A Doctoral Dissertation

The efficacy of chemokine C-X-C motif ligand 12 as a diagnostic marker for papillary thyroid carcinoma

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갑상선암 진단에 있어서 CXCL12의 유용성

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The efficacy of CXC12

as a diagnostic marker for papillary thyroid carcinoma

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ABSTRACT

The incidence of thyroid cancer is increasing worldwide, with the most dramatic growth being the up to 25% annual increase observed over the last 10 years in Korea. Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer in Korea, and it is essential to develop methods for the accurate diagnosis of PTC to avoid unnecessary surgery. Chemokine C-X-C motif ligand 12 (CXCL12) has been reported to be associated with chemokine C-X-C motif receptor 4 (CXCR4) mediated activation of G-protein-coupled signaling molecules in various cancers. We aimed to study the efficacy of CXCL12 for the diagnosis of PTC.

We prospectively collected 58 PTC samples from 2013 to 2015 and obtained 31 benign thyroid disease samples from the Korea Biobank of Jeju National University Hospital. Immunohistochemical and immunocytochemical staining for CXCL12 and B-type Raf kinase (BRAF) V600E mutant protein were performed on fine-needle aspiration (FNA) and tissue microarray (TMA). A *BRAF V600E* gene mutation study was also performed.

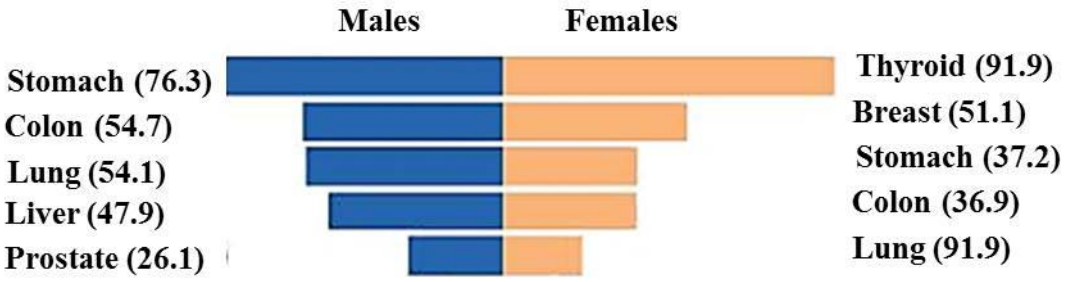
The mean age of the subjects was 49.1±1.4 years and 88.5% were wemen. CXCL12 and BRAF were positive in 6.4% and 19.4% of cases of benign disease and 98.3% and 93.1% of PTCs, respectively. The sensitivity of CXCL12 was 93.1% in FNA and 98.3% in TMA, and that of BRAF was 62.1% in FNA and 93.1% in TMA. CXCL12 showed 90.3% specificity, 94.7% positive predictive value, 87.5% negative predictive value and 89.1% accuracy for diagnosis of PTC. Among the cases of atypia of undetermined significance (AUS), CXCL12 in FNA showed 100% sensitivity and 100% specificity compared with 62.5% sensitivity and 100% for BRAF. Strong CXCL12 expression (3+) was correlated with lymph node metastasis ($p = 0.031$), but not tumor size.

In conclusion, CXCL12 in FNA and TMA may be an effective diagnostic marker for PTC and could facilitate its accurate diagnosis, especially in cases where the FNA is initially reported as AUS.

INTRODUCTION

The thyroid gland is an uncommon site of cancer, accounting for only 1.4% and 4.6% of new cases of cancer among men and women, respectively, in the United States in 2016 [1]. The incidence of thyroid cancer has increased worldwide, with the most dramatic growth being observed in developed countries [2]. In Korea, thyroid cancer is an important health problem. With an incidence of 91.9 per 100,000 peple, it was ranked as the top cancer in women by the Natioinal Information Center in Korea (Figure 1), and has shown an annual 25 % increase over the last 10 years [3]. It has been suggested that screening is the main factor driving this high incidence of thyroid cancer, although another report showed that the incidence of thyroid cancer has also increased in children, who are not subjects of routine health examinations [4]. This means that the increasing incidence of thyroid cancer cannot be fully explained as relating only to screening. Therefore, Korea is one of the countries with the highest incidence of thyroid cancer, which makes it a major health problem requiring proper screening and additional epidemiological analysis.

