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Original Article

Periodic reevaluation of our genetic pathological diagnosis by application of polymerase chain reaction (PCR) to routine formalin-fixed paraffin-embedded tissues

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Objective: We have used PCR for an accurate diagnostic procedure for 4 years rom 1999. In this study the reassessment of our routine PCR examination were done to reconfirm its profit to pathological diagnosis.

Study design: 262 specimens of various organs in 2002 were examined, 53% in which consisted of gastrointestinal tracts and lymph nodes. Paraffin-embedded tissues were rendered for PCR analysis. Examination in PCR included #1. lymphocytic monoclonality (immunoglobulin H-chain VDJ area (IgHVDJ) for B cells and γ-chain of T cell receptor (TCRγ)), #2. suppressor gene p53-PCR-single strand conformational polymorphism method (p53-PCR-SSCP) for malignancy or multiplicity in malignancy,: #3. Human androgen receptor gene (HUMARA) for multiplicity in malignancy. #4. infections: #4a: tuberculosis (tbc), #4b. cytomegalovirus (CMV), #4c. herpes simplex virus (HSV), #4d. Ebstein-Barr virus (EBV), #4e. Tsutsugamushi's disease. Each positivity was compared with those of classical pathological examination including immunohistochemistry. Pathological diagnosis was tentatively classified into three groups: strongly compatible to malignancy or specific infections (positive group), malignancy or specific infections could not be neglected (suspicious group), and benign or non-specific inflammation (negative group).

Results: As to purposes in use of PCR, 47% of specimens was used to confirm Group #1, 31% to Groups both #2 and #3, and 14% to Group #4, respectively. Lymphocytic monoclonality was confirmed in 49% in positive or suspicious groups and 31% in negative group. Rearrangement of p53-PCR-SSCP was shown in 71% in positive group and 14% in suspicious group. Rearrangement of either p53-PCR-SSCP or HUMARA could disclose multiplicity or metastasis in 56% of malignant tumors. Tbc was diagnosed in 36% of positive or suspicious groups. Clinical sarcoidosis was corrected by PCR in 2 cases.

Conclusion: The PCR was very available for routine pathological diagnosis of lymphoma, malignancy, multiplicity in cancer, and tbc. Several clinically diagnosed sarcoidosis, furthermore, were regarded to be tbc.

Key Words: PCR, SSCP, IgHVDJ, TCRγ, p53, tuberculosis

Introduction

Immunohistology was a useful technique, having taken the place of electron microscopy. In these years PCR became more reliable method than immunohistology (1-11). Genetic rearrangement as lymphocytic monoclonality and suppressor-gene p53 were the most beneficial evidence for malignancy (1, 3-6). p53-PCR-SSCP and HUMARA anomalies were also worthy of differentiating metastasis from multiplicity, i.e. non-metastasis or multiple cancers, patients with multiple cancerous foci (6, 7). Tuberculosis was easily confirmed by PCR rather than any laboratory examinations and an additional DNA-sequencing by purified tbc-PCR products differentiated tbc from other acid-fast bacilli infection (1, 2, 8). Other confirmation PCR methods against infections or rare tumors were introduced in routine laboratory works (1, 2, 9-11).

We have established new PCR methods available for routine pathological study and made official announcements on homepage for 4 years from 1999. A reassessment of our previous PCR results was thought to be valuable for an establishment of laboratory accuracy. In this study we listed a year's result of 2002 and analyzed.

Materials and Methods

262 specimens in 2002 were analyzed, which consisted of 88 specimens of gastrointestinal tract (GI tract, occupied 34% in the whole), 49 ones of lymph nodes (19%), 24 ones of skin (9%), 22 ones of lung (8%), 19 ones of bone marrow (7%), 13 ones of breast (5%), and 47 ones of other organs (Table 1). 52% of specimens were biopsied ones and 48% were derived from extirpation operation.

Nucleic acids were obtained from fromalin-fixed paraffin-embedded specimens. Formalin was buffered into 10% neutral one. Duration of fixation was shortened less than 3 days (1, 2, 11).

