Acta Med. Nagasaki 51: 73－77

Case Report

HIV- and HHV-8-negative Primary Effusion Lymphoma Harboring the Dual Rearrangement of Antigen Receptor Genes and a Translocation of *bcl-6*

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Primary effusion lymphoma (PEL) is a rare type of lymphoma occurring in HIV-seropositive persons. Since the tumor cells frequently harbor human herpes virus (HHV)-8 DNA, the virus is thought to be causative. We here report an instructive case of PEL in an HIV-seronegative elderly. The neoplastic cells in the fluid showed unusual morphology with multi-lobulated nuclei. They expressed CD19, CD20, CD79a and SmIgD/Mκ, but not CD3, CD30 or CD23. The *IgH* gene was clonally rearranged based on the results of Southern blotting and PCR for CDR3. In addition to the *IgH* clonality, the *TCR*γ-chain gene was also in the clonal rearrangements. Neither HHV8 nor EBV was detected in the cells. The *bcl-6* and *IgH* chimeric transcripts were evident. Conclusively, our case suggests the following two points: first, the *bcl-6/IgH* translocation may be associated with the pathogenesis of unusual PEL without HIV or HHV-8; and second, unusual PEL may be an extra-nodal spectrum of diffuse large-cell lymphoma with neoplastic cells harboring the *bcl-6/IgH* translocation and dual rearrangements of antigen receptor genes.

ACTA MEDICA NAGASAKIENSIA 51: 73－77, 2006

Keywords: PEL; *bcl-6/IgH* translocation; HIV; HHV-8

Introduction

Primary effusion lymphoma (PEL),[1] first described by Nador et al.,[2] is a rare type of malignant lymphoma occurring exclusively in human immunodeficiency virus (HIV)-seropositive individuals. PEL is characterized as follows: continuous lymphomatous effusion in the absence of an identifiable tumor mass, positive status for HIV and Kaposi-associated herpes virus/human herpes virus-8 (KSHV/HHV-8) infection, a distinctive morphology bridging large-cell immunoblastic lymphoma and anaplastic large cell lymphoma with atypia of the nucleus, an aberrant B-cell immunophenotype of $CD45^+$, $CD30^+$, $CD19^-/20^-$, $CD138^+$ and surface membrane immunoglobulin $(SmIg)^-$ with clonal immunoglobulin heavy (IgH) gene rearrangements, the presence of Epstein-Barr virus (EBV, HHV-4) DNA within tumor cells and the absence of *bcl-2*, *bcl-6*, *ras*, *c-myc* and *p53* genetic alterations. In particular, HHV-8 has been noted to be a causative agent for the tumorigenesis of PEL.

On the other hand, pleural effusion is well known to present the features of aggressive and advanced lymphomas without an identifiable tumor mass.[3] However, there appears to exist rare cases completely sharing the same clinico-pathological features as PEL.[4-6] In such cases, it is now controversial whether the infectious status for HIV, HHV-8 and EBV is positive or not, because the original article[2] has stressed the causative relation between the viruses and PEL.

We encountered an HIV-seronegative elderly suffering from chest pain and dyspnea with massive pleural effusion. The cytological and clinical examinations revealed the same morphological and clinical features as PEL, but differed from it in the genetic profiles, such as unusual rearrangements of *IgH*, *TCR*γ and *bcl-6* genes. Accordingly, we describe here this unique and instructive case among PEL arising in an HIV-seronegative elderly woman, not associated with HHV-4 (EBV) and HHV-8 (KSHV) infection.

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Received February 1, 2006; Accepted May 25, 2006

Case report

A 98-year-old woman was admitted to a public hospital because of dysphagia and malnutrition with hemiplegia due to vascular central nervous attack. She suffered from insidious chest pain and dyspnea. On physical examination, pleural effusion on the right side was found, but there was no peripheral lymph-adenopathy or hepatosplenomegaly. Images revealed only massive pleural effusion in the chest X-ray image (Figure 1), but no evidence of detectable tumor mass in the CT scan. The laboratory data at the time of dyspnea was as follows: Hb—10.8 g/dL; WBC—4.5×10^3/μL with no detectable atypical cells and a lymphocyte fraction of 10.0%; platelets—10×10^4/μL; total protein—5.8 g/dL; albumin—2.8 g/dL; ALT—20 IU/L; AST—7 IU/L; LDH—309 IU/L; and CRP—1.02 mg/dL. HIV, HCV, HBV and human T-lymphotropic virus type-I (HTLV-1) were serologically negative. The serum level of IgG, IgA and IgM was 670, 60 and 85 mg/dL, respectively. About two liters of pleural fluid was removed twice because of severe dyspnea. The fluid had a bloody color, total proteins of 2.3 g/dL, LDH activity of 1295 IU/L, and negative findings in bacterial culture. The removed fluid was processed to examine the morphological, immunophenotypical and genetic characteristics.