Figure 1. Incidence of top-ranked cancers per 100,000 people in 2009 according to the National Cancer Information Center in Korea



Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer (Table 1). However, in Korea the proportion of PTC is higher (more than 95%) and that of follicular thyroid cancer is lower (1.6%) than in Western countries (10-15%) [5] in Korea. Therefore, PTC is the most important subtype of thyroid cancer in Korea.

Table 1. Incidence of different histological types of thyroid cancer in 2010 according to the National Cancer Information Center in Korea

Histological group	Cases (N)	%
1. Carcinoma	35,793	99.4
Follicular carcinoma	572	1.6
Papillary thyroid carcinoma	34,869	96.8
Medullary carcinoma	155	0.4
Anaplastic carcinoma	78	0.2
Other specified carcinoma	79	0.2
Unspecified carcinoma	40	0.1
2. Sarcoma	2	0.0
3. Other specified malignant neoplasm	3	0.0
4. Unspecified malignant neoplasm	223	0.6
Totol	36,021	100.0

Recently, many researchers have become concerned about the rapidly increasing incidence and overdiagnosis of PTC in Korea [3]. Despite these concerns, we must selected thyroid cancer in thyroid nodules because the EUROCARE working group reported that in the 1990s, when doctors did not aggressively try to diagnose PTC, the five-year survival rate for thyroid cancer was reported to be 71%

for men and 79% for women, even to 37-42% in elderly patients [6]. In Korea, the five-year survival rate for thyroid cancer has improved to nearly 100% because of efforts to diagnose the cancer early and treat it aggressively using various methods [7]. However, there is still some concerns about the acute or chronic side effects of treatment of PTC, including hoarseness, vocal cord paralysis, hypoparathyroidism, bleeding, and dry mouth. These side effects could be prevented by accurate diagnosis, selection of subjects who require surgery, and the development of markers that indicate a poor prognosis. Therefore, it is necessary to develop methods for the accurate diagnosis of thyroid cancer, especially the most common pathological form, PTC, to avoid unnecessary surgery and to maintain excellent survival.

Fine-needle aspiration (FNA) has been considered to be the best diagnostic test to confirm PTC in thyroid nodules. Although FNA of PTC is usually straightforward, achieving an accurate diagnosis still remains challenging for several reasons. First, a cytological aspirate specimen containing few cells with considerable nuclear atypia is often categorized as atypia of undetermined significance (AUS) in the Bethesda system [8]. AUS has an up to 15% possibility of being thyroid cancer [8], and repeated AUS results lead to difficult decisions about whether to observe or to undertake surgery, or to use another diagnostic marker. Second, mutations in B-type Raf kinase (BRAF) are helpful to diagnose PTC, but have a low negative predictive value [9]. Thirds, genetic mutation have been studied by many researchers, but this is expensive and there are many different results of gene mutation between previous studies [10-12]. Therefore, we need to develop an accurate diagnostic marker for PTC, especially in cases of AUS.

Chemokine C-X-C motif ligand 12 (CXCL12), or stromal cell-derived factor 1, is a member of the chemokine family that functions in conjunction with the G-protein-coupled receptor chemokine C-X-C motif receptor 4 (CXCR4) [13]. The molecular mechanism underlying the biological effect of CXCL12 is associated with CXCR4-mediated activation of G-protein-coupled signaling molecules, including ERK1/2, MAPK, JNK, and AKT [14,15]. Although CXCL12 has been reported to have

prognostic significance in some cancers [16], there have been few studies of the diagnostic role of CXCL12 in thyroid cancer [17,18]. Therefore, we aimed to evaluate the efficacy of CXCL12 for diagnosis of PTC.

