซ้ำได้อย่างดี[11]

- CA 125 เป็นสาร glycoprotein ที่พบอยู่บนผิวของเซลล์ที่มีต้นกำเนิดมาจากเซลล์ตัวอ่อนทารกชนิด embryonic coelomic epithelium ค่าปกติจะอยู่ที่ 0-35 U/ml[18,30] ช่วยประเมินการเป็นซ้ำของโรคหลังผ่าตัดจากมะเร็งรังไข่[11] CA 125 มักมีค่าสูงขึ้นในผู้ป่วยมะเร็งรังไข่ รวมทั้งมะเร็งตับอ่อน มะเร็งปอด เป็นต้น นอกจากนี้ยังมีค่าสูงกว่าปกติในหญิงตั้งครรภ์ ในมะเร็งรังไข่ระยะที่ 1 และ 2 พบค่า CA 125 สูงเพียงร้อยละ 20 และน้อยกว่าร้อยละ 10 ตามลำดับ แต่ในผู้ป่วยมะเร็งรังไข่ระยะลุกลามพบค่า CA 125 สูงถึงร้อยละ 80 นอกจากนี้ค่าเฉลี่ยของ CA 125 ที่ใช้มีความแตกต่างกันไปตามเชื้อชาติ การสูบบุหรี่ และอายุ จึงไม่แนะนำให้ใช้ค่า CA 125 เพียงอย่างเดียวในการคัดกรองมะเร็งรังไข่[13]

- Calcitonin ใช้ในการติดตามการรักษามะเร็งชนิด medullary thyroid ได้ ถ้ามี calcitonin ในระดับที่สูงอาจบ่งบอกได้ว่า การรักษานั้นไม่ได้ผลหรือกลับมาเป็นมะเร็งอีกครั้งหลังการรักษา [22]

- Alpha -fetoprotein (AFP)

- CA 19-9

- CA 15-3

เทคนิคการตรวจ tumor markers[31]

ควรใช้วิธีการทดสอบที่มีความไวสูง ซึ่งจะช่วยสามารถตรวจปริมาณ tumor markers ที่มีปริมาณเพียงเล็กน้อยได้ ชุดทดสอบควรมีความจำเพาะต่อ tumor markers ให้มากที่สุด วิธีที่เหมาะสมในปัจจุบันจึงเป็น Immunoassay โดยอาจเป็นวิธี RIA/EIA/CICA การรบกวนผลการทดสอบ ในปฏิกิริยา immunoassay

ตามทฤษฎีแล้วจะมีความเสี่ยงที่จะเกิดการรบกวนของผลทดสอบได้ โดยปัญหาที่พบในห้องปฏิบัติการที่สำคัญ ได้แก่

• High dose Hook Effect

เมื่อใช้ตรวจหาแอนติเจนที่มีความเข้มข้นสูงมากเกินไป จะเกิดผลต่ำปลอม ซึ่งกรณีนี้ปฏิกิริยาการจับกันระหว่าง แอนติเจน-แอนติบอดีถูกกีดขวางโดยแอนติเจนที่มีปริมาณสูงมากเกินไป ซึ่งวิธีแก้ไขโดยการเจือจางตัวอย่างที่มีแอนติเจนสูงก่อนทำการทดสอบ

• Heterophile antibodies

ในตัวอย่างทดสอบบางรายมี heterophile antibodies อยู่ในน้ำเหลือง โดยเฉพาะ human anti mouse antibodies ซึ่งวิธีการทดสอบส่วนใหญ่จะใช้ monoclonal antibodies จากหนู ซึ่งจะทำให้เหมือนเกิดปฏิกิริยาขึ้นถึงแม้จะไม่มีแอนติเจนในน้ำเหลืองเลย ทำให้ได้ค่าผลบวกปลอมได้

สรุป (Conclusion)

การตรวจหาตัวบ่งชี้ทางชีวภาพสำหรับมะเร็งมีความสำคัญเป็นอย่างยิ่งในการตรวจหามะเร็ง แต่ทั้งนี้ยังไม่แน่นอนต้องพิจารณาผลทางคลินิกและห้องปฏิบัติการอื่น ๆ ร่วมด้วย เพื่อความแน่ชัดในการติดตามอาการและการรักษาได้อย่างแม่นยำ