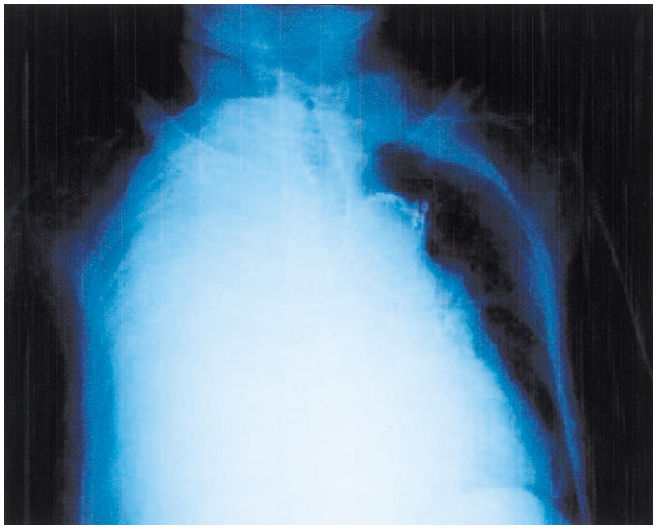

Figure 1. Chest X-ray image at the time of onset, showing the right pleural effusion.

Materials and Methods

Cyto-immunophenotypical studies

The fluid was subjected to an automated hematology analyzer and to direct smears followed by staining with May-Grünwald Giemsa dyes. Immunophenotyping was performed with a flow cytometer, FACScalibur (BD Bioscienes, San Jose, CA), following the manufacturer's instructions, and using commercially available monoclonal antibodies, such as CD3, CD4, CD5, CD7, CD8, CD19, CD20, CD22, CD23, CD79a, CD30, HLA-DR, CD25, CD38 and CD45.

Molecular analyses

Genomic DNA and total RNA were extracted from the fluid with a QIAmp DNA/RNA Blood Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions.

The status of *IgH* gene rearrangements was examined using Southern blot hybridization (SBH) and the polymerase chain reaction (PCR) for the complementarity determining region 3 (CDR3) of the *IgH* hypervariable VDJ region described by us.[7] The clonal rearrangement of the T-cell receptor (*TCR*)γ-chain was also performed with two-step PCR described by Benhattar et al.[8] The presence of HHV-4 and HHV-8 DNA sequences within the cells (total genomic DNAs) obtained from the fluid was examined with PCR using HHV consensus primers and appropriate restrictive enzymes described by us.[9] The genetic analyses were performed using either PCR or RT-PCR according to the methods previously reported by Fan et al.[10] for *bcl-2* analysis, Horsman et al.[11] for *bcl-6* analysis, and Kawamata et al.[12] and Chen et al.[18] for K-*ras* analysis, respectively.

Results

Cytological characteristics

The fluid consisted of erythrocytes and mononuclear cells with a cell density of 45,000/μL and a total protein concentration of 2.3 g/dL. Smears showed large (about 15 to 35 μm in diameter) and round lymphoid cells with abundant basophilic cytoplasm and multi-lobulated nuclei, as shown in Figure 2. The chromatin was rich and loose, and the nucleoli were fuzzy. Most neoplastic cells were positive for B-cell associated antigens including SmIgD/Mκ such as CD19, CD20, CD22 and CD79a, and were negative for T-cell associated antigens of CD3, CD4, CD8, CD5 and CD7. The other immunophenotypic status was positive for HLA-DR, CD38 and CD45, and was negative for CD25, CD10 and TdT.