SUBJECTS AND METHODS

1. Subjects

Between 2013 and 2015 we obtained 59 thyroid samples from patients scheduled for surgery because of thyroid nodules, with cytology results including benign, AUS, suspicious of malignancy, or malignant according to the Bethesda system. Thirty samples of benign thyroid diseases tissue were obtained from the Korea Biobank of Jeju National University Hospital (No. A-07). This study was conducted with approval from the Jeju National University Hospital Institutional Review Board (IRB No. 2013-01-002). Written informed consent was obtained from all subjects in this study.

2. Protocol

The patients were diagnosed by FNA using a conventional smear technique. We repeated the FNA for liquid-based cytology (LBC), which has progressively gained consensus as the best test for PTC before surgery, in an operating room under the anesthesia to avoid unnecessary pain by methos as same as initial FNA. Routine Papanicolaou staining and immunocytochemical studies for CXCL12 and BRAF V600E mutant protein were performed on the LBC specimens. Surgically resected tissue specimens were tested for routine diagnosis, immunohistochemistry (IHC) for CXCL12 and BRAF V600E mutant protein, and *BRAF* gene mutation. All slides were randomly twice reviewed by the study pathologist (Y.H.M.).

3. Tissue Microarray (TMA) Construction

Two TMAs were constructed as described previously. Briefly, hematoxylin and eosin (H&E)-

stained slides were reviewed and the most representative tumor area was marked. The area was carefully marked on both H&E-stained slides and formalin-fixed, paraffin-embedded tissue blocks. A core (2 mm in diameter) of the representative area was obtained from each of 59 cases of PTC and 30 cases of benign thyroid diseases including normal thyroid, nodular hyperplasia, follicular adenoma, and Hashimoto's thyroiditis. One section from each block was stained with H&E for tissue confirmation.

4. Immunohistochemistry and Immunocytochemistry

IHC was performed on 4-μm-thick sections from TMA blocks. Tissues were stained with anti-CXCL12 antibody [R&D systems, Minneapolis, MN, USA] (1:50 dilution) and anti-BRAF V600E monoclonal antibody [Spring Bioscience, Pleasanton, CA, USA] (1:50 dilution) using an automated immunostainer (Benchmark XT; Ventana Medical Systems Inc., Tucson, AZ, USA). For immunocytochemical (ICC) staining, LBC slides were fixed in 95% ethyl alcohol for 30 minutes before being processed as for IHC.

Immunoreactivity was evaluated by a pathologist blinded to the mutation analysis results. The IHC and ICC slides were examined independently. The extent of cytoplasmic staining was graded from 0 to 3: 0, negative; 1, focal weak staining; 2, diffuse weak or focal strong staining; 3, diffuse strong staining. Scores of 1, 2 and 3 were considered positive, and a scores of 0 was considered negative [19]. The samples were scored twice by an independent observer in a blinded manner. If discrepancies occurred, a consensus score was reached.

5. DNA extraction

A representative slide was selected from each tissue sample and the most relevant area was marked

for manual microdissection. Genomic DNA was extracted from 10-μm sections of formalin-fixed paraffin-embedded tissue using the QIAmp DNA FFPE tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

6. Detection of *BRAF V600E* mutation

The presence of the *BRAF V600E* mutation was tested using the peptide nucleic acid (PNA) Clamp BRAF Mutation Detection Kit (Panagene, Inc., Daejeon, Korea) according to the manufacturer's instructions. All reactions were performed in a total volume of 20 μl containing template DNA, a primer and PNA probe set, and real-time SYBR® Green PCR master mix. All required reagents were included with the kit. The PCR cycling conditions were 94 ℃ for 5 minutes followed by 40 cycles of four temperature steps (94 ℃ for 30 sec, 70 ℃ for 20 sec, 63 ℃ for 30 sec, and 72 ℃ for 30 sec).

The PNA probe, designed to hybridize completely to the wild type *BRAF* allele, suppresses amplification of wild-type targets but allows amplification of the mutant allele. The threshold cycle (Ct) was automatically calculated from the PCR amplification plots where fluorescence was plotted against the number of cycles. The changes in Ct values (ΔCt) were calculated as the Ct values of the PCR of the samples minus the Ct value of the PCR of the PNA control. The higher ΔCt value indicates that the mutant was efficiently amplified. The cutoff value of 2.0 was used for determining the presence of mutant DNA.

7. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS standard version 18.0; SPSS Inc., Chicago, IL, USA). We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV), and diagnostic accuracy. The χ^2 test or Fisher's exact test was used

to evaluate the statistical significance of the associations between the expression of CXCL12 and BRAF in IHC and ICC, and the *BRAF* mutation in TMA. We also used coefficient analysis to identify any association between CXCL12 or BRAF and T and N stange. All p values < 0.05 were considered significant.

RESULTS

1. The baseline characteristics of patients

The mean age of subjects in this study was 49.1 ± 1.4 years and 88.5% were women. By FNA, 12 patients had AUS, 17 suspicious of malignancy, and 40 malignant. The mean tumor size was 1.2 ± 0.1 cm, range 0.4 -4.7 cm, 59% of subjects had a single tumor and 46.2% of them had no lymph node metastasis. The subjects were recommended for surgery for various reasons including the size of tumor, repeated AUS, or suspicious of malignancy or malignant in FNA. In addition, we obtained 30 specimens of benign thyroid diseases including normal thyroid, nodular hyperplasia, follicular adenoma, and Hashimoto's thyroiditis from the Korea Biobank of Jeju National University Hospital, to supplement the small number of benign tumors. Therefore, there were final diagnoses by tissue histology of benign conditions in 31 subjects and PTC in 58 (Table 2).

The distribution of CXCL12 and BRAF immunochemical staining in benign and malignant tissue specimens is shown in Table 2. CXCL12 was detected in only two cases of benign disease. We strictly classified samples with 0+ CXCL12 only and 0+ BRAF as a negative. Therefore, CXCL12 and BRAF were positive in 6.4% and 19.4% of benign diasease samples and 98.3% and 93.1% of PTC samples, respectively.

Table 2. Distribution of CXCL12 and BRAF immunohistochemical staining, according to final diagnosis

	CXCL12		BRAF	
	Positive	**Negative**	**Positve**	**Negative**
Benign (n=31)	**2**	**29**	**6**	**25**
PTC (n=58)	**57**	**1**	**54**	**4**

CXCL12, chemokine (C-X-C motif) ligand 12; PTC, papillary thyroid carcinoma,

2. The expression of CXCL12 and BRAF V600E mutant protein in specimens of benign and malignant disease

IHC showed that CXCL12 was strongly expressed in 98.3% of FNA specimens of PTC and absent in 93.5% of samples with benign thyroid cytology (Figure 2A and 2D); expression was only weakly positive (1+) in the positive benign specimens. BRAF V600E mutant protein was expressed in 93.1% of specimens with PTC cytology and 19.4% of those with benign thyroid disease (Figure 2E and 2F). However, over 25% of the total specimens examined by ICC had inadequate results because the staining intensity was too faint or ambiguous. Therefore, CXCL12 was considered to give more consistent results for thyroid specimens.

CXCL12 showed a sensitivity of 93.1% in FNA and 98.3% in TMA specimens (Table 3), a specificity of 90.3% in FNA and 93.5% in TMA specimens, a PPV of 94.7% in FNA and 96.6% in TMA specimens, an NPV of 87.5% in FNA and 96.7% in TMA specimens, and an accuracy of 89.1% in FNA and 96.6% in TMA specimens. BRAF showed a sensitivity of 62.1% in FNA and 93.1% in TMA specimens and a specificity of 100% in FNA and 80.6% in TMA specimens after exclusion of the cases with inadequate results. Its PPV was 100% in FNAC and 90.0% in TMA specimens and its NPV was 4.3% in FNA and 96.7% in TMA specimens: these results were very different because of the inadequate results for many FNA specimens. Therefore, the accuracy of BRAF was very low: 62.7% in FNA and 88.8% in TMA specimens.

