Original Article

Epidemiology and Efficacy of Immunohistochemistry Investigation of CD34, CD117, DOG-1 and SMA for GIST in Rajavithi Hospital

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Background: Gastrointestinal Stromal Tumor (GIST), the most common gastrointestinal mesenchymal malignancy, requires immunohistochemistry (IHC) for pathological diagnosis and can be treated by targeted therapy or personalized medicine, which produce considerably fewer side-effects than conventional chemotherapy. The panel of antibodies used in different categories of hospitals and medical centers may vary, and the epidemiology of the tumor seems to be different in eastern and western patients. The present study aims to reorder suitable panels for tertiary care centers in terms of their efficacy as proven by research evidence.

Objective: To study the epidemiology and effectiveness of immunohistochemistry investigation of CD34, CD117, DOG-1 and SMA in GIST in Rajavithi Hospital (RJVH) from 2012 to 2017.

Materials and Methods: Data were retrospectively collected from all cases which had been pathologically diagnosed as GIST in the RJVH database from 2012 to 2017. Epidemiological information and effectiveness of immunohistochemistry investigation of CD34, CD117, DOG-1 and SMA were recorded.

Results: The study data consisted of 119 records. All subjects were of Thai nationality, most were female (52.9%), and their ages ranged from 27 to 84 years old, with an average of 59.35 years old. Patient expense per day varied from 3,087.5 to 130,100.0 THB (Thai Baht) and was 9,388.4 THB on average. The turnaround time (TAT) of IHC in 2, 3, 4 and 5 days was in 4, 108, 6 and 1 cases respectively, with most reported within 3 days. The most common organ involvements were stomach (41%), duodenum (8.5%) and rectum (8.5%), and the most prevalent secondary site was the liver (6.9%). The results of expression of the panel of IHC were recorded, and extragastrointestinal GIST was found 28.1% of cases.

Conclusion: Epidemiologically, there were some differences in age and sex of patients in the present study compared with those of western papers, but these data were similar to those of other eastern ones. The common primary and secondary sites, as well as the order of positive IHC expressions, were similar to those in the literature. With regard to efficacy, standard guidelines for large-scale hospitals should include DOG-1 in the first panel because of its advantages in terms of time, expense and diagnostic confidence.

Keywords: Epidemiology, Worthiness, GIST, Immunohistochemistry, IHC, CD34, CD117, DOG-1, SMA

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Gastrointestinal stromal tumor (GIST), the most common sarcoma of the gastrointestinal tract, was first described by Mazur and Clark in 1983[1]. The incidence of GIST is only 0.2% in gastrointestinal neoplasms but 80% in malignant mesenchymal neoplasms of the gastrointestinal tract[2,3]. The most common site is the stomach, and less common sites include the small intestine and colon[4]. The order of prognostic risk, from low to high, is the esophagus, stomach, duodenum, small intestine, extra-gastrointestinal GIST[5-8] and colon[9]. GIST is found most commonly in patients aged 40-50 years old, and its incidence in males is a little higher than in females[10]. The most common type of mutation in GIST is c-kit exon 11, and individualized medicine is preferred for GIST because of its high mutation rates and types[4,11-13]. GIST requires immunohistochemistry (IHC) for pathological diagnosis and preparation of treatment plans[14-18]. Recently, many related molecular studies[13,19-21] have examined targeted therapy of the tyrosine kinase group[20] which play an important role in specific treatment for this neoplasm[13,16,22], and one of these studies found that treatment by imatinib mesylate caused phenotypic change[23]. The liver has been found to be the most common metastatic organ in a number of studies[24]. One study examined the coexistence of gastrointestinal stromal tumor with esophageal and gastric cardia carcinomas[25] and poorly-differentiated neuroendocrine carcinoma[26]. The pathogenesis of GIST shows there are many features similar to interstitial cells of Cajal (ICC)[27]. Electron microscopic examination shows the mixed features of neurological and muscular tissue[21,28]. The specific antibody

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used in the initial phase was CD34, but later on, the antibody CD117 was found to yield more specificity and sensitivity than CD34. CD117 (or c-kit), is a proto-oncogene, meaning that overexpression or mutations of this protein can lead to cancer. The efficacy of imatinib (trade name Gleevec), a CD117 inhibitor, depends on the mutation status of CD117 which is therefore the companion test for individualized cancer therapy. At the present time, there is another acceptable antibody, DOG-1 (discovered on GIST-1) which is positive in GIST and in some cases of CD117-negative GIST, and which also responds to anti-KIT therapy. For reasons of cost-effectiveness, we had to decide whether to include all three antibodies in the same panel or separate them into two panels. Before the era of diagnosis of GIST by these antibodies, most cases were diagnosed as smooth muscle neoplasm, so the antibody of smooth muscle actin (SMA) ought to be part of the minimal panel. The results can serve as an evidence base for recommendation of the most effective panel, and epidemiological results of the study will be compared to those in the literature.

