Current status of the histopathological assessment, diagnosis, and reporting of colorectal neuroendocrine tumors: a Web survey from the Japanese Society for Cancer of Colon and Rectum

Koji Ikeda, MD[1, 2], Motohiro Kojima, MD, PhD[3], Norio Saito, MD, PhD [1], Naoki Sakuyama, MD[1, 2], Kenichi Koushi, MD [1, 2], Toshiaki Watanabe, MD, PhD [4], Kenichi Sugihara, MD, PhD [5],Tetsuo Akimoto, MD, PhD [2, 6], Masaaki Ito, MD, PhD [1], Atsushi Ochiai, MD, PhD[3]

[1]Division of Surgical Oncology, National Cancer Center Hospital East, Kashiwa, Japan;

[2]Juntendo University Graduate School of Medicine, Advanced Clinical Research of Cancer, Tokyo, Japan;

[3]Division of Pathology, National Cancer Center Hospital East, Kashiwa, Japan; [4]Division of Surgical Oncology, the University of Tokyo, Tokyo, Japan;

[5]Division of Surgical Oncology, Tokyo Medical and Dental University, Tokyo, Japan;

[6]Division of Radiation Oncology, National Cancer Center Hospital East, Kashiwa, Japan;

Address correspondence and reprint requests to:

Atsushi Ochiai

Division of Pathology, National Cancer Center Hospital East, Kashiwa, Japan;

Telephone: 81-4-7133-1111

Fax: 81-4-7131-4724

E-mail: aochiai@east.ncc.go.jp

Short title: **Pathological reporting of colorectal NET**

Manuscript word count: **2170 words**

Abstract

Although new classifications for neuroendocrine tumors were established by the World Health Organization, the current procedures and terms used in pathology laboratories are not known. A Web-based questionnaire was distributed to 491 institutions affiliated with the Japanese Society for Cancer of the Colon and Rectum, and 150 participated. The questionnaires included questions regarding routine pathological reporting, staining, and assessment of neuroendocrine tumors. Next, time to assess Ki-67 index and mitotic count according to recommendation was evaluated to know its feasibility. Most laboratories recorded diagnostic term, depth of invasion, size, lymph-vascular invasion, Ki-67 index, and mitotic count. However, only 32.2% reported tumor stage. Chromogranin A and synaptophysin were common neuroendocrine markers. D2-40 and elastica stain were frequently used to confirm lymph-vascular invasion. Only 62.1% counted more than 500 cells to for Ki-67 index, and only 17.0% counted more than 50 fields for mitotic count, as suggested by recommendations. Median time of 7 cases was 18.0 and 27.3 minutes to assess mitotic count in 50 fields with Ki-67 index in 500 and 2000 cells, respectively. For more standardized pathological reporting, educations about standardized staging systems are needed in Japan. Practical and standardized procedure for mitotic index and Ki-67 index is also required.

.

Key words: neuroendocrine tumor, carcinoid tumor, pathological reporting, colorectal, rectum

Introduction

Since the first proposal of Oberndorfer for the classification of carcinoid tumors [1], this lesion has been characterized by neoplastic cells with otherwise endocrine properties and a phenotype that has been recognized and studied in not only gastrointestinal organs, but also the lung, endocrine organs, and others [2]. The wide distribution of this tumor has caused a lack of standard nomenclature and staging classification; it has been reported as a carcinoid tumor, endocrine tumor, endocrine carcinoma, or small cell carcinoma with various staging systems [2-13]. The World Health Organization (WHO) Classification of the Digestive System 2010 adopted the term “neuroendocrine neoplasm” to bridge this classification gap. Using mitotic count and Ki-67 index, researchers have further classified neuroendocrine neoplasms into neuroendocrine tumor (NET) G1, NET G2, and neuroendocrine carcinoma (NEC) [14]. Next, Klimstra et al. proposed a minimum pathology data set for diagnosing gastrointestinal NET, and important pathology data that should appear in routine reports were selected using the Delphic consensus development method [15]. However, the degree of acceptance in Japan of this NET classification and reporting system in routine pathology diagnosis has not been investigated. A detailed understanding of the current practices in the diagnosis of NET may reveal underlying problems in routine practice, and therefore, the Japanese Society

for Cancer of the Colon and Rectum (JSCCR) developed a Web-based survey. We assess and report herein what is standardized and what is not in Japanese pathology laboratories to provide a more concordant data set of colorectal NET diagnosis procedures.

Material and Methods

Nationwide Web Survey

The nationwide Web survey was conducted from November 1, 2013 to December 16, 2013 within multiple pathology laboratories under institutions belonging to the JSCCR. It was performed in accordance with ethical guidelines for clinical studies and considered the patients' human rights and privacy. The study protocol was approved by the institutional review board of JSCCR. In total, 491 pathology laboratories were invited by mailed invitation letter to participate in the online Web survey. The questionnaire in this survey included questions about reporting criteria, types of routine immunohistochemical and histochemical stains used, and procedures for the assessment of colorectal NET. The questionnaire was sent to the laboratories in Japanese and the table is only the translated version (Table 1). Because the Japanese Classification of Colorectal Carcinoma (JCCC) 7th edition adopted "carcinoid tumor" as their nomenclature [16], as opposed to "neuroendocrine tumor" in the WHO 2010 Classification, one question we asked was which term was used in daily practice. The study was approved by the institutional review board of the JSCCR.

Feasibility of Mitotic Count and Ki-67 Index According to WHO 2010 recommendation

Ten rectal NETs underwent surgery from 2009 to 2013 were entered into this study. These cases were assessed retrospectively by two observer (M.K and K.I), and 7 cases were assessed as G1, 2 were G2, and 1 were G3, according to the WHO 2010. Three of them have synchronous lymph node metastasis. Time required for the assessment according to WHO recommendation was evaluated in one observer (K.I). Time required for the assessment of mitotic count in 50 field and Ki-67 index in 500 and 2000 cells were evaluated. The mitotic count and Ki-67 index were calculated by manually counting under the magnification of 400-fold with the aid of an eyepiece grid (5 X 5 squares).

Results

Respondent Profile

Of the 491 JSCCR institutions sent survey invitations, 150 (30.5%) began the survey, and 144 (29.3%) completed it. The interim report was presented at the 80th JSCCR congress in Tokyo, Japan, on January 2014.

Reporting Content

More than 70% of laboratories reported the pathological diagnostic term (98.6%), depth of tumor invasion (96.0%), tumor size (87.2%), lymph-vascular invasion (96.0%), Ki-67 index (80.5%), and mitotic count (70.5%). Conversely, the tumor stage was recorded in only 32.2% of the laboratories (Figure 1).

Pathological Diagnostic Term and Staging

Half (50.0%) of the pathology laboratories reported the NET diagnostic term according to both “carcinoid tumor” as per JCCC 7th edition nomenclature, and “neuroendocrine tumor” as per WHO 2010 Classification, in parallel; 18.0% of institutions adopted only the WHO 2010 Classification, and 29.3% used only the JCCC 7th edition. With regard to the tumor staging system, the JCCC 7th edition classification of malignant tumors (48.9%) and the International Union Against Cancer (UICC) classification (44.6%) were most commonly selected. The European Neuroendocrine Tumor Society (ENETS) classification (4.2%) and the North American Neuroendocrine Tumor Society (NANETS) classification (2.1%) were rarely adopted (Figure 2).

Staining and Immunostaining

Most institutions (88.6%) used immunohistochemical and histochemical stains in addition to hematoxylin and eosin (H&E) for their routine staining to confirm endocrine features and the Ki-67 index of the tumor. They used chromogranin A (83.9%), synaptophysin (79.9%), Ki-67 (61.1%), and CD56 (56.4%) routinely. NSE (9.4%) or histochemical stains of Fontana-Masson or Grimelius (10.1%) were also performed in some laboratories (Figure 3). For the assessment of lymph-vascular invasion, 90.3% of the institutions routinely used immunohistochemical and histochemical stains in addition to H&E stains. D2-40 stains (52.1%) and EVG stains (56.3%) were also adopted as routine stains in many laboratories. CD34 (8.3%), CD31 (5.6%), Factor VIII (2.1%), or Victoria Blue (17.4%) were also sometimes adopted as routine stains (Figure 4).