Table 1 Examined organs

organs	examined case numbers/year	%
GI tract	88	34
node	49	19
skin	24	9
lung	22	8
bone marrow	19	7
breast	13	5
others	47	18
total	262	100

Our laboratory menu of PCR and SSCP included followings: #1. lymphocytic monoclonality (immunoglobulin H-chain VDJ area (IgHVDJ) for B cells and γ-chain of T cell receptor (TCRγ)), #2. suppressor gene p53-PCR-single strand conformational polymorphism method (p53-PCR-SSCP) for malignancy or multiplicity in malignancy,: #3. Human androgen receptor gene (HUMARA) for multiplicity in malignancy, #4. infections: #4a: tuberculosis (tbc), #4b. cytomegalovirus (CMV), #4c. herpes simplex virus (HSV), #4d. Ebstein-Barr virus (EBV), #4e. Tsutsugamushi's disease (1-11). Each concrete method followed our PCR menu disclosed and rendered on E-mail (1, 2). Main PCR's as above menu #1-4 were analyzed in this study. Statistical analysis was done in accordance with Exel statistics (Microsoft) (1).

Results

Concerning purposes of PCR, 47% of specimens was assigned to lymphocytic monoclonality analysis, 31% to p53-PCR-SSCP, and 14% to tbc (Table 2).

Table 2 Examination list of PCR analysis

PCR products	examined case numbers/year	%
lymphocyte monoclonality	125	47
p53-PCR-SSCP	80	31
tbc	37	14
HUMARA	16	6
virus	4	2
total	262	100

Lymphocytic monoclonality was confirmed in 49% in positive or suspicious groups and simultaneously 31% in negative group (Table 3). There was no statistical difference of PCR-positivity between positive cases and negative cases judged with other classical examinations.

Rearrangement of p53-PCR-SSCP was shown in 71% in positive group and 14% in suspicious group (Table 4).

Rearrangement of either p53-PCR-SSCP or HUMARA could disclose multiplicity or metastasis in 56% of malignant tumors (Table 5).

Tbc was diagnosed in 36% of positive or suspicious groups (Table 6). Clinically diagnosed sarcoidosis was corrected by PCR in 2 cases (Table 6).

Discussion

PCR were done in 262 specimens in 2002, which was 1.3% of all 20225 tissue samples rendered for pathological diagnosis. This revealed one specimen a day required to PCR analysis in our research institute and PCR was inevitable tool for clinical pathology.

The most frequent PCR was examination was re-

Table 3 Results in PCR of lymphocytes for monoclonality; case numbers/year and its percentage

		diagnosed with other examinations	
		positive/suspicious	nagative
diagnosed with PCR	positive	44 (49%)	10
	negative	45	22 (69%)

Table 4 Results in PCR of p53-PCR-SSCP for malignancy; case numbers/year and its percentage

		diagnosed with other examinations		
		positive	suspicious	negative
diagnosed with PCR-PCR-SSCP	positive	5 (71%)	6 (14%)	0
		negative	2	36

Table 5 Results in PCR of Humara and p53-PCR-SSCP for multiplicity; case numbers/year and its percentage

PCR	results	
	effective	failed
HUMARA	10 (63%)	6
p53-PCR-SSCP	9 (56%)	7
others	0	2
total	19 (56%)	15

Table 6 Results in PCR of tbc; case numbers/year and its percentage

		diagnosed with other examinations		
		positive/suspicious	negative	sarcoidosis
PCR	positive	9 (36%)	0	2
	negative	16	5	1

quired to confirm lymphocytic monoclonality, and reached 47% of all examination, the high frequent requirement of which suggested that lymphoma was difficult to diagnose by classical methods. PCR was mostly used for lymphoma, malignancy, and tbc. Lymphoma can be diagnosed by PCR at the rate of 49% in positive or suspicious cases. PCR was valuable for extranodal lymphoma including GI tract. Because monoclonality was sown in 31% cases of lymph nodal swelling without definite pathology, lymphoma should not be easily denied by ordinary pathological examination.

14% cases of boderline malignancies, i.e. in suspicious group, was diagnosed malignancy by p53-PCR-SSCP, rising the possibility that a judgement of malignancy by genetic rearrangements was alternative to that by traditional subjective pathological standard, i.e. atypism.

