Int J Pharm Bio Sci 2016 Jan; 7(1): (B) 67 - 73

Research Article Pathology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IMMUNOHISTOCHEMICAL EXPRESSION OF Ki-67 IN OVARIAN TUMORS & CORRELATION WITH CLINICOPATHOLOGICAL FACTORS

LAVANYA RAJAGOPAL*[1] AND RAMESH.S[2]

[1]Assistant Professor, Department of Pathology, SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai India.-603203
[2] Associate Professor, Department of Pathology, Madurai Medical College, Madurai

ABSTRACT

Ovarian cancer is the leading cause of death from gynecological malignancies. The prognosis is poor, with an overall survival rate of about 40% in 5 years. Important prognostic factors include stage, age, histological type and grade, ploidity etc. Furthermore, high proliferative activity in the ovarian tumor has been shown to imply a poor prognosis.. The rate at which a tumor proliferates has long been considered to bear a relationship with its clinical course. Immunohistochemical detection of proliferating cells using Ki67 can be used to determine the proliferative potential of a tumor. Ki-67/MIB-1 expression was positively correlated with mitotic count, tumor grade , and stage of disease. We evaluated Ki 67 expression in 42 selected ovarian epithelial tumors which include 14 benign cystadenoma (7 serous, 7 mucinous), 14 borderline cystadenomas (7 serous,7 mucinous), 14 carcinoma (7 serous, 7 mucinous). Mean Ki 67index of benign, borderline and malignant tumors were 2.9% , 7.2% and 29.9% respectively . A statistically significant difference (p =<0.001) was obtained between these mean Ki 67 indices. Ki 67 immunostaining provides robust prognostic information in ovarian cancers. This biomarker is very much useful to identify borderline tumors which are likely to behave in a malignant fashion and may define subgroups of patients who would be more likely to benefit from cell- cycle dependent chemotherapy regimens, and may guide the development of future therapeutic strategies.

KEY WORDS: ovarian epithelial cancer, Ki 67 index, prognosis

*Corresponding author

LAVANYA RAJAGOPAL
Assistant Professor, Department of Pathology, SRM Medical College Hospital and Research Centre, Kattankulathur, Chennai India.-603203

INTRODUCTION

In India, ovarian tumors account for 8% of all gynecological malignancies[1] Ovarian cancer remains the leading cause of death among gynecological malignancies[)2]. Ovarian tumors are a puzzling group of neoplasms that do not fall neatly into benign or malignant categories. Their behaviour is enigmatic, their pathogenesis is unclear, and their diagnosis, clinical management& prognosis is controversial, especially for borderline epithelial tumors. Diagnosis of ovarian tumors has attained a significant development in recent years after the use of immunohistochemistry. Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells is often correlated with the clinical course of cancers. The purpose of this study was to evaluate the biological significance of reactivity of Ki67 antigen expression in ovarian tumors, and assess the statistical difference between benign, borderline & malignant epithelial ovarian tumors.

MATERIALS AND METHODS

This prospective 3 year study is undertaken in the Department of Pathology, Madurai medical college, Madurai.This study was conducted on 42 ovarian epithelial neoplasms. Ki 67 immunohistochemicaproliferative marker study using peroxidase-antiperoxidasetechnique was done using mouse monoclonal *Ki67* antibody [B126.1] Goat anti-mouse IgG polyclonal (1/300) was used as the *secondary antibody* in 42 selected cases which comprised benign, borderline and malignant ovarian tumors. Positive Ki 67 staining was observed as brown granular nuclear staining. For Ki 67 scoring the most positive area of the tumor was selected avoiding foci of inflammation. The number of positive nuclei is counted in 500 tumor cells in a high power field (x 400 magnification). The average of 3 counts over the same slide was taken and expressed as the percentage of Ki 67 positive cells in the tumor. This study was approved by the institutional ethical committee.