Assessment of Ki-67 index and mitotic count

The Ki-67 index or mitotic counts are influenced by their assessment methods [15, 17]; therefore, we evaluated their assessment method in routine practice. In 88.0% of respondent laboratories, the Ki-67 index was assessed using the hot spot method in

accordance with the recommended scheme in the WHO 2010. Although, 62.1% counted more than 500 cells for the Ki-67 index; variable number of cells were assessed ; 37.9% counted 501–1000 cells, 15.5% counted 1001–1500 cells, 4.3% counted 1501–2000, and 1.4% counted more than 2000 cells (Figure 5-a,b). Conversely, 37.9% of laboratories counted 500 or less cells. Similarly, mitotic count was performed by the hot spot method in 71.3% of respondent laboratories, as suggested by the WHO 2010 recommendation, but only 17.0% of those laboratories assessed in areas of more than 50 high power fields. Many institutions counted in areas of only 10 high power fields, which does not fulfill the WHO 2010 recommendation (Figure 5-c, d).

Feasibility of Mitotic Count and Ki-67 Index According to WHO 2010 recommendation

Time required for mitotic count in 50 fields was 12.0 minutes (range, 10.3-13.3 minutes). And time to count Ki-67 index in 500 and 2000 cells were 6.0 minutes (range, 4.5-7.3 minutes) and 15.3 minutes (range, 13.3-18.5), respectively. Mitotic count with Ki-67 index in 500 cells required 18.0 minutes (range, 15.3-21.7 minutes), and mitotic

count with Ki-67 index in 2000 cells required 26.2 minutes (range, 25.0-31.8) (Figure 6).

Discussions

This study presented not only a regional baseline audit of pathological reports for NET, it also evaluated the acceptance and feasibility of the WHO 2010 classification. The diagnostic terms of NET and NEC were satisfactory adopted in pathology laboratories under institutions belong to the JSCCR. Although the JCCC 7th edition adopted "carcinoid tumor" as the diagnostic term for what is referred to as "neuroendocrine neoplasm" in the WHO 2010 classification, many Japanese laboratories are dealing with this issue by using both diagnostic terms in parallel. The use of the term carcinoid tumor has been criticized [18-20], because of concerns that the term does not suitably inform about the potential malignant behavior. And the introduction of concordant term of NET would ease international clinicopathological study. On the other hand, especially in colorectal NETs, the clinicopathological utility of this classification is not fully investigated. And further investigation is required.

ENETS proposed a consensus-based staging system applicable to colorectal NETs in 2008 [12]. The 7th edition of the American Joint Committee on Cancer (AJCC) presented

a staging system applicable to G1 NETs revising the ENETS classification in 2010 [10]. On the other hands, NANETS suggested other staging system applicable to colorectal NETs from the ENETS classification in 2011 [4]. Recent analysis supports the use of the AJCC/UICC systems for G1 NETs, and the use of ENETS and NANETS systems for all NETs until there is further evidence for medication [10, 21-25]. The variation in different staging systems may result in less concordant reporting in the pathological staging in Japan. This survey revealed that many pathology laboratories are using JCCC 7th staging system which is not for NET staging, but for colorectal cancer. A unified staging system and education would be required for concordant pathological reporting

Most laboratories performed D2-40 and elastic stains to report high quality information on risk factors in addition to the conventional neuroendocrine markers. Many items included in the previously reported minimum data set were also reported in these pathology laboratories [15]. Institutions belonging to the JSCCR, including many hospitals, perform intensive clinical investigations and treatments of colorectal cancer, and this can be reflected by the policies of the respondent institutions. However, some problems were also found.

Visual estimation of the Ki-67 index can be effective to distinguish very low rates (less than 1% in a G1 NET) from very high rates (>20% in a NEC), however, the subtle differences in proliferative rates between G1 and G2 NET are difficult to recognize accurately by this method [17]. Although many laboratories assessed the Ki-67 index according to the WHO 2010 recommended scheme, this scheme suggests a wide range of 500–2000 cells for the assessment of the Ki-67 index, and accordingly, a wide range of cell numbers counted was confirmed in Japanese institutions in our survey. The assessment methods of the Ki-67 index and mitotic cell counts have been reported to influence results and can produce a discrepancy in reporting among institutions [14, 16, 26-28]. The WHO 2010 proposal for a more specific cell count number for the assessment of the Ki-67 index would be available to produce concordant data in the future [14, 29-31]. Next, we speculated that the time and effort necessary to assess the Ki-67 index and mitotic count may also be one cause of the discordant cell counts and fields. In a regional survey, it was reported that Japanese pathologists diagnose and report on 2500 cases per year on average (data not shown). We firstly elucidated that it takes 18.0 and 27.3 minutes only to assess the mitotic count in 50 fields with Ki-67 index in 500 and 2000 cells respectively. This seemed to limit the feasibility of this assessment method in pathology laboratories supporting a busy practice. Recently automatic assessment of the

Ki-67 index was reported to be available in routine practice [26, 27] and such a method would assist in producing consistent data across laboratories. Standardization of these methods will be required for the introduction into routine practice. As for the mitotic count, automatic evaluation can be more difficult. However, in addition to the widespread standardization of the assessment method for the mitotic index, investigation of automatic analysis method will facilitate the production of concordant results in the future.

In conclusion, detailed pathology reporting was performed by the laboratories surveyed, but accurate recording of tumor stage was lacking in many Japanese pathology laboratories. There was also a reasonable acceptance of the WHO classification scheme for the use of diagnostic terms. However, a large variety of assessment methods for Ki-67 index and mitotic index were found. Recommended assessment method of Ki-67 index and mitotic count seemed to be low feasibility by its long required time. Standardized and practical assessment method of Ki-67 index and mitotic count is still required to produce concordant reporting results of NETs across all laboratories.

Acknowledgments

The authors thank all members and staff of the JSCCR member institutions for collecting data for the Japanese colorectal cancer registration. This work was supported in part by the National Cancer Center Research and Development Fund (26-A-7).

Disclosure statement

All authors have no conflict of interest to disclose.

References

1. Oberndorfer S: *Karzinoide tumoren des dunndarms*. Frankf Z Pathol. 1907;1:426-32

2. Modlin IM, Oberg K, Chung DC, et al. *Gastroenteropancreatic neuroendocrine tumours*. Lancet Oncol. 2008;9:61-72.

3. Klöppel G, Couvelard A, Perren A, et al. *ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Towards a Standardized Approach to the Diagnosis of Gastroenteropancreatic Neuroendocrine Tumors and Their Prognostic Stratification.* Neuroendocrinology. 2009;90:162-6.

4. Pamela L Kunz, Diane Reidy-Lagunes, Lowell B, et al. *Consensus Guidelines for the Management and Treatment of Neuroendocrine Tumors.* Pancreas. 2013;42: 557-77.

5. Klimstra DS, Modlin IR, Coppola D, et al. *The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems.* Pancreas. 2010;39:707-12

6. Nikou GC, Lygidakis NJ, Toubanakis C, et al. *Current diagnosis and treatment of gastrointestinal carcinoids in a series of 101 patients: the significance of serum*

chromograninA, somatostatin receptor scintigraphy and somatostatin analogues. Hepatogastroenterology. 2005;52:731–41.

7. Bordi C, D'Adda T, Azzoni C, et al. *Criteria for malignancy in gastrointestinal endocrine tumors.* Endocr Pathol. 2006;17:119–29.

8. Sen N, Calli Demirkan N, Aksoy Altinboga A, et al. *Synchronous endocrine tumors of small intestine: report of a case.* Turk J Gastroenterol. 2008;19:193–6.

9. Wick MR, Graeme-Cook FM. *Pancreatic neuroendocrine neoplasms: a current summary of diagnostic, prognostic, and differential diagnostic information.* Am J Clin Pathol. 2001;115 Suppl:S28–45.

10. Edge SB, Byrd DR, Carducci MA, et al. *AJCC cancer staging manual. 7th ed.* New York: Springer; 2010.

11. Klöppel G, Couvelard A, Perren A, et al. *ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Towards a Standardized Approach to the Diagnosis of Gastroenteropancreatic Neuroendocrine Tumors and Their Prognostic Stratification.* Neuroendocrinology. 2009;90:162–6.

12. Rindi G, Kloppel G, Alhman H, et al. *TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system.* Virchows Arch. 2006;449:395-401.