In the review of literature, there has been no study of the epidemiology and efficacy of these IHC tests in RJVH or indeed in Thailand, and this may be important information to consider when adapting the local standard pathological practice.

Materials and Methods

This was a retrospective descriptive study which was approved by the Ethics Committee of Rajavithi Hospital (No.198/2561).

We collected data retrospectively from the records of all cases which were pathologically diagnosed as GIST in RJVH from 2012 to 2017. In data analysis, we use descriptive statistics and reported them by number, percent, average, and maximum and minimum.

Pathological diagnoses were performed in accordance with standard practice in almost all pathology departments. They were derived from clinical information in the request form, gross findings and microscopic findings. Microscopic examination was performed with basic Hematoxylin-Eosin (H&E) stain of 2 to 4 micron thick tissue sections on glass-slides, and cases suggestive of GIST via H&E were sent for IHC investigation. The tissue sections were taken from paraffin blocks, all of which underwent deparaffinization by Xylene and rehydration with concentrations of alcohol. The paraffin blocks make the tissue easier to cut into sections on glass-slides. The tissue was taken after gross examination.

For IHC, we increased immunoreactivity by microwave antigen retrieval at 750W for 30 minutes in a Citrate buffer (pH 6.0), after blocking the endogenous peroxidase activity by hydrogen peroxidase for 10 minutes. We performed primary antibody incubation at 4 degrees Celsius overnight, and labeling of immunoreactive staining was done with the avidin-biotin-peroxidase method using a Histostatin-plus kit (Zymed, CA, USA) Antigen-antibody reaction can be seen with 3-amino-9-ethycarbazole as Chromogen with Mayer's hematoxylin counterstain for exclusion of equivocal reactions.

Results

The results of the study were derived from the 119 records of all cases that were pathologically diagnosed as GIST from 2012 to 2017. All patients were of Thai nationality, and the majority were female (52.9%). The subjects' ages ranged from 27 to 84 years old, with an average of 59.35 years old.

The cost of hospital stay at the discharge time varied from 3,087.5 to 130,100.0 THB (Thai Baht) per day, with an average of 9,388.4 THB. Turnaround time (TAT) of IHC in 2, 3, 4 and 5 days was 4, 108, 6 and 1 case with most reported within 3 days.

The most commonly-involved organ was the stomach followed by the duodenum and rectum. Total small and large intestine involvement amounted to 13 and 20 cases respectively. Extra-gastrointestinal GIST was found in 32 cases (24.81%). Organ involvement is shown in Table 1. Some of the 119 records reviewed had more than one organ involvement. The liver has been found to be the most common metastatic site in a number of other studies.

The expression of CD34, CD117, DOG-1 and SMA is shown in Table 2.

Two cases were negative for CD117 and DOG-1, 2 cases were negative for CD117 and positive for DOG-1, and 2 cases were negative for CD117 but not tested for DOG-1, as shown in Table 3.

Discussion

In terms of epidemiology, the most common GIST sites found were similar to those in the literature[2,4,10]: stomach, small intestine and colon, while the most common metastatic site was the liver, in line with findings in the literature review[24]. The incidence of extra-gastrointestinal GIST or GIST that was not related to tubular GI-tract in this study (28.1%) was higher than the cases (10%) in another paper by Miettinen M[2], while the average age of our patients was similar (59.35 years) to the median age of those in that study (55 to 60 years)[2]. The proportion of males to females in this all-Thai study was different from that of western populations: in this study, there were slightly more females than males, while in the western literature[10] there are more males than females. Our study composition was, however, similar to that of other eastern populations, in which females slightly outnumber males[12], and this may be due to the genetic influences of different races. The order of percentage of positive results, in descending order, of the expression of the panel of IHC in this study {CD117 (94.5%), DOG-1 (90.0%), CD34 (78.9%) and SMA (25.4%)} was similar to the order of IHC expressions in the reference paper[29]: {CD117 (100%), DOG-1 (89%), CD34 (62%) and SMA (5%)}. The rate of DOG-1 positivity in CD117 negative cases reported ranged widely from 20 to 100% in the same research[29], while DOG-1 positivity was found in two CD117 negative cases in the present study. Regarding the small

Table 1. Organ involvement

Organ	Number of cases						Total cases	%
	2012	2013	2014	2015	2016	2017		
Ampulla of Vater				1			1	0.77
Ileum	1						1	0.77
Transverse colon				1			1	0.77
All bowels					1		1	0.77
Retroperitoneum				1			1	0.77
Omentum		1					1	0.77
T2 vertebra			1				1	0.77
Inguinal region			1				1	0.77
Esophagus			2				2	1.55
Abdominal cavity				1	1		2	1.55
Ovary		2					2	1.55
Peritoneum		1		1			2	1.55
Spleen		1			1		2	1.55
Pelvic mass	1	1	1				3	2.33
Pancreas			1	1	1		3	2.33
Small bowel				2	2		4	3.10
Jejunum		2	1	1	1		5	3.88
Mesentery		2	2	1			5	3.88
Colon	1	1	4		1		7	5.43
Liver		2	2	4	1		9	6.98
Duodenum		1	5	2	3		11	8.53
Rectum		5	1	4	1		11	8.53
Stomach	2	13	8	14	15	1	53	41.09
Total	5	32	29	34	28	1	129	100

