CHAPTER V

DISCUSSION

The bcl-2 oncogene was originally detected in B-cell lymphomas with a balanced chromosomal translocation t(14;18)(q32;21)[78], and was cloned by Tsujimoto[79] from the breakpoint of this translocation chromosome. In subsequent studies, it was shown that bcl-2 expression is not only specific for this translocation but also be found in normal tissues, long-lived stem cells demonstrating apoptotic turnover, postmitotic cells and in certain tumors.[22-23] Bcl-2 is well known to inhibit programmed cell death (apoptosis),[20,80] but recent studies have shown that overexpression of bcl-2 suppresses cellular proliferative activity and is associated with less aggressive biological behaviour.[29,81]

In cervical cancer, lots of studies tried to find the association between bcl-2 expression and the prognosis of the cancer without definite consensus. The present study aims to resolve the controversy in the prognostic behavior of bcl-2 expression in a well-selected group of surgical treated cervical cancer patients. All patients in this study were stage Ib or IIa who were treated in two institutions, which had the same line in management of cervical cancer. All underwent radical hysterectomy with pelvic node dissection with the same surgical technique, and same follow up protocol. All of them had negative pelvic nodes, free surgical margin and did not receive any pre- or postoperative adjuvant treatment. Theoretically, these patients would have low risk of recurrence, ranging from 7-11%.[7,9,10] To date, there has been no consensus to select these patients for an adjuvant treatment in order to decrease tumor recurrence. This study investigated the association of bcl-2 expression and recurrence in this group of patients. If bcl-2 expression has some association with tumor recurrence, in the future we will proceed to study whether bcl-2 can be a new prognostic factor to identify high-risk cases in these patients.

Generally, the most appropriate and reliable research to study the natural courses or prognostic factors of the diseases is a cohort study design. Based on the low recurrent rate in this particular group of studied patients, a cohort study requires a large number of study populations (large sample size), which would certainly increase the cost of the study from immunohistochemical staining. A case-control study design was implicated in this study due to a reasonable cost-yielding effect.

Owing to lots of well known prognostic factors for tumor recurrence in this group of patients. We chose matched case-control study, trying to match all these known prognostic factors, including age, stage, histology, and tumor size, in order to clearly see the role of bcl-2. We could not match depth of invasion, tumor grade and lymph-vascular space invasion because all these information were not consistently available in previous pathological reports. However, we had adjusted these factors (from the present pathological review) with conditional multiple logistic regression.

With the low recurrent rate in this group of patients as previously mentioned, we doubled the matched controls for each studied case to justify the number of cases studied. To avoid recruitment bias, all recurrent patients with available paraffin blocks during the study period were included in this study as "cases". Thirty-six cases had disease recurrence during the study period but four cases or 11.1% did not have available blocks and were excluded from the study. Hence, we had 32 recurrent cases in the present study. In arriving to the diagnosis of recurrence, 59% had pathological confirmation, 19% were confirmed by imaging study, while 22% were diagnosed solely on physical examination. Although the most reliable mean to provide the diagnosis of recurrence was from the pathological tissue, this was not always the situation and we have to rely on the grossly abnormal clinical examinations or imaging studies. However, the follow up information of these patients finally revealed the clinical evidence of recurrence in all patients.

To avoid the bias selection of the "controls", we recruited them from cervical cancer patients who were operated in the same hospital at the nearest date to cases,

experienced no recurrence for at least five years after surgery, and met all criteria of matching controls.

Five-year was set as a cut off point for tumor recurrence because 90-98% of recurrent cases has tumor recurrence within this period.[7, 77,82] At these figures, it can be assumed that 2-10% of tumor recurrences may occur after 5 years and some non-recurrent patients (controls) might be transposed into the recurrent group (cases) after this time. The 10-year cut off point would certainly better define "cases" and "controls". However, 5-year cut off point is more appropriate in the function of study with time limitation.

To yield the optimal reliability of the study result, the same technician performed all the immunohistochemical stainings. The immunohistochemical staining and pathological slides were interpreted by two independent experienced-pathologists without acknowledged of any clinical information.

In this study, the association between bcl-2 expression and tumor recurrence cannot be demonstrated; bcl-2 expression in cases and controls were 43.7% and 50.8% respectively [odds ratio 0.7 (95%CI: 0.3-1.6)] which was not statistically significantly different. Tumor grade and lymph-vascular space invasion were significantly associated with tumor recurrence in both uni- and multivariable analysis. Invasion of more than half of cervical thickness tended to have higher risk of recurrence but did not reach statistical significance.

One might question that the insignificant association between bcl-2 expression and tumor recurrence in this study may result from insufficient sample population. We could recruit only 32 cases and 63 controls, which was less than the calculated sample size (36 cases and 72 controls for univariable analysis). Since the difference of bcl-2 expression in cases and controls is small (43.7% vs. 50.8%) and seems to have no clinical significant difference, there was no need to expand the sample size trying to reach statistical significance from the new Po value (0.51) and new odds ratio (0.7) from this study. From the result in the present study, we could not see any advantage to detect the bcl-2 expression if the bcl-2 positive in nonrecurrent patients was about 51%

and the recurrent patients was about 44%. Moreover, simple pathological results such as tumor grade, lymph-vascular space invasion were found to be better prognostic factors, which associated well with tumor recurrence with the adjusted odds ratio of 6.9 (95%CI: 1.4-35.0) and 5.4 (95%CI: 1.4-20.1) respectively. Since simple pathological reports can predict recurrent patients better than the costly bcl-2 immunohistochemical staining, there is no need to detect the bcl-2 expression by immunohistochemical study in cervical cancer patients, who underwent radical hysterectomy with pelvic nodes dissection and has negative pelvic nodes and free surgical margin.