เอกสารอ้างอิง (References)

(1.) กรุงเทพธุรกิจ [อินเทอร์เน็ต]. กรุงเทพฯ: [ม.ป.พ]; c2017. 'มะเร็ง' สาเหตุคนไทยตายอันดับ 1; 2562 [เข้าถึงเมื่อ 20 มิ.ย. 2564]; [1 ย่อหน้า]. เข้าถึงได้จาก: https://www.bangkokbiznews.com/news/detail/838695

(2.) โรงพยาบาลพญาไท [อินเทอร์เน็ต]. กรุงเพทฯ: [ม.ป.พ.]; c2016. รู้จัก “โรคมะเร็ง” ภัยร้ายที่เป็นสาเหตุการเสียชีวิตอันดับต้นๆ; c2016. โรคมะเร็งสาเหตุการตายอันดับ 1; 2562 [เข้าถึงเมื่อ 20 มิ.ย. 2564]; [1 ย่อหน้า]. เข้าถึงได้จาก: https://www.phyathai.com/article_detail/2886/th/รู้จัก_“โรคมะเร็ง”_ภัยร้ายที่เป็นสาเหตุการเสียชีวิตอันดับต้นๆ

(3.) Smart news [อินเทอร์เน็ต]. กรุงเทพฯ: บุฟเฟต์ เฟมัส จำกัด; c2021. โรคมะเร็งถือเป็นสาเหตุการเสียชีวิตอันดับต้นๆ ของคนทั่วโลก; 2663 [เข้าถึงเมื่อ 5 ก.ค. 2564]; [1 ย่อหน้า]. เข้าถึงได้จาก: http://www.ashthailand.or.th/smartnews/post/detail/558/5

(4.) อำนาจ คงมณี [อินเตอร์เน็ต]. กรุงเทพฯ: [ม.ป.พ.]; 2564 แนวคิดเกี่ยวกับเซลล์ต้นกำเนิดของมะเร็ง; [ม.ป.ป.] [เข้าถึงเมื่อ 20 มิ.ย. 2564]; [1 ย่อหน้า.]. เข้าถึงได้จาก: https://www.si.mahidol.ac.th/department/Biochemistry/home/articles/cancer%20stem%20cells.htm

(5.) National Library of Medicine [Internet]. Maryland: The library; 2020. Cancer stem cells: models and concepts; 2007 [cited 2021 Jul 5]; [about 1 paragraph.]. Available from: https://pubmed.ncbi.nlm.nih.gov/17002552/

(6.) Cancer Hospital Wattanosoth [อินเทอร์เน็ต]. กรุงเทพฯ: ศูนย์การแพทย์กรุงเทพดุสิต; c2021. ระยะของมะเร็ง; 2564 [เข้าถึงเมื่อ 20 มิ.ย. 2564]; [11 ย่อหน้า]. เข้าถึงได้จาก: https://www.wattanosoth cancerhospital.com/all-about-cancer/general-information-about-cancer

(7.) Scimath [อินเทอร์เน็ต]. กรุงเทพฯ: สถาบันส่งเสริมการสอนวิทยาศาสตร์และโทคโนโลยี, กระทรวงศึกษาธิการ; c2018. การตรวจวัดสารบ่งชี้มะเร็ง; 2561 [เข้าถึงเมื่อ 5 ก.ค. 2564]; [3 ย่อหน้า]. เข้าถึงได้จาก: https://www.scimath.org/article-biology/item/7939-tumor-markers

(8.) Scimath [อินเทอร์เน็ต]. กรุงเทพฯ: สถาบันส่งเสริมการสอนวิทยาศาสตร์และโทคโนโลยี, กระทรวงศึกษาธิการ; c2018. สารบ่งชี้มะเร็ง (Tumor marker) คืออะไร?; 2561 [เข้าถึงเมื่อ 5 ก.ค. 2564]; [2 ย่อหน้า]. เข้าถึงได้จาก: https://www.scimath.org/article-biology/item/7939-tumor-markers