RESULTS

PROLIFERATIVE MARKER STUDY
RESULTS OF Ki 67 LABELLING INDEX

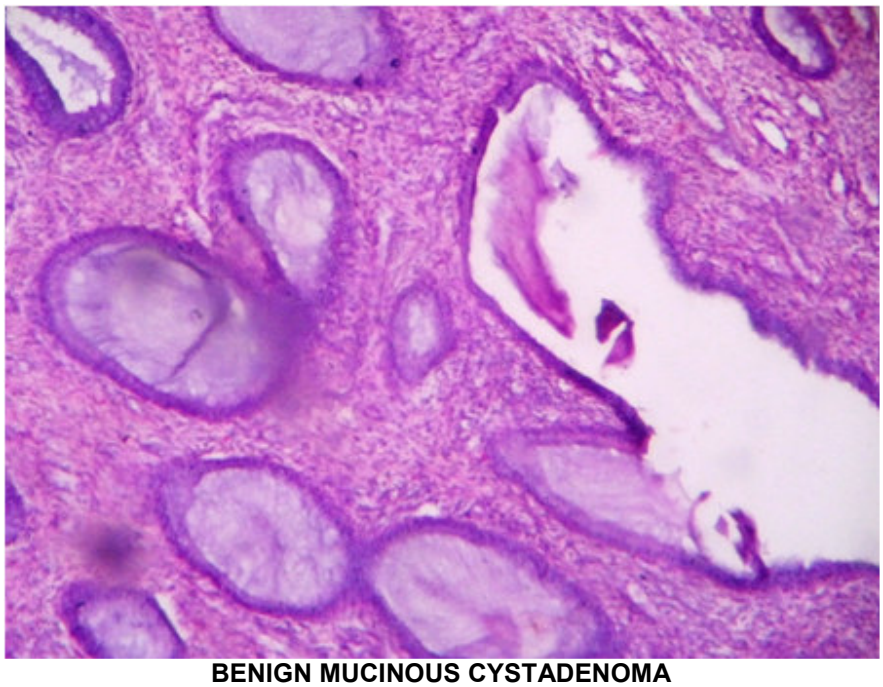
Ki 67 labelling index was studied in42selected cases which comprised of 14 benign cystadenoma (7 serous,7 mucinous), 14 borderline cystadenomas (7 serous,7 mucinous), 14 carcinoma (7 serous, 7 mucinous adenocarcinoma), One way analysis of variance test was used to assess the statistical difference between Benign, borderline & malignant epithelial ovarian tumors. The comparative analysis of Ki 67 labelling index has been shown in table 1.

Table 1
Comparative analysis of ki 67 labelling index

S. no	TYPE OF CASES	NO.OF CASES	Ki67 INDEX
1	BENIGN SEROUS CYSTADENOMA	7	2.8
2	BENIGN MUCINOUS CYSTADENOMA	7	3.0
3	BORDERLINE SEROUS CYSTADENOMA	7	8.3
4	BORDERLINE MUCINOUS CYSTADENOMA	7	6.1
5	SEROUS CYSTADENOCARCINOMA	7	29.1
6	MUCINOUS CYSTADENOCARCINOMA	7	32.4

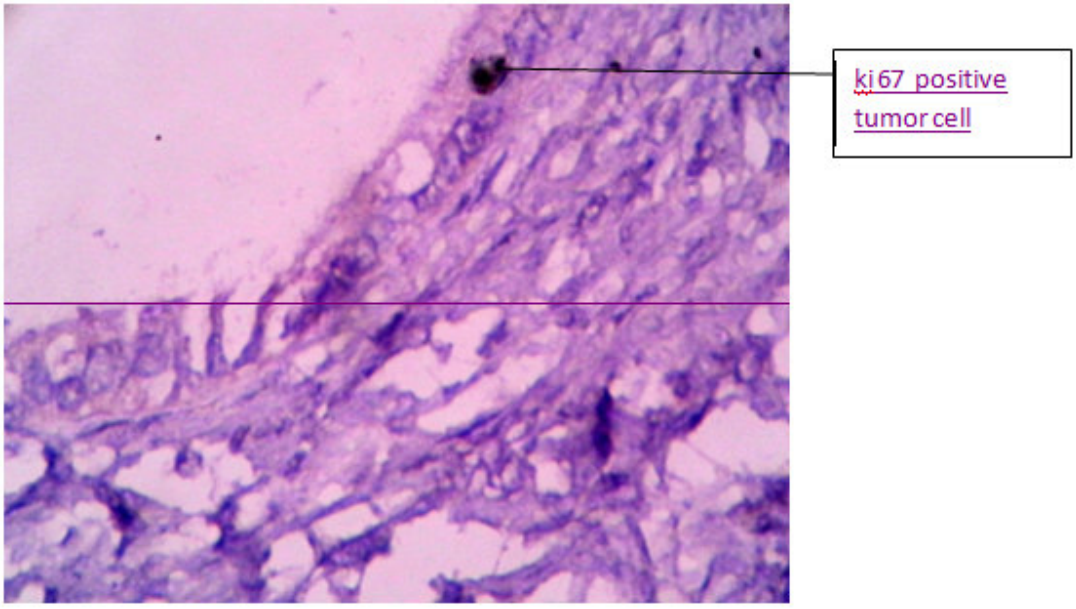
Benign tumors had a mean Ki 67index of 2.9%(Fig 1&2), borderline tumors had a mean Ki 67 index of 7.2%(Fig 3&4) while the malignant tumors had a mean Ki 67 index of 29.9%(Fig 5&6).The difference in the mean value between benign, borderline, and malignant epithelial tumors were statistically significant (p =<0.001).