13. Rindi G, Kloppel G, Couvelard A, et al. *TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system.* Virchows Arch. 2007;451:757-62.

14. Rindi G, Arnold R, Bosman F, et al. *Nomenclature and classification of neuroendocrine neoplasms of the digestive system. In WHO Classification of Tumours of the Digestive System. 4th ed.* Lyon: International Agency for Research on Cancer; 2010:13–4.

15. David S Klimstra, Irvin R Modlin, Volkan Adsay, et al. *Application of the Delphic Consensus Process to the Development of a Minimum Pathology Data Set.* Am J Surg Pathol 2010;34:300-13.

16. *General rules for clinical and pathological studies on cancer of the colon, rectum and anus. 7th ed.* Tokyo: Japanese Society for Cancer of the Colon and Rectum; 2006.

17. Tang LH, Gonen M, Hedvat C, et al. *Objective quantification of the Ki67 proliferative index in neuroendocrine tumors of the gastroenteropancreatic system: a comparison of digital image analysis with manual methods.* Am J Surg Pathol. 2012;36:1761-70.

18. Soga J. *The term "carcinoid" is a misnomer: the evidence based on local invasion.* J Exp Clin Cancer Res. 2009;28:15.

19. Washington MK, Tang LH, Berlin J, et al. *Protocol for the examination of specimens from patients with neuroendocrine tumors (carcinoid tumors) of the colon and rectum.* Arch Pathol Lab Med. 2010;134:176–80.

20. Soga J. *Reevaluation of the term "carcinoid"--how to deal with this misnomer.* Nihon Rinsho. 2012;70:1427-35

21. Yao JC, Hassan M, Phan A, et al. *One hundred years after ”carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States.* J Clin Oncol. 2008;26:3063-72.

22. Ryaz Chagpar, Yi-Ju Chiang, Yan Xing, et al. *Neuroendocrine Tumors of the Colon and Rectum: Prognostic Relevance and Comparative Performance of Current Staging Systems.* Ann Surg Oncol. 2013;20:1170-8.

23. Brett Weinstock, Stephen C Ward, Noam Harpaz, et al. *Clinical and Prognostic Features of Rectal Neuroendocrine Tumors.* Neuroendocrinology 2013;98:180-7.

24. Strosberg JR, Cheema A, Weber JM, et al. *Relapse-free survival in patients with nonmetastatic, surgically resected pancreatic neuroendocrine tumors: an analysis of the AJCC and ENETS staging classifications.* Ann Surg. 2012;256:321-5.

25. Rindi G, Falconi M, Klersy C, et al. *TNM staging of neoplasms of the endocrine pancreas: results from a large international cohort study.* J Natl Cancer Inst. 2012;104:764-77.

26. Tang, Laura H, Gonen, et al. *Objective Quantification of the Ki67 Proliferative Index in Neuroendocrine Tumors of the Gastroenteropancreatic System: A Comparison of Digital Image Analysis With Manual Methods.* Am J Surg Pathol. 2012;36:1761-70.

27. Parkins CS, Darling JL, Gill SS, et al. *Cell proliferation in serial biopsies through human malignant brain tumours: measurement using Ki67 antibody labelling.* Br J Neurosurg. 1991;5:289-98.

28. Torp SH, Alsaker M. *Ki-67 immunoreactivity, basic fibroblastic growth factor (bFGF) expression, and microvessel density as supplementary prognostic tools in*

low-grade astrocytomas. An immunohistochemical study with special reference to the reliability of different Ki-67 antibodies. Pathol Res Pract. 2002;198:261–5.

29. La Rosa S, Klersy C, Uccella S, et al. *Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors.* Hum Pathol. 2009;40:30-40.

30. Pape UF, Jann H, Müller-Nordhorn J, et al. *Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors.* Cancer. 2008;113:256-65.

31. Ekeblad S, Skogseid B, Dunder K, et al. *Prognostic factors and survival in 324 patients with pancreatic endocrine tumor treated at a single institution.* Clin Cancer Res. 2008;14:7798–803.

Table 1 Questionnaire List

Please answer the following questions about the pathology examination of surgical and endoscopic excision specimens of the large intestine and the diagnosis of colorectal neuroendocrine tumors in your facility.

Question 1. Contents of the diagnosis report

1.1 Which of the items below are listed in the pathology diagnosis?
(Multiple choice allowed)
☐ Pathology diagnostic term
☐ Depth of tumor invasion
☐ Tumor size
☐ Lymph-vascular invasion
☐ Mitotic count
☐ Ki-67 (MIB-1) index
☐ Tumor stage classification
☐ Other (Please describe)

1.2 Please select the pathology diagnosis term used in your facility.
(Select one answer)
☐ Only Japanese Classification of Colorectal Carcinoma (carcinoid tumor)
☐ Only WHO 2010 Classification (neuroendocrine tumor)
☐ Both Japanese Classification of Colorectal Carcinoma and WHO 2010 Classification in parallel
☐ Other (Please describe)

1.3 Please select the classification that is used for tumor staging.
(Select one answer)
☐ Japanese Classification of Colorectal Carcinoma
☐ TNM Classification of Malignant Tumors (UICC)
☐ ENETS Classification
☐ NANETS Classification
☐ Other (Please describe)

Question 2. Routine immunohistochemical and histochemical stains used

2.1 Which stainings are routinely performed for the diagnosis of neuroendocrine tumors, in addition to hematoxylin and eosin stainings?
(Multiple choice allowed)

☐ Only hematoxylin and eosin staining
☐ Chromogranin A
☐ Synaptophysin
☐ CD56
☐ NSE
☐ Ki-67 (MIB-1)
☐ Histochemical stains (Fontana Masson, Grimerius, etc)
☐ Other (Please describe)

2.2 Which stainings are routinely performed for the confirmation of lymph-vascular invasion in neuroendocrine tumors, in addition to hematoxylin and eosin stainings?
(Multiple choice allowed)

☐ FactorⅧ
☐ CD31
☐ CD34
☐ D2-40
☐ EVG
☐ Victoria blue staining
☐ Other (Please describe)

2.3 Please select the assessment method used for the Ki-67 index.
(Select one answer)

☐ Hot spot method
☐ At random
☐ Other (Please describe)

2.4 Please select the cell number used to assess the Ki-67 index.
(Select one answer)

☐ 500 or less
☐ 501～1000
☐ 1001～1500
☐ 1501～2000
☐ More than 2001

2.5 Please select the assessment method used for the mitotic count.
(Select one answer)

☐ Hot spot method
☐ At random
☐ Other (Please describe)

2.6 How many fields do you use to evaluate the mitotic count?
(Please describe to the nearest integer)

(　　)

Figure Legends

Figure 1. Pathology report contents

Pathology laboratories reported included pathology diagnosis (98.6%), depth of tumor invasion (96.0%), tumor size (87.2%), lymph-vascular invasion status (96.0%), Ki-67 index (80.5%), mitotic count (70.5%), and tumor staging classification system (32.2%).

Figure 2. Current status of diagnosis terms and stage classification used in pathology reports in Japan

(a) Pathology laboratories used the following nomenclature for pathology diagnosis terms: the Japanese Classification of Colorectal Carcinoma “carcinoid tumor” and WHO 2010 Classification “neuroendocrine neoplasm (NET)” in parallel (50.0%), only the Japanese Classification of Colorectal Carcinoma “carcinoid tumor” (29.3%), and only the WHO 2010 Classification“NET” (18.0%).

(b) Pathology laboratories reported the following tumor staging systems: Japanese Classification of Colorectal Carcinoma (48.9%), UICC classification (44.7%), ENETS classification (4.3%), and NANETS classification (2.1%).

Figure 3. Routine staining used for evaluation of colorectal neuroendocrine tumors

(a) Routine use of immunohistochemical and histochemical stains in addition to hematoxylin and eosin (H&E) were performed in 88.6% of the respondent institutions.

(b) The routine stains in addition to H&E were chromogranin A (83.9%), synaptophysin (79.9%), Ki-67 (61.1%), CD56 (56.4%), NSE (9.4%), and histochemical stains (10.1%).

Figure 4. Routine staining used for an evaluation of lymph-vascular invasion

(a) Routine use of immunohistochemical and histochemical stains in addition to hematoxylin and eosin (H&E) were performed in 90.3% of the respondent institutions.