(9.) LAB FINDINGS IN CANCER & TUMOR MARKERS [อินเทอร์เน็ต]. [ม.ป.ท.]: [ม.ป.พ.]; 2564. Characteristics & Classification of Tumor Marker; 2564 [เข้าถึงเมื่อ 6 ก.ค. 2564]; [1 ย่อหน้า]. เข้าถึงได้จาก: http://www.cai.md.chula.ac.th/lesson/lesson4809/html/2f.html

(10.) National Library of Medicine [Internet]. Maryland: The Library; 2020. Tumor markers in clinical practice: General principles and guidelines; 2007 [cited 2021 Jul 5]; [about 3 paragraphs]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2902207/

(11.) เฉลิมพร โรจน์รัตน์ศิริกุล [อินเตอร์เน็ต]. [ม.ป.ท.]: [ม.ป.พ.]; 2564 Clinical use of tumor marker in the follow-up of cancer patient; [ม.ป.ป.] [เข้าถึงเมื่อ 4 ก.ค. 2564]; [1,4,7,9,11-12.]. เข้าถึงได้จาก: http://medinfo.psu.ac.th/surgery/document/SerumTumorMarker.pdf

(12.) Siriraj E-Public Library Faculty of Medicine Siriraj Hospital [อินเตอร์เน็ต]. กรุงเทพฯ: [ม.ป.พ.]; [ม.ป.ป.]. ตรวจเลือด หามะเร็งได้จริงหรือ?; 2553 [เข้าถึงเมื่อ 4 ก.ค. 2564]; [1 ย่อหน้า.]. เข้าถึงได้จาก: https://www.si.mahidol.ac.th/sidoctor/e-pl/articledetail.asp?id=619

(13.) มะเร็งวิทยาสมาคมแห่งประเทศไทย [อินเตอร์เน็ต]. กรุงเทพฯ: [ม.ป.พ.]; c2559. How to approach asymptomatic patients with rising tumor marker; 2560 [เข้าถึงเมื่อ 4 ก.ค. 2564]; [6 ย่อหน้า.]. เข้าถึงได้จาก:http://www.thethaicancer.com/Webdocument/GParticle 006 .html

(14.) กฤษฎา ศิริสภาภรณ์, บรรณาธิการ. THAM-LAB. Prostate Cancer มะเร็งต่อมลูกหมาก [อินเทอร์เน็ต]. มี.ค. 2564 [เข้าถึงเมื่อ 6 ก.ค. 2564];5(1):3. เข้าถึงได้จาก: https://www.hospital.tu.ac.th/lab/PDF/ THAM-LAB%2010.pdf

(15.) Basic medical key [Internet]. [place unknown]: [publisher unknown]; 2016. TYPES OF TUMOR MARKERS; 2017 [cited 2021 Jul 5]; [about 6 paragraphs]. Available from: https://basicmedicalkey.com/circulating-tumor-markers-basic-concepts-and-clinical-applications/

(16.) Nation Comprehensive Cancer Network [Internet]. c2019. Prostate Cancer Early Detection; 2019 [cited 2021 Jul 5]; [about 6 p.]. Available from: https://www2.tri-kobe.org/nccn/guideline/urological/english/prostate detection.pdf

(17.) Stanford Health Care [Internet]. California: The Association; c2020. Prostatic Acid Phosphatase (PAP); [date unknown] [cited 2021 Jul 5]; [about 1 paragraphs.]. Available from: https://stanfordhealthcare.org/medical-tests/t/tumor-markers/types/prostate-acid-phosphatase-pap.html

(18.) Siriraj E-Public Library Faculty of Medicine Siriraj Hospital [อินเตอร์เน็ต]. กรุงเทพฯ: [ม.ป.พ.]; 2564 สารบ่งชี้มะเร็ง (tumor marker) ที่ควรรู้จัก; 2553 [เข้าถึงเมื่อ 4 ก.ค. 2564]; [6 ย่อหน้า.]. เข้าถึงได้จาก: https://www.si.mahidol.ac.th/sidoctor/e-pl/articledetail.asp?id=618