Figure 1
Cyst lined by columnar cells with bland basal nuclei and apical mucin (H&E x 100X)



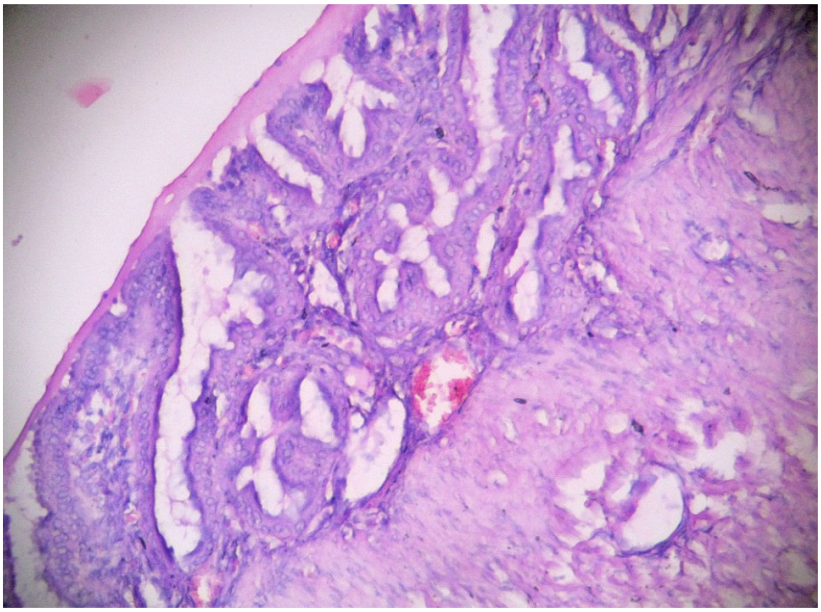
BENIGN MUCINOUS CYSTADENOMA

Figure 2
Ki-67 index 3.1 % (H& E x 400X)