(b) The stains routinely used in addition to H&E were D2-40 (52.1%), CD34 (8.3%), CD31 (5.6%), Factor Ⅷ (2.1%), EVG (56.3%), and VB (17.4%).

Figure 5. Criteria of Ki-67 index and mitotic

(a) The assessment methods for Ki-67 index were hot spot method (88.0%) and at random (9.4%).

(b) The number of cells counted to assess the Ki-67 index was 500 or less (37.9%), 501～1000 (37.9%), 1001～1500 (15.5%), 1501～2000 (4.3%), and more than 2000 (4.3%).

(c) The assessment methods of mitotic count were hot spot method (88.0%) and at random (9.4%).

(d) The number of high-power fields counted to evaluate mitotic count was 10 (71.0%), 20 (8.0%), and more than 50 (17.0%).

Figure 6. Time to assess Ki-67 index and mitotic count according to WHO recommendation

Time to count Ki-67 index in 500 and 2000 cells were 6.0 minutes (range, 4.5-7.3 minutes) and 14.3 minutes (range, 13.2-18.4). Time to count mitotic count in 50 fields was 11.5 minutes (range, 10.3-13.3 minutes). And Mitotic count with Ki-67 index in 500 cells required 17.6 minutes (range, 15.3-21.6 minutes), and mitotic count with Ki-67 index in 2000 cells required 26.2 minutes (range, 21.4-31.8)

Table 1 Questionnaire List

Please answer the following questions about the pathology examination of surgical and endoscopic excision specimens of the large intestine and the diagnosis of colorectal neuroendocrine tumors in your facility.

Question 1. Contents of the diagnosis report

1.1 Which of the items below are listed in the pathology diagnosis?
(Multiple choice allowed)
☐ Pathology diagnostic term
☐ Depth of tumor invasion
☐ Tumor size
☐ Lymph-vascular invasion
☐ Mitotic count
☐ Ki-67 (MIB-1) index
☐ Tumor stage classification
☐ Other (Please describe)

1.2 Please select the pathology diagnosis term used in your facility.
(Select one answer)
☐ Only Japanese Classification of Colorectal Carcinoma (carcinoid tumor)
☐ Only WHO 2010 Classification (neuroendocrine tumor)
☐ Both Japanese Classification of Colorectal Carcinoma and WHO 2010 Classification in parallel
☐ Other (Please describe)

1.3 Please select the classification that is used for tumor staging.
(Select one answer)
☐ Japanese Classification of Colorectal Carcinoma
☐ TNM Classification of Malignant Tumors (UICC)
☐ ENETS Classification
☐ NANETS Classification
☐ Other (Please describe)

Question 2. Routine immunohistochemical and histochemical stains used

2.1 Which stainings are routinely performed for the diagnosis of neuroendocrine tumors, in addition to hematoxylin and eosin stainings?
(Multiple choice allowed)

☐ Only hematoxylin and eosin staining
☐ Chromogranin A
☐ Synaptophysin
☐ CD56
☐ NSE
☐ Ki-67 (MIB-1)
☐ Histochemical stains (Fontana Masson, Grimerius, etc)
☐ Other (Please describe)

2.2 Which stainings are routinely performed for the confirmation of lymph-vascular invasion in neuroendocrine tumors, in addition to hematoxylin and eosin stainings?
(Multiple choice allowed)

☐ FactorⅧ
☐ CD31
☐ CD34
☐ D2-40
☐ EVG
☐ Victoria blue staining
☐ Other (Please describe)

2.3 Please select the assessment method used for the Ki-67 index.
(Select one answer)

☐ Hot spot method
☐ At random
☐ Other (Please describe)

2.4 Please select the cell number used to assess the Ki-67 index.
(Select one answer)

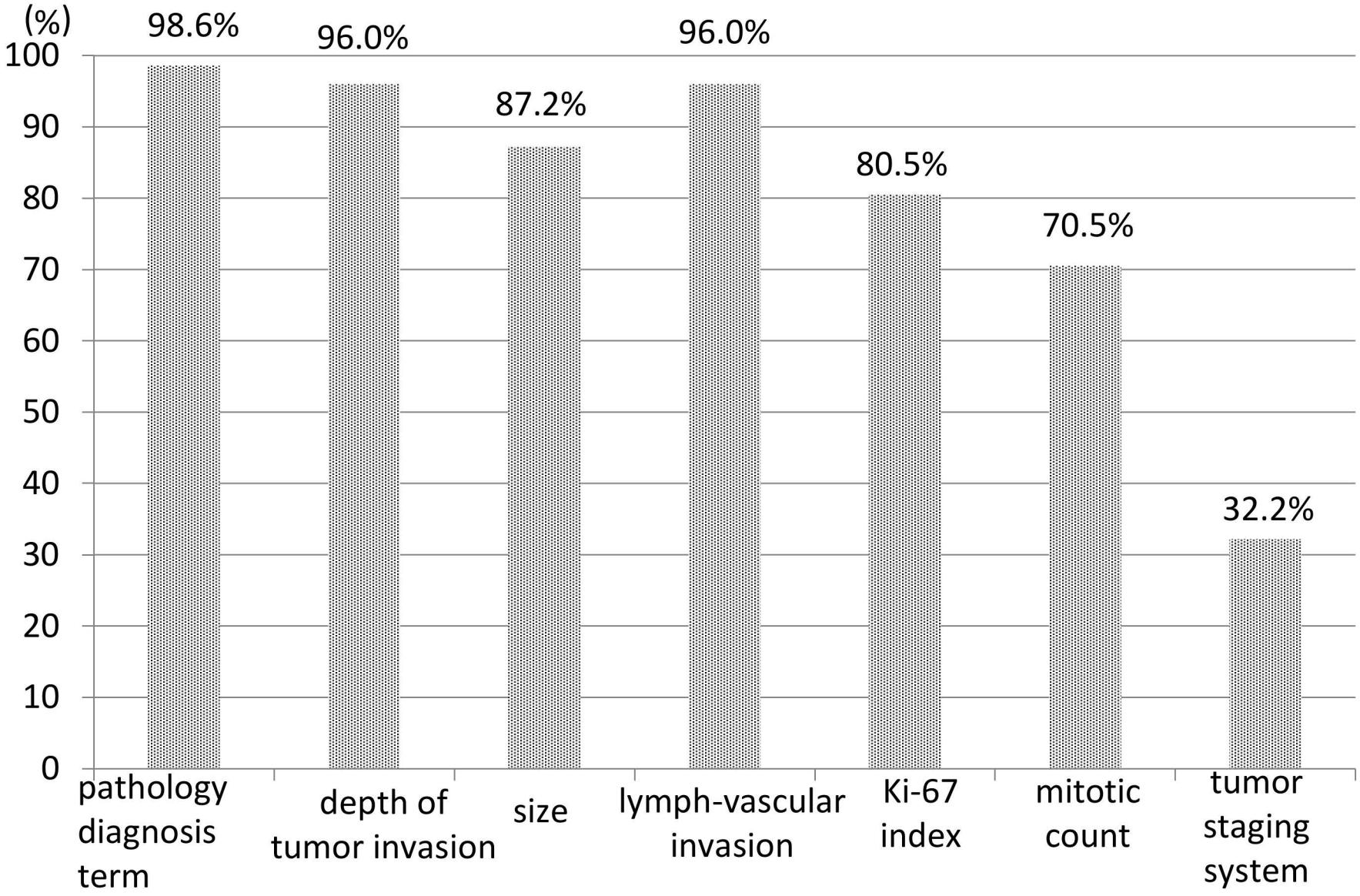
☐ 500 or less
☐ 501～1000
☐ 1001～1500
☐ 1501～2000
☐ More than 2001