(19.) สมจินต์ จินดาวิจักษณ์, วิศณุ ปานจันทร์, อาคม ชัยวีระวัฒนะ, วีรวุฒิ อิ่มสำราญ, บรรณาธิการ. แนวทางการตรวจวินิจฉัยและรักษาโรคมะเร็งต่อมไทรอยด์ [อินเทอร์เน็ต]. พิมพ์ครั้งที่ 1. กรุงเทพฯ: สถาบันมะเร็งแห่งชาติ, กรมการแพทย์, กระทรวงสาธารณสุข; 2558 [เข้าถึงเมื่อ 5 ก.ค. 2564]. เข้าถึงได้จาก: https://www.nci.go.th/th/cpg/Cervical_Cancer4.pdf

(20.) The New England Journal of Medicine [Internet]. Massachusetts: The Association; c2021. Calcitonin as a Tumor Marker; [date unknown] [cited 2021 Jul 5]; [about 1 paragraphs.]. Available from: https://www.nejm.org/doi/full/10.1056/NEJM197405092901901

(21.) Medthai [อินเทอร์เน็ต]. [ม.ป.ท.]: [ม.ป.พ].; c2014-2021. การตรวจหาสารบ่งชี้มะเร็งตับ (AFP:Alpha-Fetoprotein); 2018 [ปรับปรุงเมื่อ 25 พ.ค. 2018; เข้าถึงเมื่อ 6 ก.ค. 2564]; [4 ย่อหน้า]. เข้าถึงได้จาก: https://medthai.com/%E0%B8%81%E0%B8%B2%E0%B8%A3%E0%B8%95%E0%B8%A3%E0%B8%A7%E0%B8%88-afp/

(22.) National Library of Medicaine [Internet]. Maryland: The library; 2020. Calcitonin Test; 2021 [updated 2021 Feb 18; cited 2021 Jul 5]; [about 1 paragraph.]. Available from: https://medlineplus.gov/lab-tests/calcitonin-test/

(23.) AMPROHEALTH [อินเทอร์เน็ต]. [ม.ป.ท.]: [ม.ป.พ].; 2561. การตรวจเลือดวิเคราะห์ฮอร์โมน (Hormone Assays); 2561 [เข้าถึงเมื่อ 6 ก.ค. 2564]; [1 ย่อหน้า]. เข้าถึงได้จาก: https://amprohealth.com/ checkup/hormone-assays/

(24.) National Library of Medicaine [Internet]. Maryland: The Library; 2020. Beta 2 Microglobulin (B2M) Tumor Marker Test; 2020 [updated 2020 Dec 10; cited 2021 Jul 7]; [about 2 paragraphs.]. Available from: https://medlineplus.gov/lab-tests/beta-2-microglobulin-b2m-tumor-marker-test/

(25.) อารีย์ ปะสิทธิพยงค์, อนันต์ กรลักษณ์, วิชุดา ไตรรัตน์อภิชาต, ผ่องพรรณ รมหิรัญ. ความสัมพันธ์ระหว่างระดับ AFP ในซีรั่มกับไวรัสตับอักเสบบี อายุและเพศของผู้ที่มารับบริการตรวจที่สถาบันมะเร็งแห่งชาติ. วารสารมะเร็ง THAI CANCER JOURAL. [อินเทอร์เน็ต]. กรกฎาคม-กันยายน 2552 [เข้าถึงเมื่อ 6 ก.ค. 2564]; 39: 109.
เข้าถึงได้จาก: http://ams2.kku.ac.th/fileaf/suchat/Cholangiocarcinoma/_AFP .pdf

(26.) โสภณ เคหาไสย, บรรณาธิการ. จุลสารโรงพยาบาลมะเร็งอุดรธานี. องค์ความรู้เรื่องสารสัมพันธ์มะเร็ง [อินเทอร์เน็ต]. ต.ค.-ธ.ค. 2558 [เข้าถึงเมื่อ 6 ก.ค. 2564]; 13: 5 - 7. เข้าถึงได้จาก: https://www.udch.go.th/uploads/doc/journal/2558/v59.pdf