Analysis of the combination of CXCL12 and BRAF in TMA specimens showed that when both were positive, the sensitivity and the specificity were 91.4% and 100%, the PPV and NPV were 100% and 85.7%, respectively, and the accuracy was 94.3%. Therefore, positivity for both CXCL12 and BRAF in TMA showed the highest specificity with a high PPV and also higher sensitivity, NPV and accuracy.

Figure 2. Immunocytochemical findings in fine needle aspiration of benign thyroid disease and papillary thyroid cancer. Benign thyroid disease showing A) cytological finding (Papanicolaou stain), B) no CXCL12 expression, C) no BRAF expession. Papillary thyroid carcinoma showing D) cytological finding (Papanicolaou stain), E) positive CXCL12, and F) positive BRAF staining (original magnification, x400).

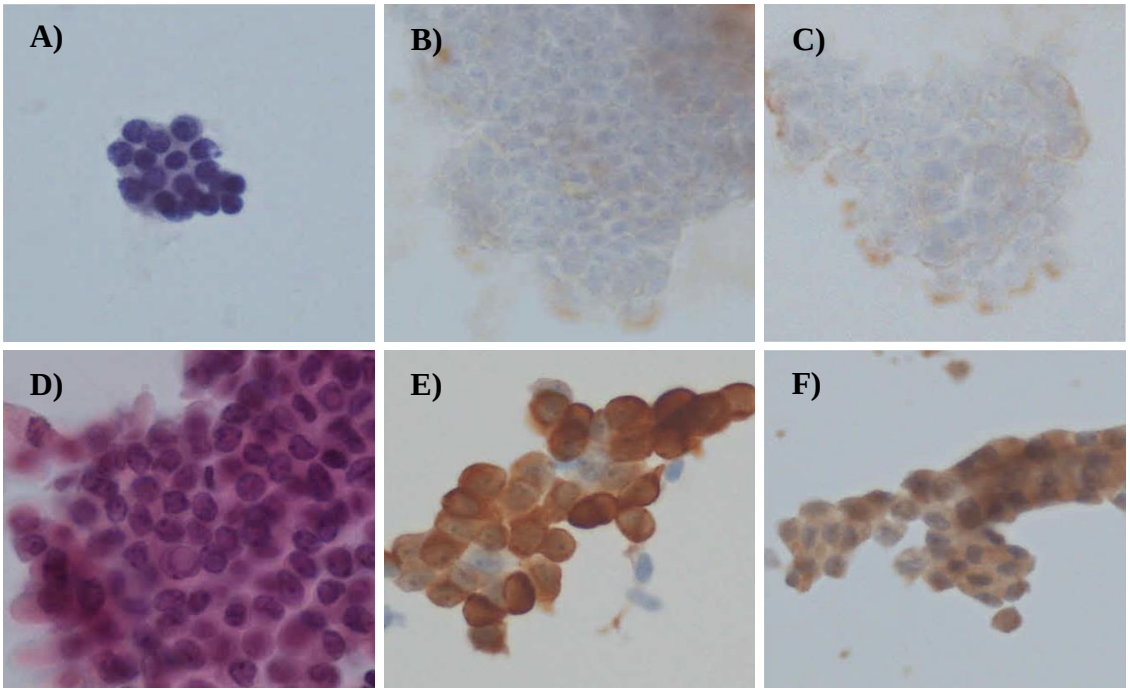


Figure 3. Immunohistochemical findings in tissue microarray of benign tumor and papillary thyroid cancer. Benign thyroid disease showing A) histological finding (H&E stain), B) no CXCL12 expression, C) no BRAF expression. Papillary thyroid carcinoma showing D) histological finding (H&E stain), E) positive CXCL12, and F) positive BRAF staining (original magnification, x200).