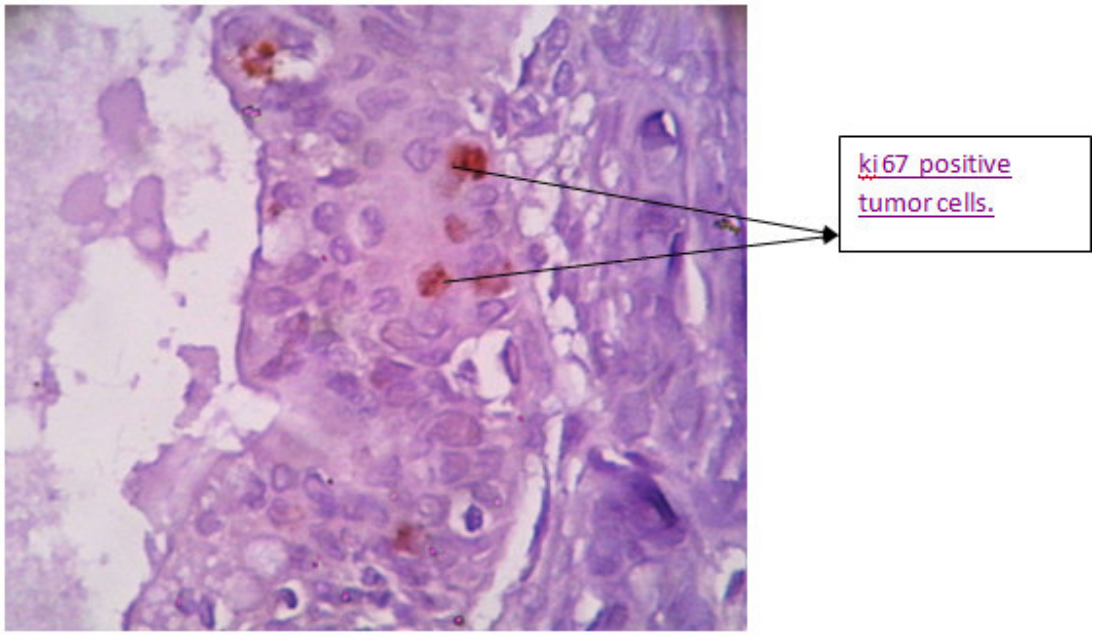
BENIGN MUCINOUS CYSTADENOMA

Figure 3
(H&E x 100X)



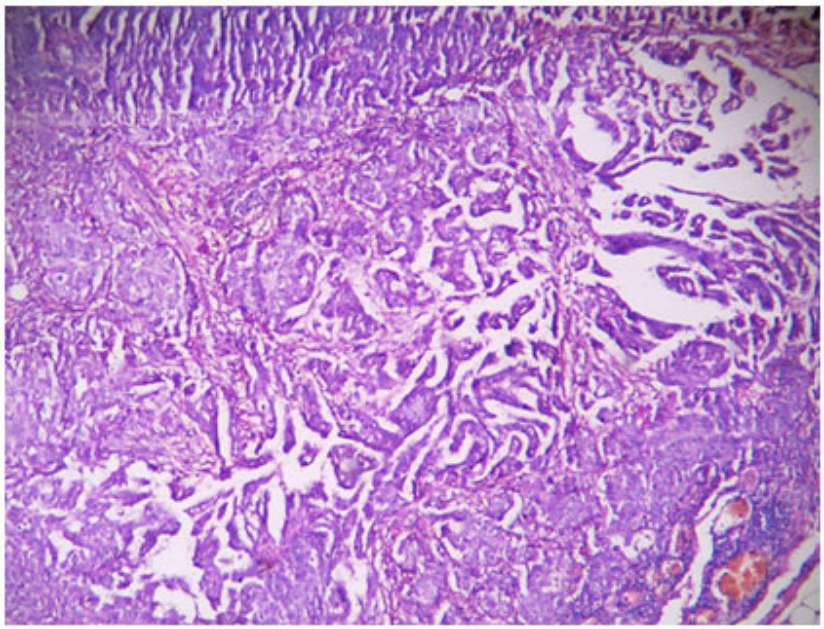
BORDERLINE MUCINOUS CYSTADENOMA

Figure4
(H&E x 100X) Ki-67 index 6 %(H& E x 400X)



