HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2020.54837
Haydarpasa Numune Med J 2022;62(3):276–281

hnhtipdergisi.com

ORIGINAL ARTICLE

The Impact of Transrectal Needle Biopsy Core Length on Prostate Cancer Diagnosis

Serdar Aykan[1], Aykut Çolakerol[2], Serkan Gönültaş[3], Yavuz Baştuğ[1], Enes Kılıç[2], Mustafa Zafer Temiz[2], Atilla Semercioöz[2]

[1]Deparment of Urology, Health Sciences University Haydarpaşa Numune Training and Research Hospital, Istanbul, Türkiye
[2]Deparment of Urology, Health Sciences University Bağcılar Training and Research Hospital, Istanbul, Türkiye
[3]Deparment of Urology, Health Sciences University Gaziosmanpaşa Training and Research Hospital, Istanbul, Türkiye

Abstract

Introduction: Prostate cancer is diagnosed by histological evaluation of the prostatic glandular structure, and ultrasound-guided needle biopsy is the most commonly used method in diagnosis. Various strategies have been developed to overcome diagnostic limitations of prostate biopsy and to increase the rate of cancer detection. In this study, it was aimed to reveal the relationship between biopsy core lengths and cancer detection rates and to establish standardization criteria that can increase the diagnostic value of biopsy core length.

Methods: Between January 2016 and September 2017, 394 patients who underwent transrectal ultrasound-guided prostate biopsy for abnormal digital rectal examination and/or prostate-specific antigen (PSA) >2.5 ng/mL were retrospectively evaluated. Under transrectal ultrasound, a total of 12 core biopsies were performed from each patient from the apical, medial, and basal regions of the both sides of the prostate. Each core length and tumor length were noted. Core lengths were compared in cancer and non-cancer patients and divided into Groups A and B, respectively. Statistical analysis was performed to determine an acceptable limit for biopsy length.

Results: The mean age of the patients was 63.84±7.26, mean PSA was 15.88±7.40 ng/dl, and the mean prostate volume was 54.30±28.48 ml. Prostate cancer was seen in 24% of patients. Average core length was 12.7 mm in cancer group and 12.3 mm in non-cancer group. Although the core lengths are high in cancer patients, the relationship between average core length and cancer detection rates and also cancer grade was compared with the Kruskal–Wallis test and no statistically significant difference was found (p=0.232).

Discussion and Conclusion: In pathological evaluation, the relationship between the biopsy core length of prostate tissue and the diagnosis of prostate cancer could not be shown, and therefore, a cutoff value for the sufficient glandular tissue could not be determined. The presence of studies with similar and opposite results, relevant to this subject, showed that the need for further clinical studies.

Keywords: Biopsy core length; prostate cancer; prostate needle biopsy.

Prostate cancer is diagnosed by histological evaluation of the prostatic glandular structure, and ultrasound-guided needle biopsy is the most commonly used method in diagnosis[1]. The most common indications for prostate biopsy are abnormal rectal examination and/or high serum prostate-specific antigen (PSA) values. Six-core systemic

Correspondence (İletişim): Serdar Aykan, M.D. Saglik Bilimleri Universitesi Haydarpasa Numune Egitim ve Arastirma Hastanesi, Uroloji Klinigi, Istanbul, Türkiye
Phone (Telefon): +90 555 821 21 40 **E-mail (E-posta):** drserdaraykan@hotmail.com
Submitted Date (Başvuru Tarihi): 19.09.2020 **Accepted Date (Kabul Tarihi):** 28.09.2020

random prostate biopsy became extremely popular and made progress in the diagnosis of prostate cancer after the studies of transrectal ultrasound-guided prostate biopsy performed by Hodge et al.[2] in 1989.

