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Applying Ki67, Bcl2 and CD117 Immuno-Histochemical Expression in the Grading of Bronchopulmonary Neuroendocrine Tumors

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Abstract

Background: Ki67, Bcl2 and CD117 are expressed significantly greater in high-grade Bronchopulmonary Neuroendocrine Tumors (BP-NETs). This study tries to evaluate the co-expression of these three markers in small Cell Neuroendocrine Tumors (SCNETs) and Typical Carcinoid Tumors (CTs).

Method: Formalin Fixed Paraffin Embedded (FFPE) blocks from the specimen repository of the Department of Pathology at Masih Daneshvari Hospital were evaluated. IHC stain was used to assess the expression of ki67, Bcl-2 and CD117 in 17 SCNETs and 19 cases of CTs. Staining percentage of neoplastic cells was multiplied by intensity of the stain to produce the Staining Index (SI). SI higher than 6 for Ki67 and 25 for both Bcl2 and CD117 were considered positive.

Result: Expression of ki67 and Bcl2 was significantly higher in SCNETs. CD117 expression and Bcl2/Ki67/CD117 co-expression were not significantly different between SCNETs and CTs. Bcl2/Ki67 and CD117/Bcl2 but not ki67/CD117 were significantly higher in SCNETs ($P< 0.05$). CD117 was positive in 52.6% of CTs and 65% of SCNETs. Its positivity was not significantly greater in bronchial biopsies of SCNETs devoid of crush artifacts.

Conclusion: A statistically significant difference was documented in single and paired expression of Ki67 and Bcl-2 as well as co-expression of CD117/Bcl-2 which could assist in discriminating low versus high grade Bronchopulmonary neuroendocrine tumors; however, using CD117 expression for this purpose requires more investigation.

Keywords: Pulmonary; Bronchial; Neuroendocrine; Ki67; Bcl2; CD117; Expression

Introduction

Although the incidence of Bronchopulmonary neuroendocrine tumors (BP-NETs) is not high, a recent rise of incidence has been noted [1]. Four principal types of these tumors include Typical Carcinoid tumor (TC), Atypical Carcinoid tumor (AC), Large Cell Neuroendocrine Carcinoma (LCNEC) and Small Cell Lung Carcinoma (SCLC). This classification is according to the grade of biological aggressiveness (Grade 1 to 3) and the extent of differentiation (well versus poorly differentiated). The well-differentiated neoplasms consist of typical (G1: Low Grade) and atypical (G2: Intermediate Grade) carcinoids, while LCNEC and SCLC (G3: high grade) are poorly differentiated [2]. This grading also is essential for treatment options [3, 4]. Grading is based on histologic findings in the endobronchial or transthoracic biopsy, e.g. mitosis and necrosis [5, 6]. There is a considerable interobserver variability in counting mitosis since it is a time consuming and erroneous job; Immuno-histochemical staining can help in this regard by differentiating the extent of the antigen expression among various grades of these neoplasms [7-8].

Ki67 is a proliferation marker which stains cell nuclei through all phases of cell cycle except G0. The mean expression of it in Bronchopulmonary Neuroendocrine Tumors (BPNETs) was variable due to different positivity criteria used for evaluating this Immuno Histo Chemistry (IHC) marker [9]. C-Kit was further expressed in a significantly higher number of Small Cells Neuroendocrine Tumors (SCNETs) compared to Carcinoid Tumors (CTs) with respect to previous studies. This protein, also known as CD117, is a type III tyrosine kinase receptor which interacts with the steel factor and results in paracrine growth stimulation of the neoplastic cells [10,11]. Bcl-2 is a nuclear protein which inhibits apoptosis and assists the survival of malignant cells. The expression of this protein was proved to increase gradually from low, through intermediate, to high grade BPNETs [12].

Previously studied co-expression of Bcl-2 and CD117 in BPNETs revealed negative results in low grade and intermediate grade BPNETs, whereas seventy percent of high grade BPNETs did express both markers [13].

According to our knowledge there is no research comparing the expression of these three IHC markers in low and high grade BPNETs. In this research we aim to study the co-expression of Ki67, Bcl-2 and CD117 in SCLC (high grade) and typical CT (low grade) in order to discriminate the two groups, regarding the difficulties of the job in small biopsies especially in the presence of crush artifact.

Method

Formalin Fixed Paraffin Embedded (FFPE) human tissues of biopsy (for CTs and SCNETs) or resection (for CTs) specimens were obtained from the archives of the department of pathology, Masih Daneshvari Hospital- a pulmonary tertiary referral hospital in Tehran-Iran. Inclusion criteria consisted of low and high grade BP-NETs with confirmed clinic pathologic diagnosis which contained adequate

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material for IHC staining. The accuracy of primary diagnosis was rechecked blindly by two expert pathologists, considering clinical and radiological evaluation. The diagnosis was according to 2004 WHO criteria. Total number of 19 carcinoid (5 of which had following resection performed in this center) tumors and 17 SCNETs were treated to inactivate endogenous peroxidase and stained with monoclonal, ready to use CD117, Ki67 and Bcl-2 antibodies (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom). The external control tissue stained for CD117 was gastrointestinal stromal tumor while tonsillar lymphoid tissue was considered for Ki67 and Bcl2. The intensity of CD117, Bcl-2 and Ki67 staining was compared with background mast cells, lymphocytes and respiratory epithelium basal cells respectively and graded as null, 0.5 and 1. For harmonization purposes this grade was taken into account when assessing the percentage of stained neoplastic cells by multiplying the grade number and the percentage and reporting a Staining Index (SI). For Bcl-2 (nuclear) and CD117 (cytoplasmic/membranous), a SI of less than 26 was negative, while both 26-75 (weak) and above 75 (strong) were considered as positive. As for Ki67 (nuclear), a SI of less than 6 was negative while both 6-20 (weak) and above 20 (strong) were interpreted as positive. Two expert pathologists blindly evaluated the staining index of every case.