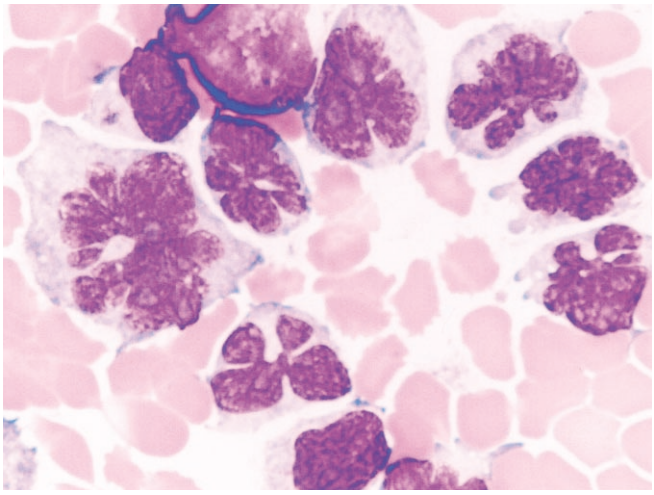

Figure 2. Morphological features of neoplastic cells from the pleural fluid stained with May-Grünwald Giemsa dyes, showing the polymorphic cells with multi-lobulated nuclei.

Molecular characteristics

To define the linearity and clonality of neoplastic cells, both SBH and PCR analyses were applied. SBH analysis, using the JH probe and restriction enzymes, *Bam*HI and *Hind*III, produced the rearrangement bands, showing the presence of a predominant B-cell clone, as shown in Figure 3 A. PCR analysis for *IgH* CDR3 revealed the presence of a monoclonal amplicon, demonstrating a sharp band in capillary microchip analysis (Figure 3 B). In addition to the clonal *IgH* gene rearrangement, they exhibited clonal *TCRγ* rearrangement (Figure 3 C), indicating bi-genotypsim. As for the configuration status of oncogenic genes relating to the pathogenesis of lymphoma, PCR analysis gave negative results for *bcl-1* and *bcl-2* gene rearrangements (Figure 4 A and B) and K-*ras* mutation at codons 12/13, whereas semi-nested RT-PCR for *bcl-6/IgH* chimeric transcripts revealed a positive band, as shown in Figure 4 C, indicating that the *bcl-6* gene is in the translocation with the *IgH* gene. Finally, this case was considered to be primary effusion lymphoma with B-phenotypic cells harboring the dual rearrangement of the *IgH* and *TCRγ* genes and the translocation of the *bcl-6* gene.

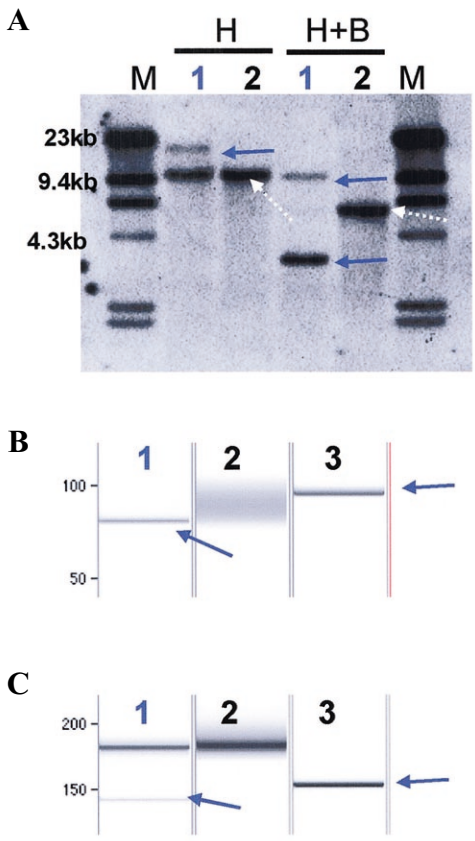

Figure 3. Demonstration of the positive *IgH* clonal rearranged bands in Southern blot hybridization (A), and PCR analysis for *IgH* CDR3 (B) and the *TCRγ* clonal band (C). **A.** Solid arrows and dotted arrows indicate the rearranged bands and the germline bands, respectively. Lanes 1 and 2 correspond to the patient and negative control samples, respectively. M: marker; H: *Hind*III; H+B: *Hind*III+*Bam*HI. **B** and **C.** The arrows represent the clonal bands. Lanes 1, 2 and 3 correspond to the patient, negative and positive control samples, respectively.

Then, oncogenic viruses relating to human lymphomas were examined using the mononuclear cells in the fluid. Neither the KSHV/HHV-8 nor the EBV/HHV-4 DNA sequence was detected with PCR analysis, as shown in Figure 5. No HTLV-1 was evident using either serology or DNA analysis for the provirus. We unfortunately had no opportunity to perform chromosomal analysis.