Although prostate biopsy is the gold standard in cancer diagnosis, the diagnostic yield of a single biopsy is not more than 25–45%[3]. Various strategies have been developed to overcome this limitations and to increase the rate of cancer detection. Samples were also taken from the far-lateral, and the number of core in a single biopsy set was increased 2–3 times[3-6]. Nowadays, besides increasing the number of core taken, MR-fusion biopsy is used to increase the diagnostic value[7]. Increasing the number of cores resulted in a higher cancer detection rate. In addition to the increase in the number of samples, obtaining more tissue may have contributed to the increase in cancer detection rate. In pathological evaluation, the core length is important in addition to the number of cores taken to evaluate the sufficient amount of prostate tissue. These studies suggest that taking longer core samples may increase the cancer detection rates and the diagnostic value of the cores[8,9]. In this study, we hypothesized that a larger core length would increase the rate of prostate cancer diagnosis by providing sufficient tissue for a pathological evaluation. For this purpose, we analyzed the data of prostate biopsy sample lengths. We also tried to create a cutoff value for biopsy core length that would increase the rate of cancer detection.

Materials and Methods

Four hundred and twenty-seven patients who underwent transrectal ultrasound-guided prostate biopsy between January 2016 and September 2017 for abnormal digital rectal examination and/or PSA >2.5 ng/mL were retrospectively evaluated. Patients were included in the study if it was first biopsy and a 12-core biopsy from the peripheral region. Patients were excluded if any biopsy core did not contain prostate tissue (only rectal mucosa, blood, or periprostatic tissue) and/or if the pathology diagnosis was atypical small acinar proliferation. Patients diagnosed with high-grade prostatic intraepithelial neoplasia were evaluated in the non-malign category. Thirty-five patients who did not meet the criteria of the study were excluded from the study, and the study continued with the remaining 392 patients. Prostate volume was calculated using ellipsoid formula before biopsy. All patients were given 10 mL of 2% lidocaine gel intrarectally and biopsy was performed in the lateral decubitus position using an 18-gauge biopsy gun. Under transrectal ultrasound, a total of 12 core biopsies were performed from each patient from the apical, medial, and basal regions of the both sides of the prostate. If the samples were too small, fragmented, or lacking tissue, another biopsy was taken at the same spot. All biopsies were performed standardized.

Each core was gently removed from the biopsy needle. Each core was numbered, the prostate lobe and its region were labeled, and placed in different tubes containing 10% formaldehyde solution and sent for pathological evaluation. In the macroscopic examination, each core was measured in millimeters. All samples were fixed in 10% formaldehyde solution for a minimum of 2 h and a maximum of 6 h. Cassettes are placed in an automated texture processor (Leica ASP 300S) and processed according to manufacturer's instructions. Samples were parafinized, and then, sections were cut in 4 mm thickness. Afterward, it was deparaffinized and stained with the routine hematoxylin-eosin procedure.

In pathology reports, the length of each core is explained as mm and tumor percentages were specified. From these reports, the diagnoses and core lengths were collected for statistical analysis. In the presence of multiple fragments from a single site due to fragmented tissue, or when a second core is obtained after a poor quality first core, the pathologist reported the length of each tissue fragment. In this case, the length of the long core was recorded and analyzed, and the smaller pieces were ignored.

Core lengths of patients diagnosed with cancer and patients with benign pathological findings were compared. In addition, statistical analyzes were performed to determine a minimum acceptable cutoff value for biopsy core length.

Results

The mean age of the patients was 63.84±7.26, mean PSA was 15.88±7.40 ng/dl, and the mean prostate volume was 54.30±28.48 ml. A total of 4704 biopsy cores from 392 patients were evaluated. One hundred and three cores with a prostate tissue length of 7 mm and less in the biopsy core were not included in the study and a total of 4601 cores were examined. Overall, cancer detection rate was 24.6% (94/392). Cancer was detected in 257 cores and 4344 cores were reported as benign. Average core length was 12.7 mm in malignant group and 12.3 mm in non-malignant group (Table 1). The relationship between average core length and cancer detection rates and also cancer grade was compared with the Kruskal–Wallis test and no statistically significant difference was found (p=0.232). According to the D-Amico classification, 45 patients were identified as low