(27.) Stanford Health Care [Internet]. California: The Association; c2020. CA 27-29 Tumor Marker; [date unknown] [cited 2021 Jul 5]; [about 1 paragraph.]. Available from: https://stanfordhealthcare.org/medical-tests/t/tumor-markers/types/ca-27-29.html

(28.) University of Rochester medical center [Internet]. New York: [publisher unknown]; c2021. CA 27-29; 2021 [cited 2021 Jul 7]; [about 1 paragraph.]. Available from: https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=ca_27_29

(29.) Stanford Health Care [Internet]. California: The Association; c2020. Lactate Dehydrogenase (LDH); [date unknown] [cited 2021 Jul 5]; [about 1 paragraph.]. Available from: https://stanfordhealthcare.org/medical-tests/t/tumor-markers/types/lactate-dehydrogenase-ldh.html

(30.) ลักขณา สุวรรณน้อย. มหาวิทยาลัยมหิดล คณะเภสัชศาสตร์ [อินเทอร์เน็ต]. กรุงเทพฯ: งานเทคโนโลยีสารสนเทศและสื่อการเรียนการสอน คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล; c2013-2020 [เข้าถึงเมื่อ 6 ก.ค. 2564] เข้าถึงได้จาก: https://pharmacy.mahidol.ac.th/th/knowledge/article/416/%E0%B8%AA%E0%B8%B2%E0%B8%A3%E0%B8%9A%E0%B9%88%E0%B8%87%E0%B8%8A%E0%B8%B5%E0%B9%89%E0%B8%A1%E0%B8%B0%E0%B9%80%E0%B8%A3%E0%B9%87%E0%B8%87-tumormarkers/

(31.) เส้นทางสุขภาพ [อินเทอร์เน็ต]. [ม.ป.ท]: [ม.ป.พ]; c2007-2009. เทคนิคการตรวจ tumor makers; [ม.ป.ป] [เข้าถึงเมื่อ 5 ก.ค. 2564]; [4 ย่อหน้า]. เข้าถึงได้จาก: http://yourhealthyguide.com/article/ac-tumor-markers.html

CASE REPORT

METANEPHRIC ADENOMA WITH METASTASIS AFTER 13 YEARS

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Abstract

Metanephric adenoma is a rare epithelial tumor of kidney. Even though it is considered benign with an excellent prognosis, a few cases of metastatic disease have been reported. Therefore, the exact biological behavior of metanephric adenoma is not well established. Herein we report a case of 51-year-old male with metastatic disease after thirteen years of diagnosis.

Keywords: Metanephric adenoma, renal cell carcinoma, immunohistochemistry, metastasis, Wilms' tumor

Introduction

Metanephric adenoma (MA) is a rare epithelial neoplasm of kidney first described by Bove in 1979[(1-2)]. It comprises of approximately 0.2% of adult renal epithelial neoplasms [(3)]. Less than 200 cases of MA have been reported in literature globally till date and have limited

clinical data [4]. MA is considered as a benign tumor with excellent prognosis [5-6]. However, few cases showing its potential to become malignant and metastasize have been reported. Therefore, the nature and biological behavior of this tumor is not clear. We report a case metanephric adenoma with multiple lymph nodes and bone metastasis after 13 years of diagnosis.