Half of cases with multiple cancerous foci could be genetically judged as either cancer with metastasis or multiple cancers by HUMARA or p53-PCR-SSCP. In the decision of a multiple cancer case, genetic analysis took the place of conventional determination standards by comparisons of their tumor size, histology, progress degree, and correlation in time and space.

Cases suggestive of tbc strongly were reconfirmed at the rate of 36%. An interesting feature of PCR for tbc was that DNA of tbc could be confirmed in classical sarcoidosis without caceous granuloma. There might be a large admixture of tbc in sarcoidosis, and, conversely, we could not deny that sarcoidosis might arise from various causes, including tbc.

PCR was also used in our cytopathological diagnosis. There were 32716 cytological specimens in 2002, and eight cases were rendered into PCR (article was in press). Analyzed DNA was retrieved from few desquamed cells from the slide glass, having already stained with Papanicolaou's method, our examined specimens were all derived from fluid samples as cerebrospinal fluid, bronchial lavage, pleural effusion, and ascites. 2 cases were examined for tbc and other 6 cases for lymphoma, respectively. All specimens were accurately diagnosed in spite of their scanty cellularity. PCR became more valuable than immunocytopathology in these days.

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原著

当病理検査センターにおける通常ホルマリン固定パラフィン包埋組織切片を使った遺伝子検査（ポリメラーゼ連鎖反応、PCR）の検査精度の定期的再評価

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目的：私達は、1999年以来４年間、正確な病理診断の目的の為に、PCRを日常診断業務に応用してきた。この取り組みにおいて、定期的なPCR検査の再評価は、病理学的診断において有効と判断される。

方法：2002年の単年度PCR検査症例262検体について検討した。検査対象臓器で、胃腸管とリンパ装置が53％であった。PCR材料は、ホルマリン固定パラフィン包埋組織より抽出された核酸である。PCR検査項目メニューは以下の通りである：#1：リンパ球の単一クロナリティ（B細胞として免疫グロブリンH鎖VDJ領域（IgHVDJ）およびT細胞受容体γ鎖（TCRγ））、#2：悪性診断のための癌抑制遺伝子p53－ポリメラーアゼ連鎖反応－一本鎖コンフォメーション多型（p53-PCR-SSCP)、#3：多発性癌症例における転移または原発性多重癌の鑑別としてのp53-PCR-SSCPまたはアンドロゲン受容体遺伝子多型（HUMARA)、#4：感染症同定として、#4a：結核（tbc)、#4b：サイトメガロウイルス（CMV)、#4c：単純疱疹ウイルス（HSV)、#4d：エプシタイン－バールウイルス（EBV)、#4e：ツツガムシ病。個々の陽性所見は、免疫組織化学検査を含む伝統的な病理学検査と比較された。日常的病理診断は３群に試験的に分類された：1．陽性群：悪性または具体的な伝染が明瞭な症例群、2．疑い群:悪性のまたは具体的な伝染が疑われる症例群、3．陰性群:明瞭な証拠が無い症例群。

結果：PCRの使用の目的について、グループ#1が47％、グループ#2または#3が31％、#4が14％であった。リンパ球の単一クロナリティは、陽性・疑い症例群の49％、および陰性症例群の31％において確認された。p53-PCR-SSCP組換えは、悪性陽性群の71％、および悪性疑い群の14％に示された。p53-PCR-SSCPまたはHUMARAの組み換え確認による癌の転移または多重癌の鑑別は、56％の症例において可能であった。結核に関して、陽性または疑い群の36％が診断されました。臨床的ならびに病理学的に診断されたサルコイドーシスにおいて、２症例に結核遺伝子が確認された。

結論：PCRは、リンパ腫、悪性、多重癌、および結核の遺伝学的病理診断で非常に有効であった。結核遺伝子検査において、サルコイドーシスと診断されている症例に結核の遺伝子が確認されたことは興味深いことであった。

キーワード：診断精度、ポリメラーゼ連鎖反応、p53－ポリメラーアゼ連鎖反応－一本鎖コンフォメーション多型、免疫グロブリンH鎖VDJ領域、T細胞受容体γ鎖、癌抑制遺伝子、アンドロゲン受容体遺伝子多型、結核