Table 1. Relationship between biopsy lengths and prostate cancer

Biopsy no	Cancer	Number of patients	X (mm)	SS	t	p
1	No	342	14.348	4.3294	0.264	0.792
	Yes	39	14.154	4.4812		
2	No	339	13.51	3.764	–0.518	0.605
	Yes	41	13.83	3.338		
3	No	340	13.6	3.852	1.133	0.258
	Yes	41	12.88	4.007		
4	No	337	13.5	4.019	0.702	0.483
	Yes	44	13.02	5.437		
5	No	340	13.06	4.079	379	0.928
	Yes	41	13	4.588		
6	No	337	12.26	4.253	2.412	0.016
	Yes	42	10.55	5.062		
7	No	348	13.32	3.995	–1.093	0.275
	Yes	33	14.12	4.56		
8	No	345	13.16	3.726	1.028	0.305
	Yes	36	12.47	4.76		
9	No	348	13.1	4.318	–0.881	0.384
	Yes	33	14.18	6.939		
10	No	348	12.683	4.2711	1.593	0.118
	Yes	33	11.848	2.7056		
11	No	353	12.12	4.39	–0.644	0.52
	Yes	28	12.68	4.304		
12	No	357	11.59	4.071	0.831	0.406
	Yes	24	10.88	3.745		

n: Number of patients; x: average core length.

risk (gleason 2–6), 33 patients as intermediate risk (gleason 7), and 16 patients as high risk (gleason 8–10) prostate cancer (Table 2). Radical prostatectomy was performed in 77 patients (63 laparoscopic-14 open). According to pathological results, cancer detection rates and PSA values were compared by independent sample t test. It was determined that there was a statistically significant difference (p=0.004) and positive individuals had higher PSA values than negative individuals. Cancer detection rates and prostate volume values were compared with the independent sample t-test, it was found that there was a statistically significant difference (p=0.001) and positive individuals had lower prostate volume values than negative individuals. There was no statistically significant difference between the right and left prostate lobes in terms of cancer detection rates (p=0.211). According to the rectal examination findings, it was found that the rate of diagnosis was statistically significantly higher in patients with pathological examination findings (p<0.001). The relationship between mean core length and cancer detection rates and cancer grade was compared with the Kruskal–Wallis test and no statistically significant difference was found (p=0.232).

Discussion

Prostate cancer, one of the most common cancers in men, continues to be an important health problem. Although prostate biopsy is the gold standard in cancer diagnosis, the diagnostic yield of a single biopsy is low[10]. The technique has been modified many times since Hodge et al.'s[11] first definition in 1989. Despite the modifications made, the diagnostic efficiency of a single prostate biopsy is not more than 25–45%[3-6,10,11].

Various strategies have been implemented to overcome this limitations and to increase the rate of cancer detection. First, the number of cores was increased to evaluate more prostate regions and more total prostate tissue. Studies have shown that as the number of core taken increases, prostate cancer detection rates increase[10,12,13]. In the Guidelines of the American Urology Association, it is recommended that prostate biopsy should be performed in 12 cores with transrectal ultrasound guidance, including the far lateral region and the apex region of the prostate. However, in a study by Ceylan et al.,[13] in which 1120 patients were evaluated, the cancer detection rate of 12 core biopsies was 24%, while this rate was 30.3% in 20 cores.

Table 2. Relationship between biopsy lengths and gleason score

	Pathology	Number of patients	Med (mm)	Min (mm)	Maks (mm)	Ki-Kare	p
Biopsy lengths and gleason score							
	No Cancer	288	11.00	3	27	5.589	0.232
	3+3	45	12.00	1	24		
	3+4	15	9.00	6	19		
	4+3	18	14.00	8	22		
	More than 4+3	16	13.00	1	22		

Another method to increase diagnostic efficiency is obtaining longer tissue pieces for each biopsy core. Especially in large prostates, the short length of the core makes it difficult to sample the anterior part of the prostate gland and its apex. In the present studies, sampling of the anterior apex of the prostate increases the cancer detection rate by 4–6%[14].

In addition to improving the diagnostic efficiency of prostate biopsy, discussions about overdiagnosis and overtreatment of detected prostate cancer have prompted urologists to seek to improve patient risk stratification. Magnetic resonance imaging (MRI) and MRI-targeted biopsy (MRITB) have emerged as promising solutions. MRITB can reduce unnecessary treatment by providing a more accurate assessment of cancer location, grade, and size. In the study of Siddiqui et al.[15] comparing MR/ultrasound fusion biopsy and standard biopsy, high-risk prostate cancer was detected 30% more with MR/ultrasound fusion biopsy, while a 17% reduction was achieved in the diagnosis of low-risk prostate cancer[15,16].