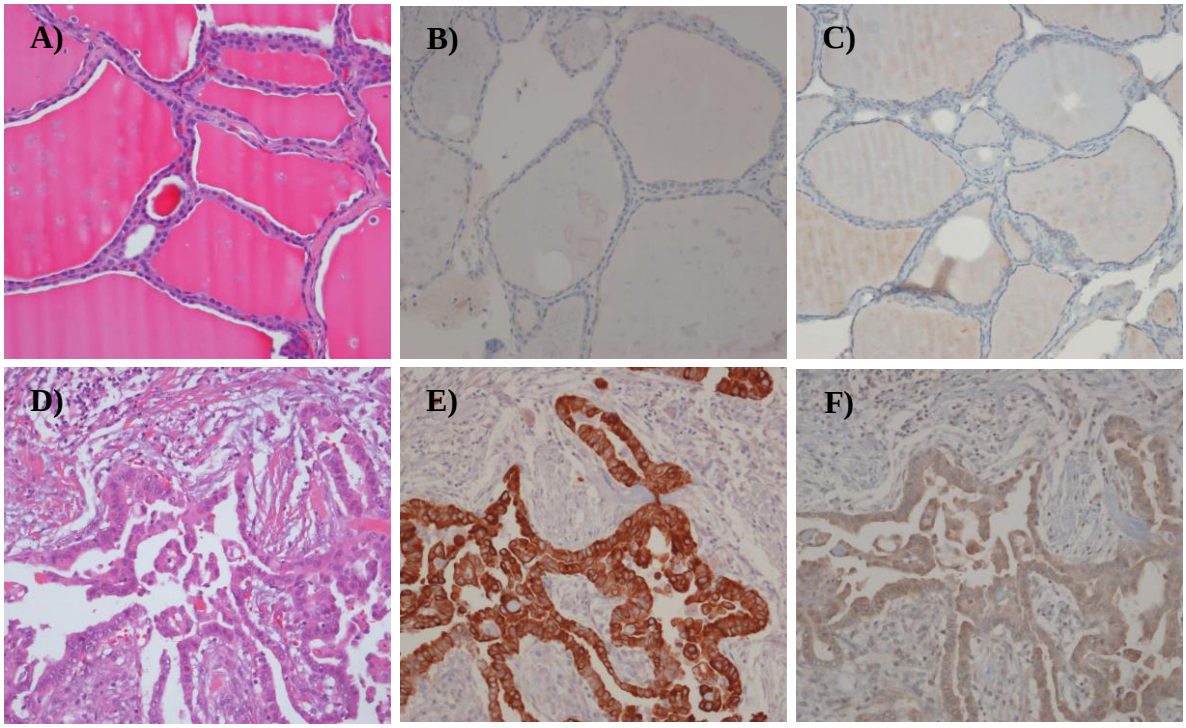


Table 3. The sensitivity, specificity, PPV, NPV, and accuracy of two immunochemical markers for papillary thyroid carcinoma

	Sensitivity	Specificity	PPV	NPV	Accuracy
CXCL12 (FNA)	**93.1 %**	**90.3 %**	**94.7 %**	**87.5 %**	**89.1 %**
BRAF (FNA)	**62.1 %**	**100 %**	**100 %**	**4.3 %**	**62.7 %**
CXCL12 (TMA)	**98.3 %**	**93.5 %**	**96.6 %**	**96.7 %**	**96.6 %**
BRAF (TMA)	**93.1 %**	**80.6 %**	**90.0 %**	**89.7 %**	**88.8 %**
Both CXCL12 and BRAF (TMA)	**91.4 %**	**100 %**	**100 %**	**85.7 %**	**94.3 %**
CXCL12 (-) and BRAF (+) (TMA)	**1.7 %**	**80.0 %**	**14.3 %**	**29.6 %**	**28.4 %**
CXCL12 (+) and BRAF (-) (TMA)	**6.9 %**	**93.3%**	**66.7 %**	**34.1 %**	**36.4 %**

PPV, positive predictive value; NPV, negative predictive value; CXCL12, chemokine C-X-C motif ligand 12; BRAF, B-type Raf kinase; FNA, fine needle aspiration; TMA, tissue microarray.

3. The *BRAF V600E* gene mutation in thyroid specimens

In this study, the prevalence of the *BRAF V600E* mutation in PTC was 58.9%. Although this is a low prevalence, the IHC staining for BRAF in TMA specimens showed good sensitivity. BRAF IHC staining was weakly positive in some PTC specimens without *BRAF V600E* gene mutations.