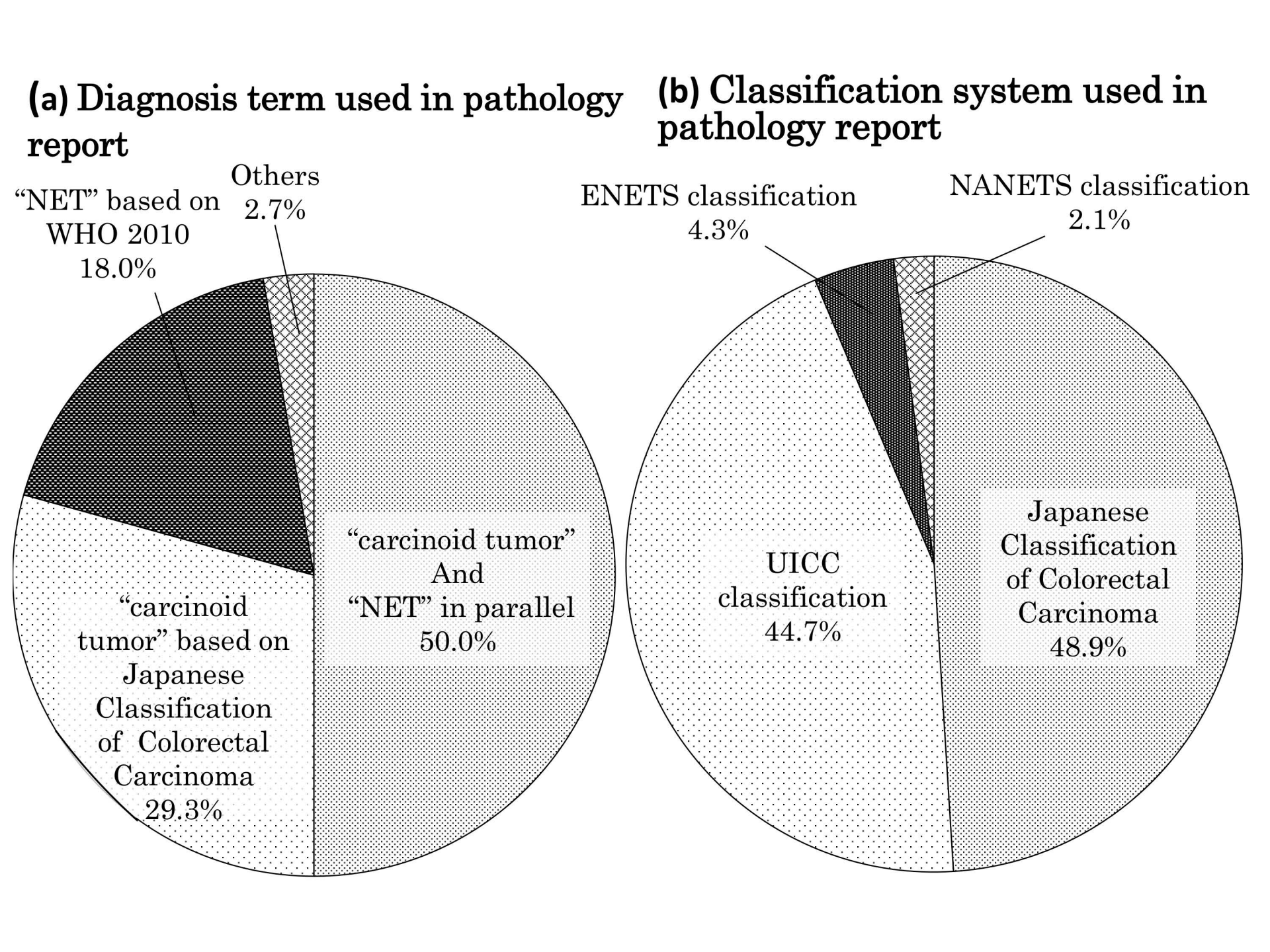
2.5 Please select the assessment method used for the mitotic count.
(Select one answer)

☐ Hot spot method
☐ At random
☐ Other (Please describe)

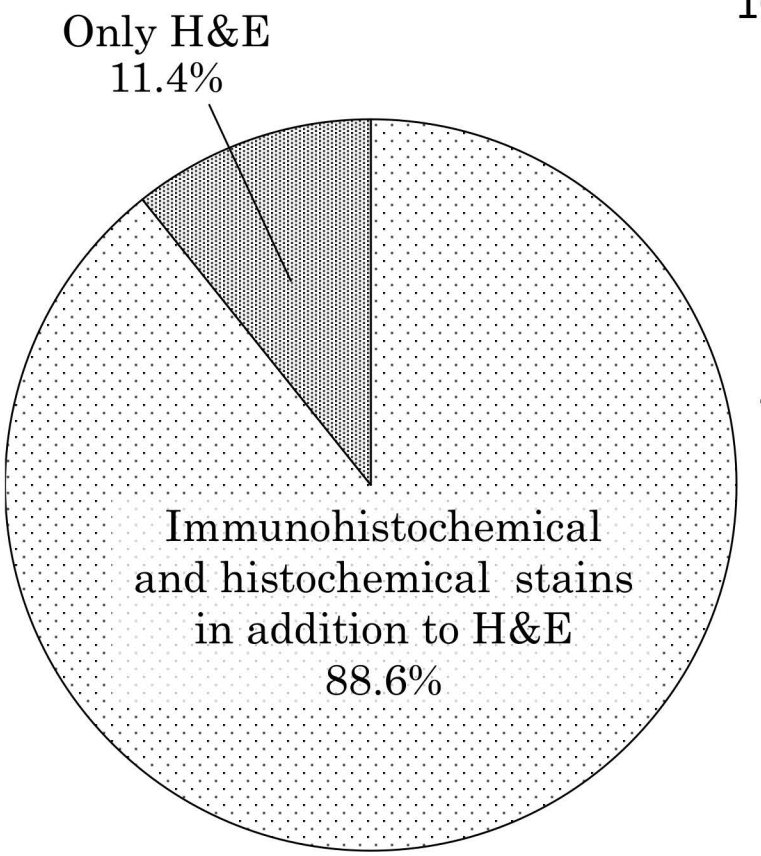
2.6 How many fields do you use to evaluate the mitotic count?
(Please describe to the nearest integer)

(　　)