In another study conducted by Pepe et al.,[17] MpMRI was performed on 100 patients whose first biopsies had no cancer. In all patients, additional biopsy was performed from suspicious areas in mpMRI as saturation biopsy. Prostate cancer was detected in 37 patients. While 29 of these 37 patients were diagnosed with mpMRI, eight of them were overlooked. However, eight overlooked patients were diagnosed with clinically insignificant cancer. According to the results of the study, the researchers concluded that cancers that cannot be detected by mpMRI are clinically insignificant. In another study, where mpMRI was applied to patients with one or more negative prostate biopsies and biopsy from suspicious areas, prostate cancer was detected in 41% of the patients, and 87% of them were defined as clinically important cancer.[18] Despite these benefits of prostate biopsy with MRI and MR/ultrasound fusion guided biopsy in the diagnosis and evaluation of prostate cancer, its use is limited due to the lack of trained personnel and appropriate technical equipment. For this reason, transrectal ultrasound-guided prostate biopsy is still the most widely used diagnostic method in prostate cancer.

Another method that will increase the diagnostic efficiency of biopsy is to obtain longer tissue pieces for each biopsy core[19]. While concerns about the optimal approach for prostate biopsy have focused on core count, prostate site, labeling, and pathological examination, core length has been neglected in the literature. Even in the official report prepared by the American Urological Association based on the literature review of more than 500 articles, the core quality providing recommendations for the best prostate biopsy could not be revealed[20]. In addition, the biopsy core quality is not mentioned in the European Association of Urology guidelines, and the European Randomized Prostate Cancer Screening Study pathology committee gives the shortest acceptable core length at the time of biopsy as 10 mm[21]. The short length of the core makes it difficult to sample the anterior part and apex of the prostate gland, especially in large prostates. In studies conducted, sampling of the anterior apex of the prostate increases the cancer detection rate by 4–6%[22].

Although there are few studies on core count and core localization, the number of studies on core length is limited. In addition to studies showing that core length does not affect the diagnosis of prostate cancer,[23] there are also studies showing that a larger core length increases the rates of prostate cancer diagnosis[8,9,24]. Dell'Atti et al.,[24] reported that the optimal biopsy core length should be longer than 11.8 mm in biopsy in the diagnosis of prostate cancer. Boccon-Gibod et al.[25] suggested that the mean biopsy length be used as a quality control measure with 10 mm tissue as the shortest acceptable length.

Recent studies focusing on cancer detection rate have also shown variation in core length between different regions of the world. In a study by Obek et al.,[8] including 245 patients, core length over 11.9 mm was found to be associated with increased prostate cancer detection rate (OR 2.57). Fiset et al.[9] examined 197 Canadian patients with an average of 11 core biopsies, they found that cancerous cores (mean length 14.1 mm) were significantly longer than benign cores (mean length 13.2 mm) ($p<0.001$) and a core length of 13 mm was the optimal sensitivity (42.8%) and specificity (76.5%) for detecting carcinoma (OR 2.43).

In a study by Reis et al.,[26] the compatibility of final gleason scores with biopsy gleason scores of 178 patients who underwent radical prostatectomy was examined. While the average core length of patients with low gleason scoring in biopsy was found to be 11.61 mm, this length was found to be 13.52 mm in patients with full compliance with biopsy, and it was stated that the difference between them was statistically significant. In this study, the authors proposed mean core length as an independent predictor of biopsy Gleason score reduction. They emphasized that longer optimal core cause less sampling inaccuracy and the importance of core length in terms of informing patients and guiding them to the correct treatment.

Prostate biopsy guidelines recommend reporting of prostate biopsy as insufficient if without glandular tissue. However, this finding can only be described in microscopic histological evaluation[21]. Yilmaz et al.[27] retrospectively examined 1712 patients who underwent 12 core biopsies to determine whether there is a relationship between core length and the presence of prostate glandular tissue. They showed that a minimum core length of 6 mm can predict correct glandular sampling with 80.2% sensitivity and 78.7% specificity. In the study, it has been proven that almost one-third (28.2%) of the core lengths below 6 mm are non-glandular.