Some of the 119 records had more than one organ involvement

Table 2. IHC expression

	Positive	Negative	No test	Total cases	% Positive
CD34	71	19	29	119	78.9
CD117	103	6	10	119	94.5
DOG-1	27	3	89	119	90.0
SMA	16	47	56	119	25.4

SMA was weakly or focally positive

Table 3. The cases of CD117 negative GIST

	Cases
CD117 neg DOG-1 neg	2
CD117 neg DOG-1 pos	2
CD117 neg DOG-1 no test	2

number of such cases, the benefits of DOG-1 in CD117 negative GIST cannot be determined by this study due to the limitation of untested data.

In terms of effectiveness, the patient expense per day at the time of discharge was 9,388.4 THB, while the most common TAT was 3 days. When we considered the formerly-recommended first panel of IHC (CD34, CD117 and SMA), we found that if the CD34 and CD117 were negative, DOG-1 needed to be tested in a second step, and this would take 6 days and incur a cost of 56,330.4 (9,388.4 x 6) THB while DOG-1 costs less than 1,000 THB. So, using the proposed first panel of IHC (CD34, CD117, DOG-1 and SMA) in the larger hospitals or medical centers such as tertiary hospitals will cut the time required by half, and it will cost less than 1,000 THB; in addition, the diagnosing pathologists will have more confidence. The proposed panel of IHC from this study for supratertiary centers, compared to the former standard panel of IHC in smaller scale medical centers, is displayed in Figure 1.

Conclusion

In terms of epidemiology, the population of this study was exclusively Thai (or Southeast Asian), and showed some difference in patients' age and sex from those of the western literature; however, these data were similar to those in the eastern literature. The incidence of extra-gastrointestinal GIST was also slightly higher than that reported in the review, but the common primary and metastatic sites were similar. The order of expressions of the panel of IHC (CD117, DOG-1, CD34 and SMA) was the same as in most papers. The diagnostic helpfulness of DOG-1 in CD117 negative cases of GIST cannot be concluded from the present study. In terms of efficacy, the standard guidelines of the large-scale

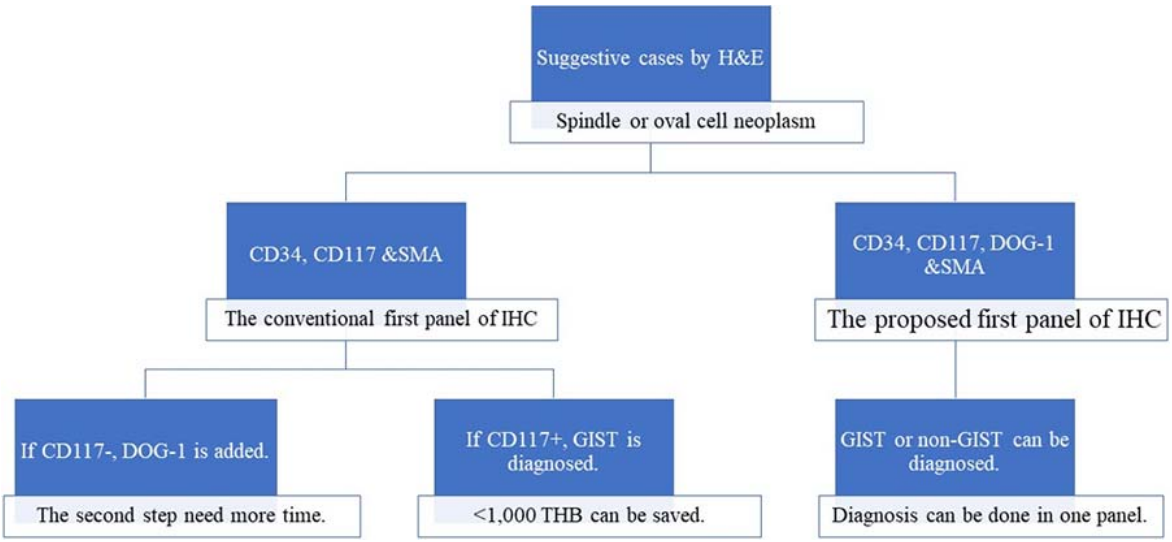

Figure 1. The conventional and proposed first panels of IHC.

hospitals or centers should include DOG-1 in the first panel, as it more effective is in terms of time, expense and diagnostic confidence.

What is already known on this topic?

The epidemiology of eastern and western countries and the suggested panel of approach.

What this study adds?

The results of the present study in RJVH are partly compatible with the conclusions of the literature; however, some epidemiological factors are different. The study also provides an evidence base for setting up standard guidelines for necessary IHC investigations in large-scale or super-tertiary hospitals, at least in Thailand.

Potential conflicts of interest

The authors declare no conflicts of interest.

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