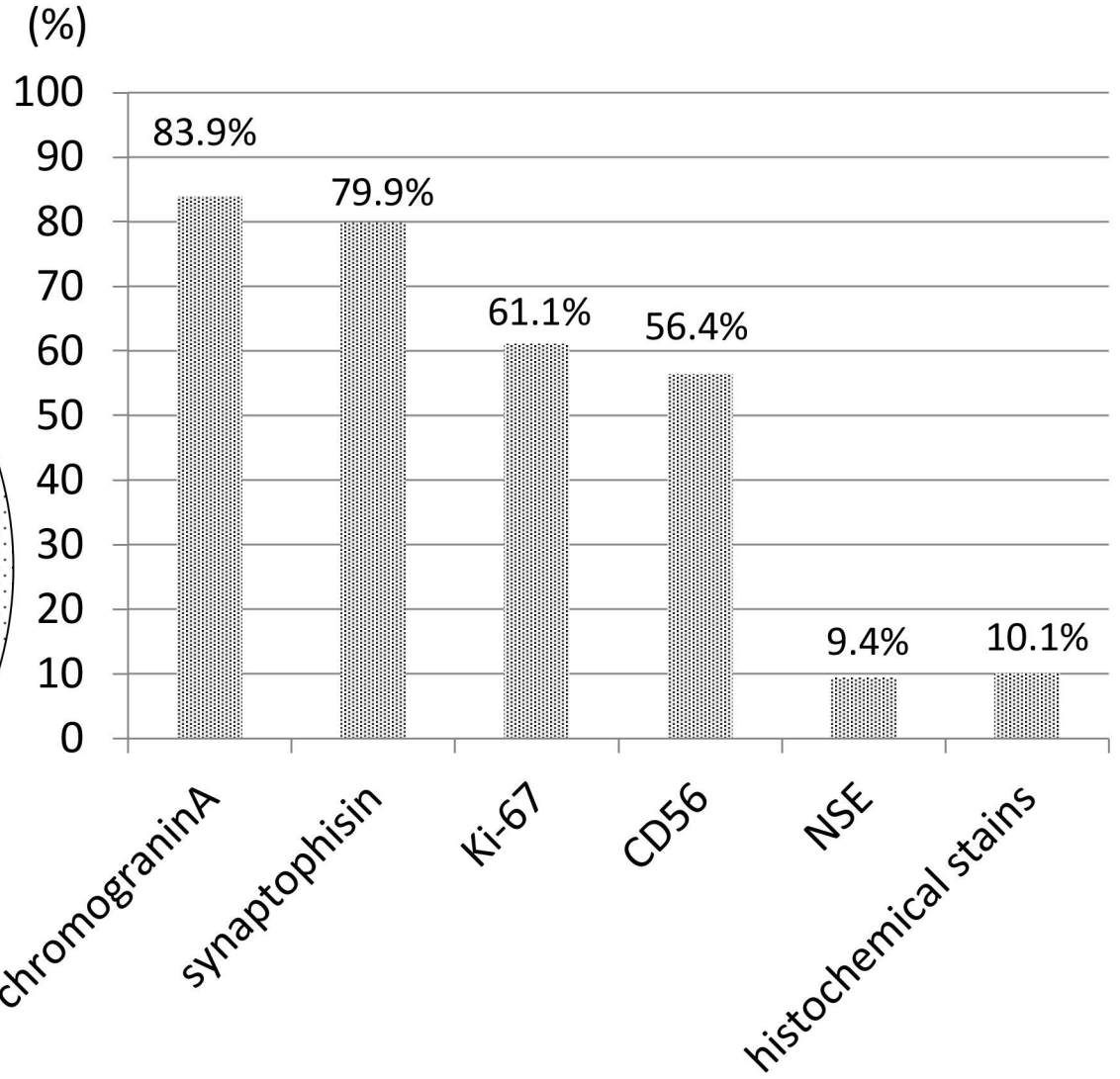

(%)
100
90
80
70
60
50
40
30
20
10
0
98.6%
96.0%
87.2%
96.0%
80.5%
70.5%
32.2%
pathology diagnosis term
depth of tumor invasion
size
lymph-vascular invasion
Ki-67 index
mitotic count
tumor staging system


(a) Diagnosis term used in pathology report
Others
2.7%
"NET" based on
WHO 2010
18.0%
"carcinoid tumor"
And
"NET" in parallel
50.0%
"carcinoid
tumor" based on
Japanese
Classification
of Colorectal
Carcinoma
29.3%
(b) Classification system used in pathology report
ENETS classification
4.3%
NANETS classification
2.1%
UICC
classification
44.7%
Japanese
Classification
of Colorectal
Carcinoma
48.9%

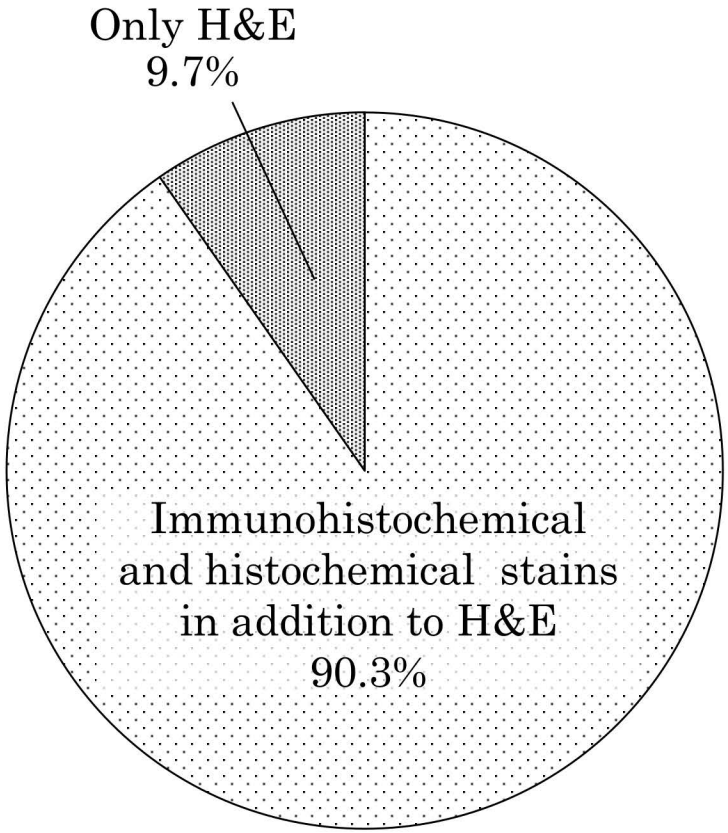
(a) Routine use of immunohistochemical and histochemical stains



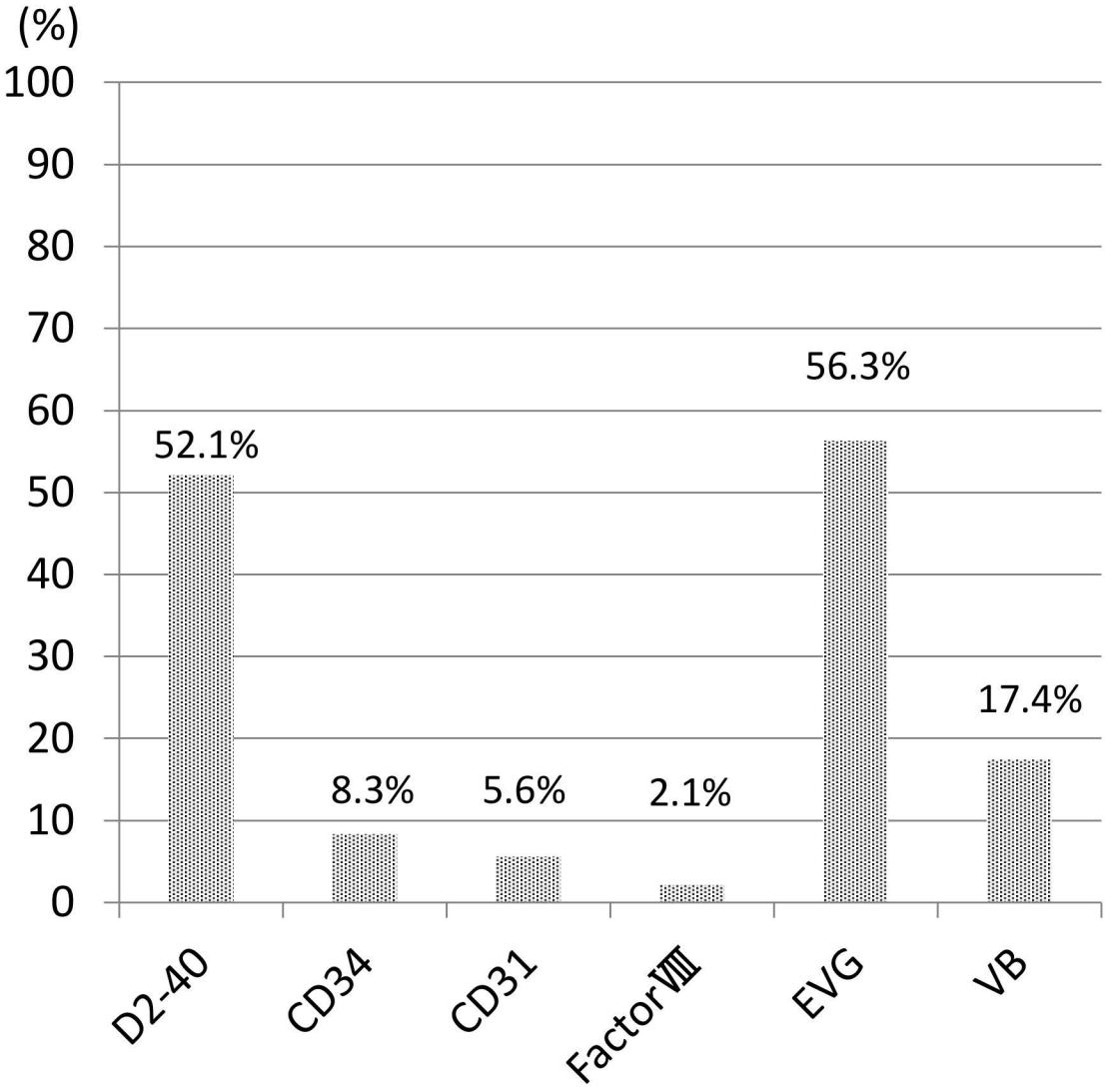
(b) Diagnosis term used in pathology report