4. The clinical efficacy for diagnosis of PTC in specimens classified as atypia of undetermined significance

In our study, FNA for 12 patients showed AUS. The final diagnosis was benign disease in 1 patient and PTC in 11 patients. To identify the clinical usefulness of CXCL12 and BRAF in AUS, we also analyzed the sensitivity, specificity, PPV, NPV, and accuracy of CXCL12 and BRAF ICC in FNA with AUC (Table 4). The sensitivity was 100% for CXCL12 and 62.5% for BRAF, the NPV was 100% for CXCL12, and 25.0% for BRAF, and the accuracy was 100% for CXCL12 and 66.7% for BRAF. Therefore, CXCL12 was a more useful marker than BRAF to diagnose PTC in FNA, especially AUS.

Table 4. Clinical effectiveness of CXCL12 and BRAF immunocytochemical staining in atypia of undetermined significance

	Sensitivity	Specificity	PPV	NPV	Accuracy
CXCL12	**100 %**	**100 %**	**100 %**	**100 %**	**100 %**
BRAF	**62.5 %**	**100 %**	**100 %**	**25.0 %**	**66.7 %**

PPV, positive predictive value; NPV, negative predictive value; CXCL12, chemokine C-X-C motif ligand 12; BRAF, B-type Raf kinase

5. The correlation of CXCL12 and BRAF expression with tumor stage

We analyzed the correlation of CXCL12 and BRAF expression with tumor stage (Table 5). CXCL12 positivity greater than 1+ in FNA was not correlated with N stage (p=0.323) or T stage (p=0.565). However, CXCL12 3+ was correlated with N stage (p=0.031) but not T stage (p=0.952). Therefore, strong positive staining for CXCL12 was clinically associated with lymph node metastasis. BRAF positivity over 1+ in TMA was correlated with N stage (p=0.028) and T stage (p=0.037), but not that in FNA (N stage p=0.145, T stage p=0.557).

Table 5. Correlation of tumor stage with CXCL12 and BRAF immunochemial staining in FNA or TMA

	CXCL12		BRAF
	FNA≥1+	**FNA≥3+**	**TMA≥1+**
N stage	**0.122 (*p*=0.323)**	**0.262 (*p*=0.031)**	**0.289 (*p*=0.028)**
T stage	**0.073 (*p*=0.565)**	**0.008 (*p*=0.952)**	**0.280 (*p*=0.037)**

N stage, regional lymph node metastasis; T stage, tumor size; FNA, fine needle aspiration; BRAF, B-type Raf kinase; TMA, tissue microarray

DISCUSSION

Our results indicate that CXCL12 is a useful marker to diagnose PTC in thyroid nodules and is a better marker than BRAF IHC or *BRAF V600E* gene mutation, even in nodules classified as AUS. In addition, strong CXCL12 expression was correlated with worse N stage.

Jung et al. suggested that CXCL12 may be an effective supplementary diagnostic marker for PTC in preoperative FNA using the cell block method [18]. Their results showed 93.6% sensitivity, 88.6% specificity, 91.7% PPV, 91.2% NPV, and 91.5% accuracy of CXCL12 staining in PTC. In another study, CXCL12 showed 90.8% sensitivity, 96.8% specificity, 98.9% PPV, 76.9% NPV, and 92.2% accuracy in tissue specimens of PTC [17]. These previous results were similar to those of this study, which showed that CXCL12 had 93.1% sensitivity, 90.3% specificity, 94.7% PPV, 87.5% NPV, and 89.1% accuracy in FNA and 98.3% sensitivity, 93.5% specificity, 96.6% PPV, 96.7% NPV, and 96.6% accuracy in TMA. Therefore, this study and the previous two studies all indicate that CXCL12 may be a useful marker for diagnosis of PTC; however, there are important differences between these studies. The two previous studies were retrospective studies that seleced only specimens with sufficient cellularity, such as cell blocks or tissue specimens, and so were limited in their ability to assess the efficacy of CXCL12 for diagnosis of PTC because they differed from the real clinical setting. However, we performed a prospective study to collect FNA and tissue specimens from subjects in the operating room under anesthesia, and added CXCL12 and BRAF IHC staining to their routine processing. Therefore, the results of our study showed the value of CXCL12 for diagnosis of PTC in a real clinical setting.