The results of the study we have presented are in line with the findings of previous studies. Most factors were systematized to minimize the factors that may affect the study results. All biopsies were performed transrectally using the same model biopsy gun and biopsy needle, 12 cores were included in the study, fragmented tissue samples and biopsy series without prostate tissue were excluded from the study. Pathological evaluation was done by the same expert using the same tissue processing methods. Although biopsies were performed by different urologists, it is known that although there may be small differences in detecting prostate cancer among operators according to the data obtained from previous studies in the literature, it is not statistically significant[28].

In our study, based on the biopsy core length, to predict the cancer detection rates more accurately, the relationship between cancer grade and average core lengths was compared and no statistically significant difference was found.

Another option offered to prostate cancer patients with localized disease besides curative treatment is active surveillance. In these patients, active treatment decision is made according to the biopsies performed during the follow-up. Therefore, biopsy quality is very important in these patients. The biopsy quality should be increased by standardizing the core length along with the number and localization of the cores in biopsies.

Conclusion

In pathological evaluation, the relationship between the biopsy core length of prostate tissue and the diagnosis of prostate cancer could not be shown, and therefore, a cutoff value for the sufficient glandular tissue could not be determined. The presence of studies with similar and opposite results, relevant to this subject, showed that the need for further clinical studies.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: S.A.; Design: S.A., A.S.; Data Collection or Processing: S.A., E.K., M.Z.T., A.S.; Analysis or Interpretation: S.A., S.G., M.Z.T., Y.B., A.S.; Literature Search: S.A.; M.Z.T.; A.Ç.; Writing: S.A., M.Z.T.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Ozden E, Göğüş C, Tulunay O, Baltaci S. The long core needle with an end-cut technique for prostate biopsy: Does it really have advantages when compared with standard needles? Eur Urol 2004;45:287–91. [CrossRef]
2. Hodge KK, McNeal JE, Terris MK, Stamey TA. Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate. J Urol 1989;142:71–5. [CrossRef]
3. Chun FK, Epstein JI, Ficarra V, Freedland SJ, Montironi R, Montorsi F, et al. Optimizing performance and interpretation of prostate biopsy: A critical analysis of the literature. Eur Urol 2010;58:851–64. [CrossRef]
4. Presti JC Jr, O'Dowd GJ, Miller MC, Mattu R, Veltri RW. Extended peripheral zone biopsy schemes increase cancer detection rates and minimize variance in prostate specific antigen and age related cancer rates: Results of a community multi-practice study. J Urol 2003;169:125–9. [CrossRef]
5. Brössner C, Bayer G, Madersbacher S, Kuber W, Klingler C, Pycha A. Twelve prostate biopsies detect significant cancer volumes (> 0.5 mL). BJU Int 2000;85:705–7. [CrossRef]
6. de la Rosette JJ, Wink MH, Mamoulakis C, Wondergem N, ten Kate FJ, Zwinderman K, et al. Optimizing prostate cancer detection: 8 versus 12-core biopsy protocol. J Urol 2009;182:1329–36. [CrossRef]
7. Sonn GA, Margolis DJ, Marks LS. Target detection: Magnetic resonance imaging-ultrasound fusion-guided prostate biopsy. Urol Oncol 2014;32:903–11. [CrossRef]
8. Öbek C, Doğanca T, Erdal S, Erdoğan S, Durak H. Core length in prostate biopsy: Size matters. J Urol 2012;187:2051–5. [CrossRef]
9. Fiset PO, Aprikian A, Brimo F. Length of prostate biopsy cores: Does it impact cancer detection? Can J Urol 2013;20:6848–53.
10. Eskew LA, Bare RL, McCullough DL. Systematic 5 region prostate biopsy is superior to sextant method for diagnosing carcinoma of the prostate. J Urol 1997;157:199–203. [CrossRef]
11. Hodge KK, McNeal JE, Stamey TA. Ultrasound guided transrectal core biopsies of the palpably abnormal prostate. J Urol 1989;142:66–70. [CrossRef]
12. Eskicorapci SY, Baydar DE, Akbal C, Sofikerim M, Günay M, Ekici S, et al. An extended 10-core transrectal ultrasonography guided prostate biopsy protocol improves the detection of prostate cancer. Eur Urol 2004;45:444–9. [CrossRef]
13. Ceylan C, Doluoglu OG, Aglamis E, Baytok O. Comparison of 8, 10, 12, 16, 20 cores prostate biopsies in the determination of prostate cancer and the importance of prostate volume. Can