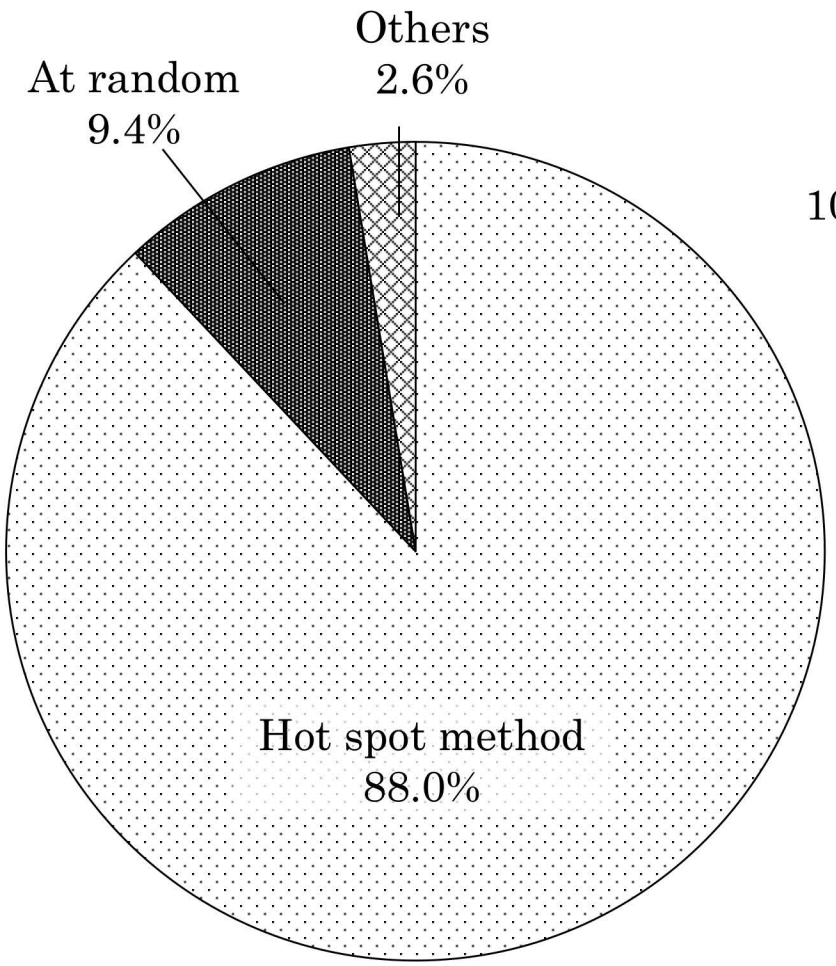
(a) Routine use of immunohistochemical and histochemical stains



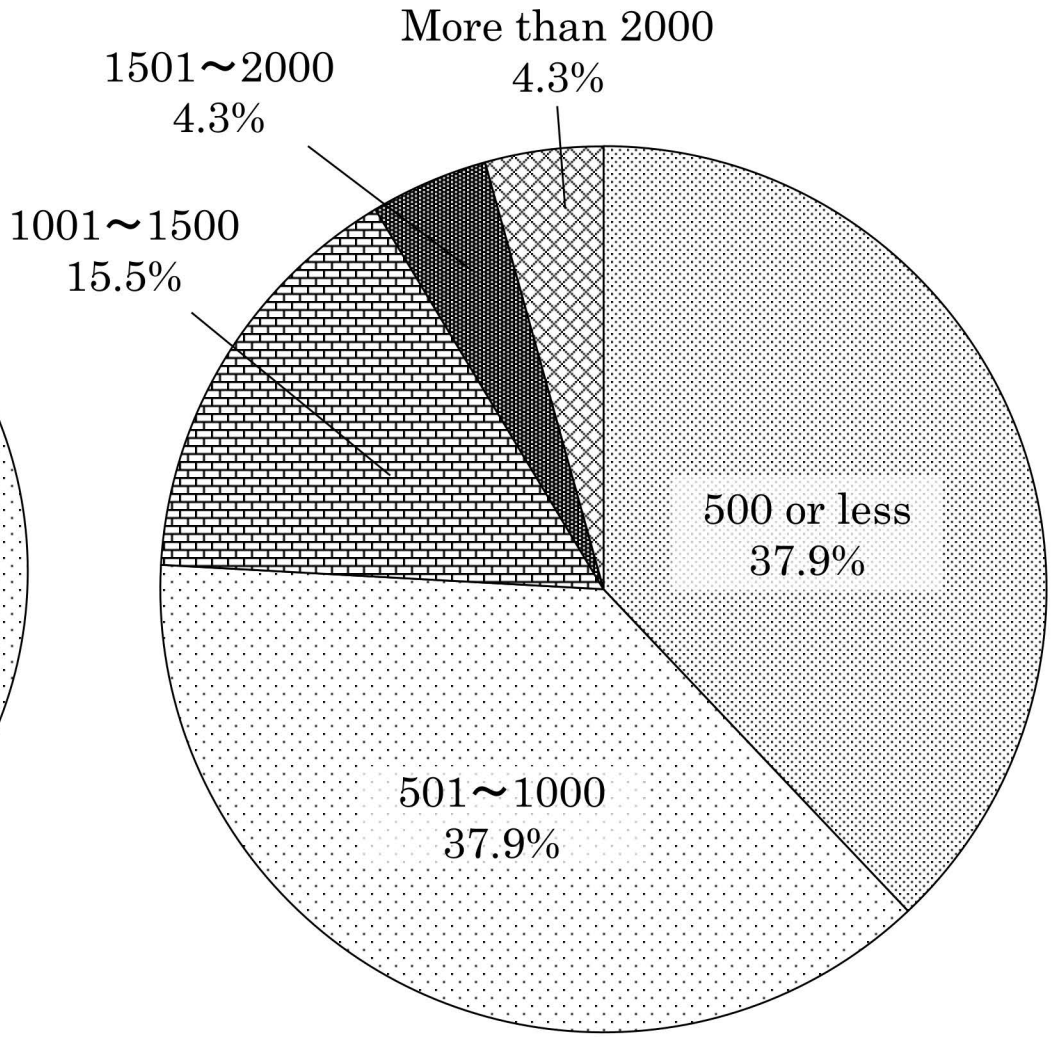
(b) The stains routinely used in addition to H&E