Categorical variables were compared by chi-square (fisher exact test with any expected frequency less than 1 or with 20% of expected frequencies $\leqq$ 5), while continuous variables were compared using the Student t-test. All statistical analyses were performed using Excel software 2010. This study was conducted with the approval of Masih Daneshvari Hospital ethics committee and utilizing protocols approved by the respective institutional review boards (SBMU1.REC.1394.109).

Result

Among the seventeen patients in the SCNET group, 11 cases (65%) were male with a mean age 0f 61y ± 9.5. Nineteen patients in the CT group (mean age 44.3y ± 16.6) were recruited, 8 (42%) of which were men.

Our findings indicated that 15 (88.2%) of SCNETs were positively stained for Ki67 (fourteen strongly and one weakly positive) while only 4 (21%) of CTs were positive (four weakly positive). Studying Bcl-2 expression revealed fourteen (82.3%) positive cases in the SCNET group (ten strongly and four weakly positive), whereas the CT group had just 2 (10.5%) cases of weak positivity. We found that 11 (64.7%) cases of SCNETs showed positive staining for CD117 (six strong and five weak in intensity) however, 10 (53%) of cases in the CT group were also positive for CD117 (two with strong and eight with weak expression). Based on the findings, despite the fact that 15 (88.2%) cases of SCNETs and (42.1%) of CTs showed crush artifacts, CD117 expression in SCNETs without artifact was not significantly higher. As seen in Table 1, the positivity difference between the SCNET and CT groups was significant for Ki67 and Bcl2 but not for CD117. The results for paired and tripled markers are also summarized in Table 1. Co-expression of Ki67/Bcl2 and Bcl2/CD117 between these two groups is significantly different, while Co-expression of all three markers was not statistically significant.

Table 1: Demographic data, single and coexpression of Ki67, Bcl2 and CD117 in BPNETs.

	Total(36)	SCNET(17)	CT(19)	P value
Sex: Male/female	36	11/6	8/11	0.17†
Age: Mean± SD	52.1±17.3	61 ± 9.5	44.3 ± 16.6	<0.001*
Smoking	15(41.7%)	11(64.7%)	4(21%)	<0.05†
Ki67	19(52.7%)	15(88.2%)	4(21%)	<0.001†
Bcl2	16 (44.4%)	14(82.3%)	2(10.5%)	<0.001†
CD117	21(58.3%)	11(64.7%)	10(52.6%)	0.4†
Ki67/Bcl2	21(58.3%)	16(94.1%)	5(26.3%)	<0.001†
Ki67/CD117	28(77.7%)	15(88.2%)	13(68.4%)	0.23‡
CD117/Bcl2	26(72.2%)	16(94.1%)	10(52.6%)	<0.05‡
Ki67/Bcl2/CD117	29(80.5%)	16(94.1%)	13(68.4%)	0.09‡
Crush Artifact				
CD117 expression with crush artifact Strong				
Weak				
Positive (strong+weak)				

Discussion

Single and paired expressions of Ki67 and Bcl-2 as well as CD117/Bcl-2 were higher in the SCNET group compared to CT (P< 0.001), which indicates they can be used to discriminate them from one another. The result of IHC staining for Ki67 and Bcl2 alone is consistent with previous research [6-9]. On the other hand, in the present study CD117 was positive in 53% cases of CTs which was higher than previous studies. Internal control was positive in all of these cases and the staining pattern was as defined in the methodology, thus undeniable. The antibody used was monoclonal and ready to use antibody, thus no need for dilution. No background staining was noticed. Mean area of cut sections in the 5 resected CTs were twice bigger than SCNETs, so volume/surface ratio in CTs was lower than SCNETs indicating excess antibody was not the matter and that adequate antibody supply was available for SCNETs. Overall applying CD117 for the means of discriminating SCNET and CT neoplasms was not useful and calls for further evaluation to determine the reason for the adverse results of this study compared to previous one [6-8]; Crush artifacts were suspected as one possible explanation and assessed, but no significant difference was demonstrated in the expression of this marker between crushed and uncrushed SCNETs. On reviewing the methodology of previous studies, other proposed reasons could be the manufacturing company, positivity criteria, antibody titration and pathologist's viewpoint all of which were beyond the scope of this work and could not be studied. The CD117 expression in BPNETs has a variable range (40-70 percent) [7, 8, 14-17]. It may be worth mentioning that the antibody used in Erler's study and their positivity criteria were the most similar to our study and revealed a ten percent expression for CD117 in CTs, higher than other [18].

There are several limitations in this study including no cases of atypical CTs and LCNETs and lack of financial support to confirm IHC results with a reference method like gene expression.

Conclusion

According to the present work co-expression of Ki67/Bcl-2/CD117 is not significantly higher in SCNETs versus CTs and expanding the diagnostic panel by CD117 does not improve histologic grading.

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Competing interests

The authors declare that they have no competing interest for this study.

Availability of data and materials

The datasets generated/analyzed during the current study are available through the corresponding author on reasonable request.

Ethical approval and consent to participate

This study was conducted with the approval of Masih Daneshvari Hospital ethics committee and utilizing protocols approved by the respective institutional review boards (SBMU1.REC.1394.109).

All patients are informed about the possibility of using archived FFPE blocks for future research purposes through a written consent. Nonetheless informed oral consent was obtained at the time of the study through phone calls.

Consent for publication

There is no patient data in the present study.

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