Urol Assoc J 2014;8:E81–5. [CrossRef]
14. Meng MV, Franks JH, Presti JC Jr, Shinohara K. The utility of apical anterior horn biopsies in prostate cancer detection. Urol Oncol 2003;21:361–5. [CrossRef]
15. Siddiqui MM, George AK, Rubin R, Rais-Bahrami S, Parnes HL, Merino MJ, et al. Efficiency of prostate cancer diagnosis by MR/ultrasound fusion-guided biopsy vs standard extended-sextant biopsy for MR-visible lesions. J Natl Cancer Inst 2016;108:djw039. [CrossRef]
16. Siddiqui MM, Rais-Bahrami S, Turkbey B, George AK, Rothwax J, Shakir N, et al. Comparison of MR/ultrasound fusion-guided biopsy with ultrasound-guided biopsy for the diagnosis of prostate cancer. JAMA 2015;313:390–7. [CrossRef]
17. Pepe P, Garufi A, Priolo G, Pennisi M. Can 3-Tesla pelvic phased-array multiparametric MRI avoid unnecessary repeat prostate biopsy in patients with PSA < 10 ng/mL? Clin Genitourin Cancer 2015;13:e27–30. [CrossRef]
18. Hoeks CM, Schouten MG, Bomers JG, Hoogendoorn SP, Hulsbergen-van de Kaa CA, Hambrock T, et al. Three-Tesla magnetic resonance-guided prostate biopsy in men with increased prostate-specific antigen and repeated, negative, random, systematic, transrectal ultrasound biopsies: Detection of clinically significant prostate cancers. Eur Urol 2012;62:902–9.
19. Reis LO, Reinato JA, Silva DC, Matheus WE, Denardi F, Ferreira U. The impact of core biopsy fragmentation in prostate cancer. Int Urol Nephrol 2010;42:965–9. [CrossRef]
20. Bjurlin MA, Carter HB, Schellhammer P, Cookson MS, Gomella LG, Troyer D, et al. Optimization of initial prostate biopsy in clinical practice: Sampling, labeling and specimen processing. J Urol 2013;189:2039–46. [CrossRef]
21. Van der Kwast T, Bubendorf L, Mazerolles C, Raspollini MR, Van Leenders GJ, Pihl CG, et al. Guidelines on processing and reporting of prostate biopsies: The 2013 update of the pathology committee of the European Randomized Study of Screening for Prostate Cancer (ERSPC). Virchows Arch 2013;463:367–77. [CrossRef]
22. Meng MV, Franks JH, Presti JC Jr, Shinohara K. The utility of apical anterior horn biopsies in prostate cancer detection. Urol Oncol 2003;21:361–5. [CrossRef]
23. Lee S, Jeong SJ, Hwang SI, Hong SK, Lee HJ, Byun SS, et al. Clinical value of core length in contemporary multicore prostate biopsy. PLoS One 2015;10:e0123704. [CrossRef]
24. Dell'Atti L, Ippolito C. The impact of core length on prostate cancer diagnosis during a standard 14-core prostate biopsy scheme. Urologia 2016;83:186–9. [CrossRef]
25. Boccon-Gibod L, van der Kwast TH, Montironi R, Boccon-Gibod L, Bono A; European Society of Uropathology; European Society of Pathology Uropathology Working Group. Handling and pathology reporting of prostate biopsies. Eur Urol 2004;46:177–81. [CrossRef]
26. Reis LO, Sanches BC, de Mendonça GB, Silva DM, Aguiar T, Menezes OP, et al. Gleason underestimation is predicted by prostate biopsy core length. World J Urol 2015;33:821–6.
27. Yilmaz H, Ciftci S, Ustuner M, Yavuz U, Saribacak A, Muezzinoglu B, et al. Minimum 6 mm core length is strongly predictive for the presence of glandular tissue in transrectal prostate biopsy. World J Urol 2015;33:1715–20. [CrossRef]
28. Lawrentschuk N, Toi A, Lockwood GA, Evans A, Finelli A, O'Malley M, et al. Operator is an independent predictor of detecting prostate cancer at transrectal ultrasound guided prostate biopsy. J Urol 2009;182:2659–63. [CrossRef]