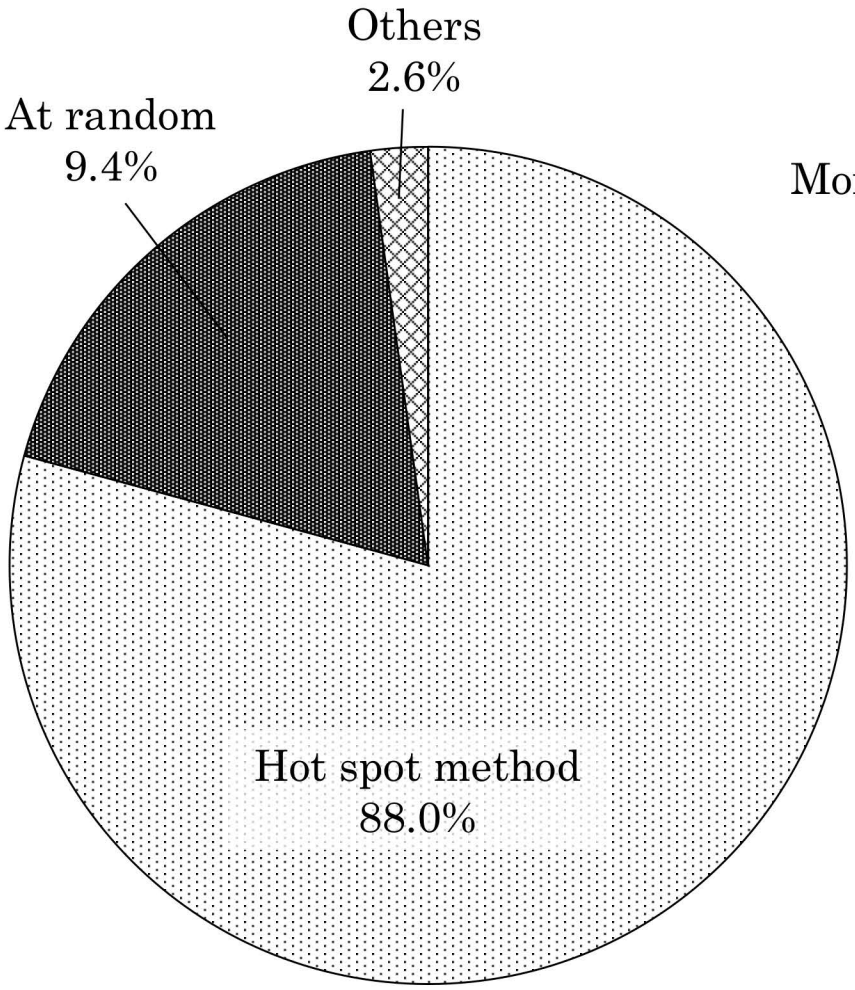
(a) Counting method for Ki-67 index



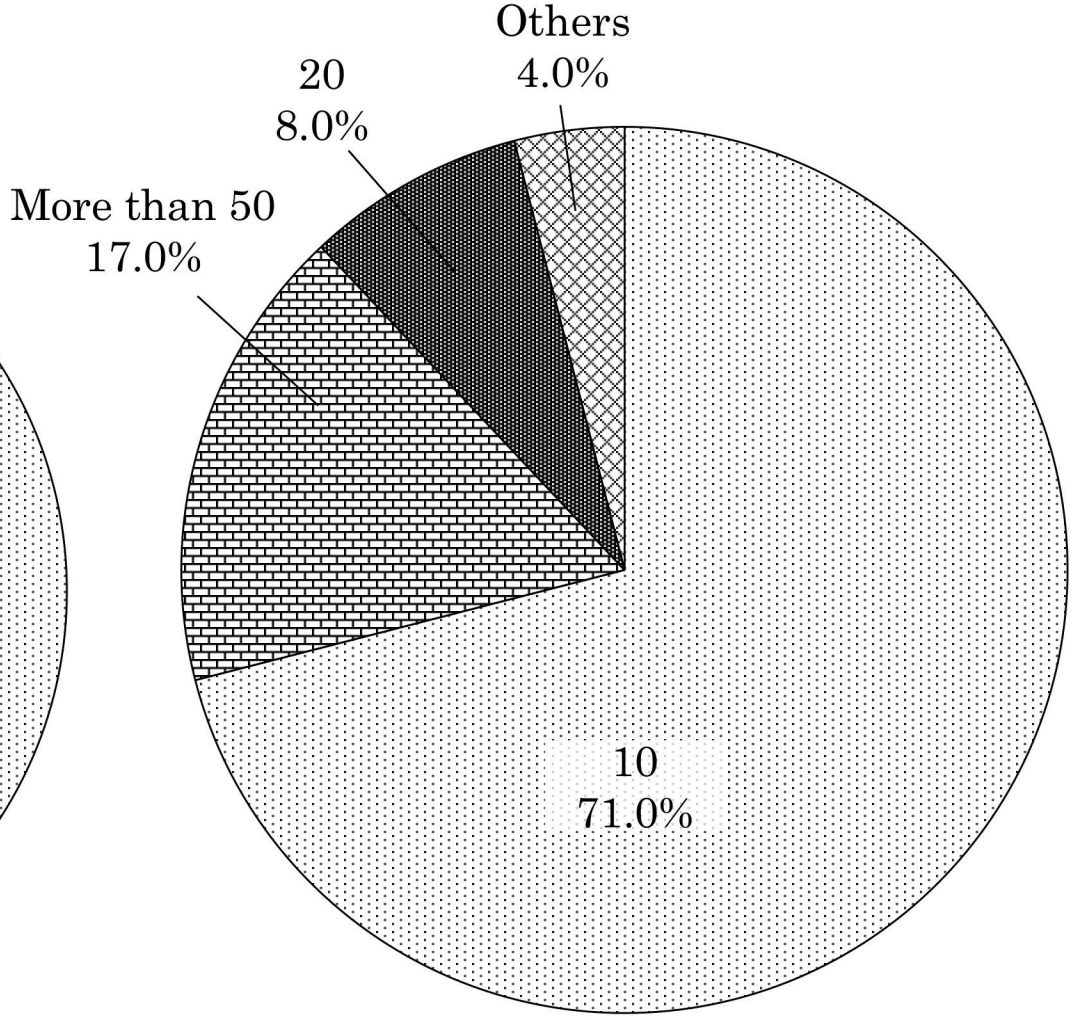
(b) Counting cells for Ki-67 index

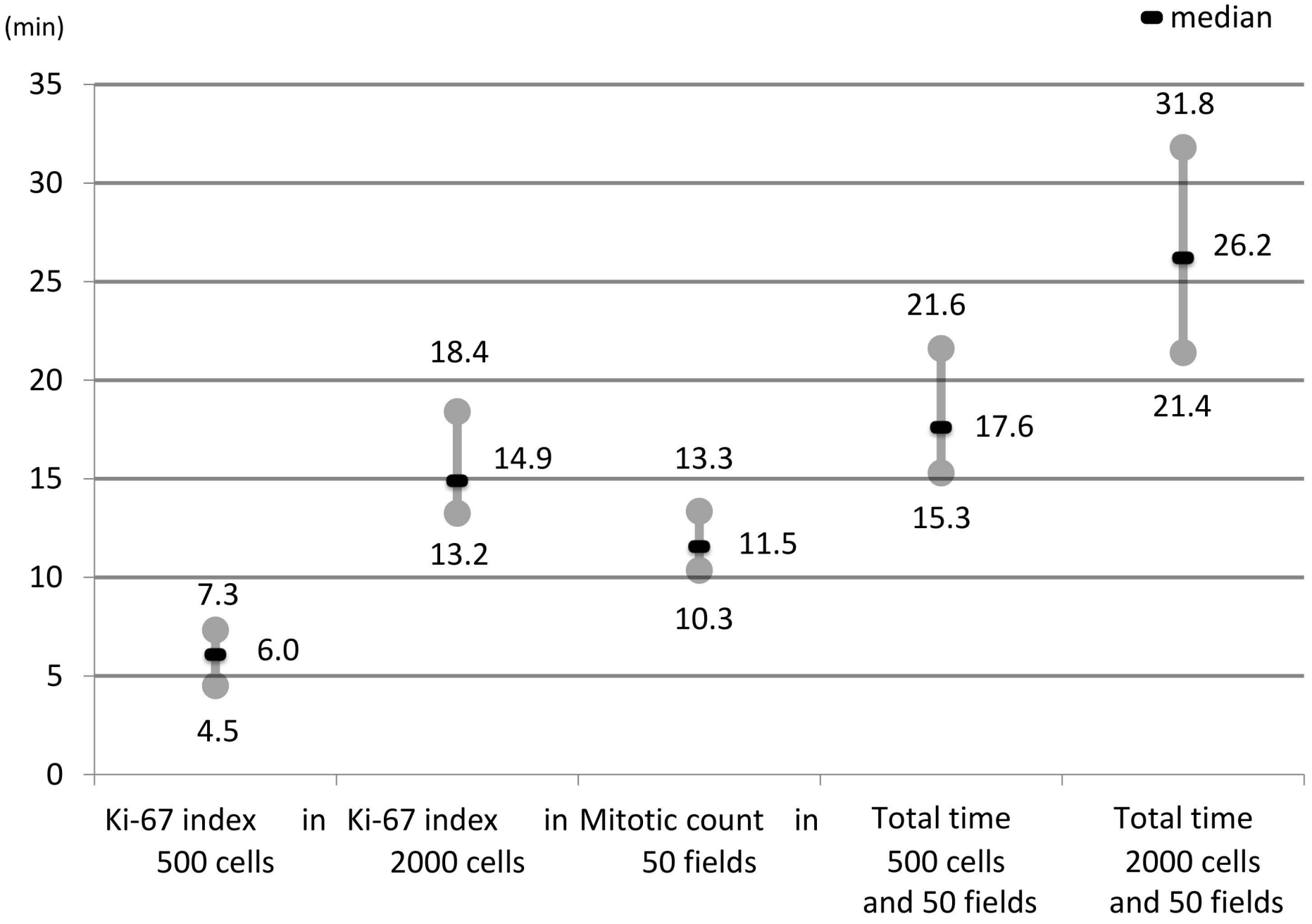


(c) Counting Method for mitotic count



(d) Counting Fields for mitotic count




(min)
median
35
30
25
20
15
10
5
0
7.3
6.0
4.5
18.4
14.9
13.2
13.3
11.5
10.3
21.6
17.6
15.3
31.8
26.2
21.4
Ki-67 index in 500 cells
Ki-67 index in 2000 cells
Mitotic count in 50 fields
Total time 500 cells and 50 fields
Total time 2000 cells and 50 fields