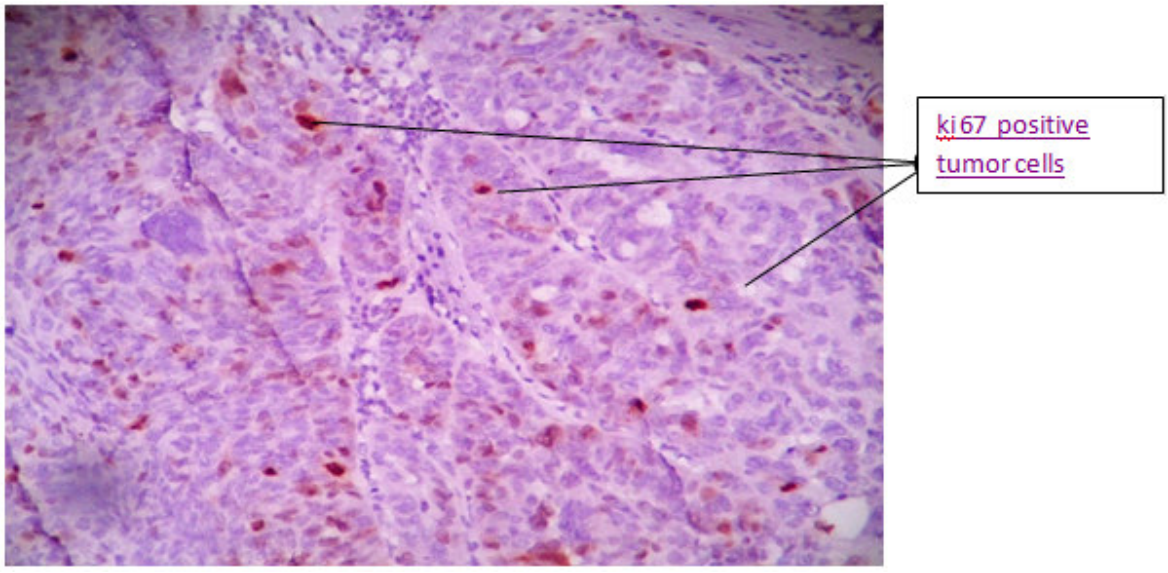
BORDERLINE MUCINOUS CYSTADENOMA

Figure 5
Tumor cells arranged in solid nests and papillary pattern (H& E x 100X)



PAPILLARY SEROUS CYSTADENOCARCINOMA

Figure 40
G 104/10(H& E x 400X) Ki-67 index 29



PAPILLARY SEROUS CYSTADENOCARCINOMA

DISCUSSION

Ovarian cancer is the third most common malignancy among women accounting for 5.5% of all cancers[3].About 80% of all ovarian tumors are benign and occur mostly in young women between 25 and 40 years of age[4]. The importance lies in distinguishing benign and malignant tumors of the ovary in the reproductive age group where the conservation of other ovary is important[4].About 75% of them are benign and 25% are malignant. The prognosis is poor, with an overall survival rate of about 40% in 5 years. Over 70% of the women diagnosed with ovarian carcinoma have advanced disease at the time of diagnosis. Important prognostic factors include stage of disease, age at diagnosis, histological type and grade, ploidity, and the amount of residual disease after primary surgery. Furthermore, high proliferative activity in the ovarian tumor has been shown to imply a poor prognosis. Mitotic count a traditional and practical method to determine proliferative activity, but is hampered by

several disturbing factors. Immunohistochemical detection of proliferating cells is an alternative way to determine the proliferative potential of a tumor[5]. Ki-67 is the most studied proliferation marker in cancer research. The gene for Ki67 protein is located on chromosome 10q25. Ki67 protein expression is strictly connected with cellular cycle. It is a nuclear protein expressed in cells during the G1, S, G2 and M cell cycle phases and is absent in quiescent cells (phase G0). Ki67 is overexpressed in malignant tissues compared to benign or borderline tissues. The Ki-67/MIB-1 immunostaining was
confined to the nucleus. The positive tumor cell nuclei were heterogeneously distributed.

Ki-67 LABELLING INDEX

The rate at which a tumor proliferates has long been considered to bear a relationship with its clinical course. The determination of growth fraction using Ki- 67 index is a simple method and has long been shown to have a prognostic value in a variety of malignancies like CNS tumors, Lymphoproliferative diseases, connective tissue tumors and breast tumors ()[6]. Ki67 expression in different ovarian tumors has long been studied by various authors across the world. However, there is a paucity of such a study in the Indian literature. Few literature clearly document the importance of Ki 67 proliferative marker in assessing the prognosis of ovarian cancer