Case Report

Fifty one year old male presented to our OPD with pain abdomen for a week. His general and systemic examination was unremarkable. The patient had undergone right nephrectomy 13 years back at our institute and was diagnosed as metanephric adenoma on the basis of histomorphology and immunohistochemistry (CK7 and Chromogranin Negative). Laboratory examination at present revealed normal Complete Blood Count, liver function tests and kidney function tests. His serum amylase and lipase were elevated. The patient was diagnosed clinically as pancreatitis. CECT abdomen was advised to see any pancreatic abnormalities and revealed mild inflammation in the pancreas with no intrinsic pancreatic abnormality. Multiple enlarged retroperitoneal lymph nodes and multiple sclerotic lesions in vertebra, lower ribs and bilateral pelvic bones were seen; suggestive of metastasis. Further investigations were also done to see other organ involvement, which did not reveal any abnormality. So, CT guided FNAC was done to investigate the primary site and to rule out metastasis of MA. The aspirated smears were highly cellular and showed a monotonous population of tumor cells arranged in sheets, cords, clusters, acini and dispersed singly (Figure 1). These cells showed bland nuclear chromatin and scant amount of cytoplasm. Cell block preparation revealed similar features with no evidence of atypia or mitosis (Figure 2). Immunohistochemically it showed diffuse positive stain for CD57 (Figure 3a) and negative stain for CK7, CK20, CD 56, WT1, AMACR (Figure 3b) and PAX8. Negative staining for CK7, AMACR ruled out papillary RCC whereas WT1 as well as CD 56 negativity ruled out Wilms' tumor. On the basis of morphological features and immunohistochemistry, diagnosis of metastatic disease was suggested.

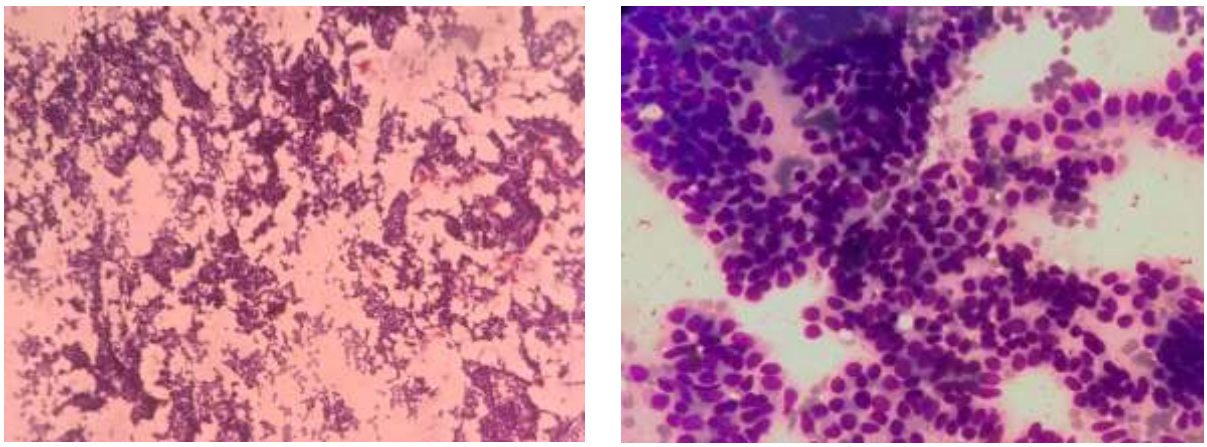

Figure 1. Photomicrograph showing uniform tumor cells; a) Pap stain(400X); b) Giemsa Stain. (40X)

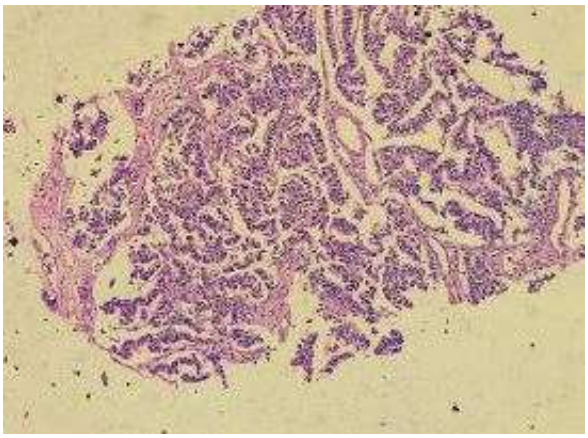

Figure 2. Cell block preparation showing tumor arranged in cords, tubules and acini; HE stain. (400X)