The AUS category of the Bethesda system is a major problem for clinicians making the decision whether to operate. However, we have no supplementary markers to allow accurate diagnosis of AUS, and the guidelines state that when the result of the first FNA is AUS, the FNA must be repeated being

able to use molecular markers such as *BRAF* gene mutation to obtain accurate results [23]. However, such molecular markers have clear limitations for diagnosing PTC becauase of their low NPV: we can diagnose PTC if the sample is positive for a *BRAF* gene mutation, but this is impossible to classify benign if it is negative for a *BRAF* gene mutation. The cause of the low NPV of *BRAF* gene mutations is their low prevalence to allow accurate diagnosis of PTC. In this study, the prevalence of *BRAF V600E* gene mutation was 58.9%, which is lower than the approximately 70% prevalence reported in a previous study of Korean subjects [24,25], which also concluded that it had limited ability to diagnose PTC in AUS samples. In this study, CXCL12 showed 100% sensitivity, specificity, PPV, NPV, and accuracy in FNA categotized as AUS. Although this might be an overestimate because of the small number of samples, it does suggest that CXCL12 may be a good option for accurate diagnosis of thyroid nodules classified as AUS.

Despite its limitations, the *BRAF V600E* mutation has been suggested in the clinical guideline as a supplementary molecular marker for the diagnosis of PTC [23], and some clinicians have reported that BRAF V600E immunochemical staining showed a good correlation with the presence of a *BRAF V600E* gene mutation [26,27]. Therefore, we also performed BRAF IHC staining in FNA and TMA samples. The results showed 62.1% sensitivity in FNA and 93.1% sensitivity, 80.6% specificity, 90.0% PPV, 89.7% NPV, and 88.8% accuracy in TMA samples. Therefore, the sensitivity of BRAF staining was lower than that of CXCL12 staining for the diagnosis of PTC. The sensitivity of the combination of CXCL12 and BRAF staining was lower than that of CXCL12 staining alone, as was the sensitivity of BRAF staining in samples categorized as AUS. Therefore, BRAF staining was not a better supplementary marker than CXCL12 staining for diagnosis of PTC.

The *BRAF V600E* mutation is well known as a prognostic marker [28-31]. It was reported in previous studies to be a poor prognostic factor for the persistence of the disease, independent of other clinicopathological features including multifocality, aggressive variant, absence or infiltration of the tumoral capsule, tumor size, and lymph node metastasis. We found that strong expression (3+) of

CXCL12 also correlated with lymph node metastasis, but we could find no correlation with multifocality, infiltration of the tumoral capsule, or tumor size. This indicated the possibility that CXCL12 could also be used as a prognostic marker. If the sample size in the study was large enough, a clinical correlation may have been found between many clinicopathological features and CXCL12 expression.

This study had some limitations. First, our sample size was small. However, we performed this study prospectively to mimic the clinical situation. Despite the small number of samples, we included specimens diagnosed as AUS, suspicious PTC, and PTC by FNA, and showed that CXCL12 was an effective diagnostic marker of PTC in FNA, a very valuable finding. Second, the BRAF ICC staining was inadequate in many specimens. Therefore, we could not achieve a precise comparison of CXCL12 and BRAF in the same FNA samples. BRAF IHC staining showed results consistent with thosd for CXCL12 in TMA. And the combination of BRAF IHC and CXCL12 was not a superior diagnostic marker of PTC compared with CXCL12 alone in FNA. Therefore, the inadequate results of BRAF ICC were an obstacle to identifying an effective diagnostic marker for PTC.

In conclusion, CXCL12 may be an effective diagnostic marker for PTC in FNA or TMA, and may facilitate more accurate diagnosis of PTC, especially in samples categorized as AUS or suspicious PTC compared with the *BRAF* gene mutation suggested by the guideline.

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