There are many previous studies reported the associtation between bcl-2 expression and the prognosis of cervical cancer. Many studies have associated bcl-2 expression in cervical carcinoma with a good prognosis.[25,40-44] Others have shown no survival advantage,[47-52] while some studies have associated bcl-2 expression with unfavorable outcome.[45-46] These varieties of results could be partially explained by the population heterogeneity with various treatment modalities.

Overexpression of bcl-2 specifically prevents cells from initiating apoptosis (programmed cell death) in response to a number of stimuli including radiotherapy and chemotherapy.[83] Patients with overexpression of bcl-2 should resist to radiotherapy and had worse prognosis. This was confirmed by the study of Pillai[45] and Rajkumar.[46] In the study of Pillai[46] in 101 stage IIb-IIIb cervical cancer patients treated by radiotherapy, those with residual and/or recurrent disease showed higher levels of bcl-2 expression than those who remained disease free. The study of Rajkumar[45] in 40 stage IIb and IIIb cervical cancer patients treated by radiotherapy concluded that bcl-2 expression significantly affected the shorter disease-free and overall survival of only in stage IIb patients. Other studies failed to demonstrate the association between bcl-2 expression and prognosis in cervical cancer patients treated solely by radiotherapy,[48,52] and claimed that apoptosis is regulated by an intricate interaction between several genes including pro-apoptotic genes (bax, bak, bcl-xs, bad, p53) and antiapoptotic genes (bcl-2, bcl-xl, bcl-wq, mcl-1).[83-84] These interactions are quite complex and can not be completely understood by simple expression on immunohistochemical study of bcl-2.

For patients treated by surgery, the association between bcl-2 and prognosis cannot be explained by antiapoptotic function of bcl-2. However, many studies in various cancers treated by surgery demonstrated that patients with bcl-2 expression had better prognosis.[28-30,32,35,36] The explanation of bcl-2 and good prognosis may be from the relationship between the loss of bcl-2 expression and the biologic aggressiveness of the cancer. In various cancers, bcl-2 showed a significant negative correlation with tumor stage,[33] tumor grade,[36,37,85] tumor size,[86] depth of invasion,[87] and lymph node metastasis.[38,87] In cervical cancer, Tjalma et al[41] reported a significantly higher fraction of bcl-2 positive cells in early-stage, and in tumor showing no lymphatic or vascular space invasion. This is contrast to our study that bcl-2 expression, though not significant, tend to have some association with the presence of lymph-vascular space invasion. Aletra et al[88] also demonstrated more frequently bcl-2 immunopositive in well-differentiated cervical cancer than in poorly differentiated cancer with borderline significance. Our study also showed that patients with grade 1 had significantly higher incidence of positive bcl-2 compared to grade 2-3 in univariable and multivariable analysis.

Regarding bcl-2 expression in surgically treated cervical cancer; most studies also included patients who received adjuvant treatment with radiotherapy and/or chemotherapy. Some of these studies failed to detect the association between bcl-2 and prognosis[47,50,51] while the study of Podovan et al[42] and the study of Tjalma et al[25] reported the association between bcl-2 and longer survival. In the study of Tjalma et al[25], subgroup analysis revealed that both in patients who received adjuvant radiotherapy and in patients, who were only treated surgically, bcl-2 overexpression remained a significant marker of good prognosis.

The only study that included cervical cancer patients who treated solely by surgery is the study of Dimitrakakis.[43] This study also demonstrated that 5-year survival rate of patients with bcl-2 positive.was 74% compared to 49% in those with bcl-2 negative, which was statistically significant difference both in uni- and multivariable

analysis.[43] Our study, which also included cervical cancer patients who solely treated by surgery can not demonstrate the association of bcl-2 and tumor recurrence.

The difference of our result compared to other studies may be explained by many reasons. Different studies used different techniques, different antigen-retrieval methods, different reagents for immunohistochemical staining, and different anti-bcl-2 antibody. The interobserver and intraobserver reliability might be another reason. Our study showed modest interobserver and intraobserver reliability of bcl-2 expression. From only reading the set up definition of positive bcl-2, the kappa value of interobserver and intraobserver reliability in the preparatory phase were quite low at 0.4-0.5. (Appendix A: Table 1, Table 2) However, after both pathologists had talked and clarified the definition, the kappa value has increased to an acceptable level (Appendix A: Table 3). This subjective interpretation of bcl-2 immunohistochemical staining might be the significant source of different results in various studies.

The other main issue of different results was the definition of positive staining in the published studies. Many studies reported the positive bcl-2 according to the percentage of positive cells compared to all tumor cells. However, most studies did not clarify how to differentiate the positive from negative cells. In our study, the positive cells were the cells that had the same intensity as positive control. The cut off point for positive bcl-2 were also different, varying from > 0%, >5%, >10% and >30%. Even with the same cut off point of 5% as in our study, the percentage of positive bcl-2 in cervical cancer still varies among studies from 54% to 77%.[24,25,41,42,44,50] This variation may result from the immunohistochemical technique including the interpretation and the heterogeneity of the study population or the intrinsic factors of the tumors themselves, such as tumor histology [28,36,37,89-92] tumor stage[27,28,33], and tumor grade. [27,36,37,85]

Due to the heterogeneity of bcl-2 expression in different area of tumor,[25,26,41,44,45,88] in order to accurately define the score of bcl-2 expression, all blocks of tumor tissues should be serially cut and immunohistochemical stained and the result of bcl-2 expression should be calculated from proportion of all stained tumor cells out of all tumor cells. However, this is impractical, labor intense, and high cost. In order to

simulate the routine technique, single section from 1 block of primary cervical cancer tissue that contained representative tumor tissue was prepared for the immunohistochemical staining in our study as were the practice of many other studies.