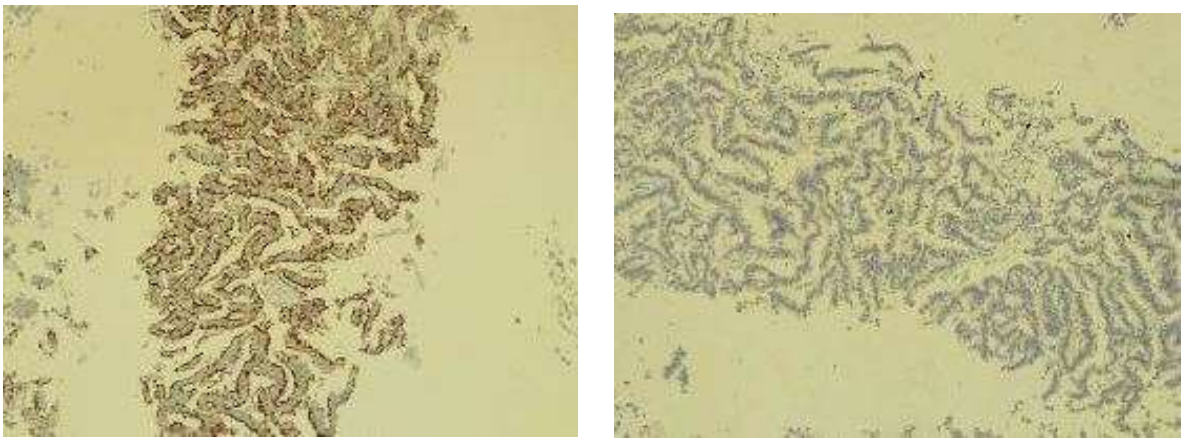

Figure 3. a) Diffuse positive immunostain for CD57; b) Negative immunostain for AMACR.

Discussions

Metanephric adenoma is a rare benign epithelial tumor of the kidney which can clinically and histologically mimic other malignant neoplasms of kidney [1-3]. It has a wide age distribution, but has a peak age occurrence in 5th to 6th decade of life with female preponderance [7]. However, its occurrence in children as young as 7 years has also been reported [2,8]. The majority of the patients are asymptomatic and discovered incidentally while undergoing examination for other reasons [5]. Some patients have presented with flank pain, hematuria or abdominal mass. There is an increase in incidence of polycythemia in MA [4].

Some theories have postulated that metanephric adenoma is derived from remnants of metanephric blastemal or embryonal renal tissue and is considered to be hyperdifferentiated form of Wilms' tumor and can sometimes co-exist with it [1,9]. Microscopically, it shows closely packed tubules, acini, solid patterns having uniform cells with bland nuclear chromatin, inconspicuous nucleoli and scant amount of cytoplasm [4]. Small glomeruloid bodies with papillary projections are also seen. Some cases may present with atypical features showing pleomorphism, increased mitosis and lacking typical architecture of tubules and acini seen in classical MA making the diagnosis more challenging [1]. It has been reported in some studies, that these subsets of tumor with atypical features may have some potential for metastasis [8]. However, the criteria for classification into these subtypes have not been established. These MA with atypical features should be differentiated from other malignant tumors like Wilms' tumor, especially in children and solid variant of papillary renal cell carcinoma (RCC) in adults [3,6-7]. The concept of composite MA with the co-existing malignant component such as RCC has been emphasized in the literature [1]. The presence of macrophages, hemosiderin deposits in the stroma, cytologic atypia, more amount of cytoplasm, prominent nucleoli should be carefully looked for. Metastatic deposits from thyroid and lung should also be considered. Therefore, careful morphological examination with immunohistochemical evaluation is necessary for accurate diagnosis. Immunopositivity for WT1 and CD57 as well as negativity for CK7 and AMACR are characteristic markers of MA [5,10]. However, our case exhibited discordance with this finding being negative for WT1. Negative stains for AMACR and CK 7 helps in excluding the diagnosis of papillary RCC. Wilms' tumor shows positive stain for WT1 and negative stain for CD57. Regarding molecular and genetic studies, BRAF V600E mutation is present in about 85% of MA [3].