Ki 67 INDEXIN BENIGN, BORDERLINE AND MALIGNANT OVARIAN TUMORS

We evaluated Ki 67 expression in 42 selected cases which included 14 benign cystadenoma (7 serous,7 mucinous), 14 borderline cystadenomas (7 serous,7 mucinous), 14 carcinoma (7 serous, 7 mucinous adenocarcinoma).The Ki 67 index gradually increases from benign to malignant tumors()[6]. We observed the same in our study with ki-67marker. Benign tumors had a mean Ki 67index of 2.9%, borderline tumors had a mean Ki 67 index of 7.2% ,while the malignant tumors had a mean Ki 67 index of 29.9% . A statistically significant difference (p =<0.001) was obtained between the mean Ki 67 indices of benign, borderline & malignant tumors. These findings are in close agreement with Garzetti et al ()[7] and with Monisha chowdhury et al[6]Ki 67 index is especially useful in borderline epithelial ovarian tumors of low malignant potential. These are seen in younger premenopausal women between 30-50 years of age. They remain confined to ovary for a longer time. Overall about 15-25 % of borderline tumors behave in a malignant fashion, invading locally and even metastasizing. It is thus important to identify those borderline neoplasms which are likely to behave in a malignant fashion, for it is possible that histological grading of the degree of severity of the epithelial proliferation and atypical may prove to have some prognostic benefits()[8]For the most part, the clinical behaviour of these histologically intermediate or borderline tumors is benign, with the exception of serous borderline surface epithelial stromal tumors, which are frequently associated with extra ovarian disease and exhibit a clinical behaviour intermediate between benign serous tumors and invasive serous carcinomas[][6,8] Ki-67/MIB-1 expression was positively correlated with mitotic count tumor grade , and stage of disease .The high proliferation rate has been associated with tumor aggressiveness and correlates with the prognosis and other known clinicopathological features of the tumor()[9] .In advanced disease, women with complete response to first-line chemotherapy had significantly higher Ki-67/MIB-1 expression as compared to women with only partial response or stable disease after first line chemotherapy (p=0.003). Ki-67/MIB-1 expression is an established method for evaluation of proliferation in ovarian tumors. In accordance with previous studies, we found higher expression of Ki-67/MIB-1 in carcinomas than in borderline and benign ovarian tumors. Furthermore, the expression of Ki-67/MIB-1 was positively correlated to stage of disease tumor grade in the group of carcinomas. This has also been reported by others .Troublesome aspects of this immunostaining are the lack of standardization of the procedure and the pronounced inter-laboratory variation of indices. These factors make it difficult to establish cutoff values for identification of more aggressive ovarian tumor.

CONCLUSION

Until now, the heterogeneous group of ovarian carcinomas has been treated with the same chemotherapy regimens. In the future, sub-classification of ovarian carcinomas will be important in order to provide a more tailored therapy for this malignancy. Thus, the cellular proliferation status of a tumor may be a diagnostic, as well as a prognostic tool. Ki 67 immunostaining is a simple method which provides robust prognostic information for patients with ovarian cancers. This biomarker is very much useful to identify borderline tumors which are likely to behave in a malignant fashion and may define subgroups of patients who would be more likely to benefit from cell- cycle dependent chemotherapy regimens, and may guide the development of future therapeutic strategies.

REFERENCES

1. Jagadeeshwari N., Reddy R.S., Raw K.S. Incidence of Ovarian tumors. J ObstetGynaecol India, 21: 727-32, (1971).
2. Benedicta D., Sreekantha, Vivian D. Hyperamylasemia in ovarian tumors- serum amylase as a marker for ovarian cancers. Int J Pharm Biosci, 2(1): B-445-449, (2011).
3. Gupta M.C., Mahajan B.K. In Epidemiology of non-communicable diseases. Textbook of

Preventive and Social Medicine. 3rd Edn.:Jaypee Brothers: New Delhi, 306-328, (2003).
4. Kumar V., Abaas A.K., Fausto N. Robbins and Cotran Pathologic Basis of Disease, 7rdEdn:: WB Saunders Company, Philadelphia;. 1059-11, (2004).
5. Christopher D.M., Fletcher. Diagnostic Histopathology of Tumors, Churchill Livingstone, Elsevier,publisher, 567-576, (2007).
6. Choudhury M., Goyal S., Pujani M. A cytohistological study of Ki-67 expression in ovarian tumors. Ind J Pathol Microbiol, 54(1) : 21-24,(2011).
7. Garzetti G.G., Ciavattini A., Goteri G., De Nictolis M., Stramazzotti D., Lucarini G. Ki-67 antigen immunostaining (MIB 1 monoclonal antibody) in serous ovarian tumors: Index of proliferative activity with prognostic significance. GynecolOncol, 56:169-74,(1995).
8. Jonathan S., Berek . Berek and Novak's gynecology,14th Edn,Lippincott Williams & Wilkins publisher: 1522-1523, (2007).
9. Wong W.S., Tattersall M.H. Immunohistochemical determination of tumor growth fraction in human ovariancarcinoma. Br J Obstet Gynaecol, 96:720-4, (1989).