Even though metanephric adenoma is considered benign, few cases of metastatic disease have been reported in literature [4,7]. Renshaw et al reported a case of MA with metastasis to para-aortic, hilar and aortic bifurcation lymph nodes and concluded that tumors with histological, immunohistochemical and genetic features of metanephric adenoma can present with metastasis [8]. Some authors have attributed to regional lymph node involvement as passive mechanical seedling of tumor into the lymph nodes rather than true metastasis [11]. Nevertheless, one case of MA with bone metastasis and other case with lung metastasis after 46 months of diagnosis have been mentioned in literature [1,4]. The most unique aspect of our patient is incidental detection of lymph node and bone metastasis after 13 years, which is by far the longest duration till date.

Conclusion

Even though MA is considered a benign tumor, its potential to metastasize has been discovered requiring regular follow up and long term surveillance of the patients. The significance of such findings on prognosis and survival of the patients is not known. Therefore, more studies are required to establish the exact biological behavior of these tumors.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

(1). Li G, Tang Y, Zhang R, et al. Adult metanephric adenoma presumed to be all benign? A clinical perspective. BMC Cancer [Internet]. 2015; 15: 1–8.

(2). Kuroda N, Toi M, Hiroi M, et al. Review on metanephric adenoma of kidney with focus on clinical and pathobiological aspects. Histol Histopathol. 2003; 18: 935 –42.

(3). Shangpliang D, Baishya P, Mishra J, et al. Metanephric Adenoma with cystic changes- An uncommon presentation of a rare tumor in a young adult. Autops Case Rep [Internet]. 2020;10(3):e2020148. https://doi.org/10.4322/ acr.2020.148

(4). Patil P, Bhatia V, Arole V, et al. Metanephric adenoma of kidney: a rare and distinct entity. Int J Res Med Sci. 2016; 4: 950–2.

(5). Zhang L, Gao X, Li R, et al. Experience of diagnosis and management of metanephric adenoma: Retrospectively analysis of 10 cases and a literature review. Transl Androl Urol. 2020; 9: 1661–9.
(6). Yu L, Hao D, Man F, et al. Metanephric adenoma: Two cases report and review of the literature. Int J Clin Exp Med. 2017; 10: 1424–8.
(7). Obulareddy SJ, Xin J, Truskinovsky AM, et al. Metanephric adenoma of the kidney: an unusual diagnostic challenge. Rare Tumors. 2010; 2: 103–5.
(8). Renshaw AA, Freyer DR, Hammers YA. Metastatic metanephric adenoma in a child. Am J Surg Pathol. 2000; 24: 570–4.
(9). Jia D, Liu H, Kong Q, et al. Pure epithelial wilms' tumor in a 57-year-old patient: a case report and literature review. Int J Clin Exp Med. 2019; 12: 6223–30.
(10). Kinney SN, Eble JN, Hes O, et al. Metanephric adenoma: The utility of immunohistochemical and cytogenetic analyses in differential diagnosis, including solid variant papillary renal cell carcinoma and epithelial-predominant nephroblastoma. Mod Pathol. 2015; 28: 1236–48.
(11). Katan R, Zizlavska M. Metanephric Adenoma. A case report and literature review. Cesko Patol. 2018; 55: 165-9.

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*
- *Keywords: 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*
  3. Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Bloom SL, Wenstrom KD. Hypertensive disorders in pregnancy. In: Cunningham FG, Hauth JC, Leveno KJ, Gilstrap L III, Brom SL, Wenstrom KD, eds. Williams Obstetrics, 22nd ed. New York: McGraw-Hill, 2005: 761 – 808.

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.

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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.

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Step 6: Upload your manuscript file (s).

Step 7: Re-check the content of your manuscript (s) and the uploaded file (s) more carefully prior to the submission. If you have submitted your manuscript file (s) incorrectly, you must contact Editor-in-Chief of Asian Archives of Pathology immediately. The Editor-in-Chief can clear the incorrect attempt and allow you another submission.

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ACADEMIC MEETINGS AND CONFERENCES

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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