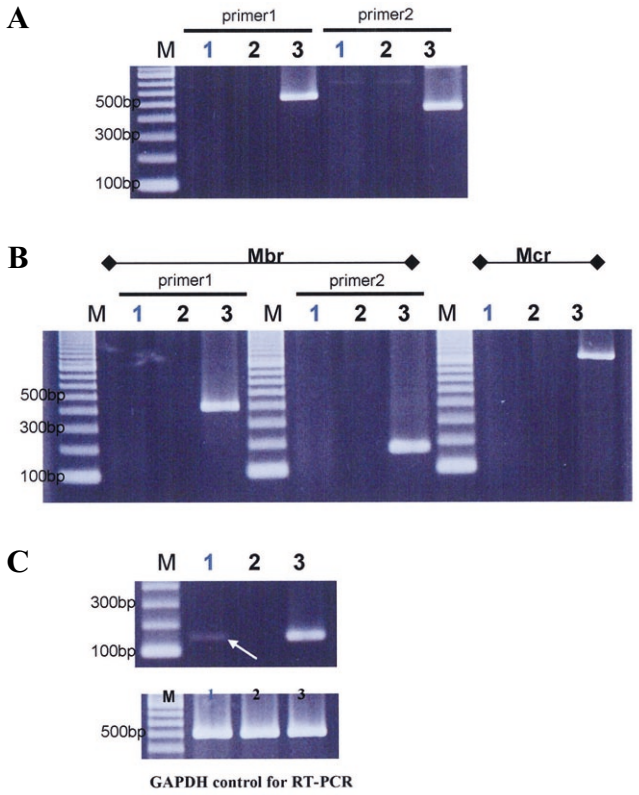

Figure 4. Results of the PCR amplicon analyses; lanes 1, 2 and 3 denote the patient, negative-control and positive-control samples, respectively, while M denotes the marker. **A.** PCR amplicon analysis for the *bcl-1/JH* rearrangement at the major translocation cluster (MTC) region, showing no rearrangement in *bcl-1*; duplicate PCR was performed using different primers 1 and 2 to avoid miss-annealing. **B.** PCR amplicon analysis for the *bcl-2/IgH* rearrangement at the major breakpoint region (Mbr) and the minor cluster regions (Mcr) regions, showing no rearrangement in *bcl-2*; duplicate PCR was performed using different primers 1 and 2 to avoid miss-annealing. **C.** PCR amplicon analysis for the *bcl-6/IgH* chimeric transcript using cDNA; *bcl-6/IgH* transcripts were identified.

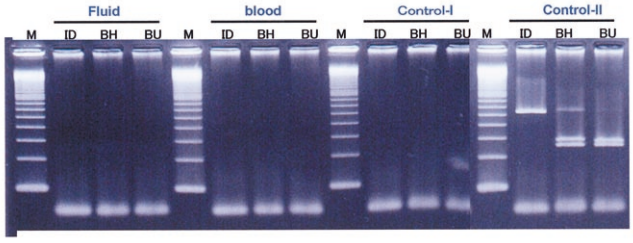

Figure 5. Results of electrophoretic analyses for fluid cells and peripheral blood using the amplicons digested with *Bam*HI and *Bst*UI. Neither HHV-4 nor HHV-8 DNA sequence was detected. Control-I was HHV-4 negative and HHV-8 negative, while Control-II was HHV-4 positive. ID: indigested; BH: digested with *Bam*HI; BU: digested with *Bst*UI.

Discussion

PEL was originally considered to be a specific subtype of lymphoma, predominantly involving the serous body cavities in HIV-positive immunodeficient individuals with the HHV-8 and EBV imposed-infection. Recently, however, unusual PEL cases clinically mimicking the prototype have been reported increasingly. The unusual issues of such cases in comparison with the prototypic PEL are mainly the absence of HIV infection, including KSHV and EBV, the nature of the aberrant T-cell immunophenotype and the presence of either c-*myc* or other oncogenic gene alterations.[5,13]

Thus, our case is also instructive because of seronegativity for HIV and no demonstration of HHV-8 or EBV DNA sequences within the fluid cells, the dual rearrangement of the *TCRγ* and *IgH* genes, and the translocation of the *bcl-6* gene. The neoplastic cells were morphologically similar to those of PEL, bridging large cell immunoblastic and anaplastic large cell lymphoma with lobulated-nuclei. The immunophenotype is fundamentally of B-cell lineage, but somewhat different from that of the prototypic PEL in terms of $SmIg^+$, $CD19^+$, $CD20^+$ and $CD30^-$ in our case. This indicates a more mature stage of B-cell ontogeny, rather than that of $SmIg^-$, $CD19^-$, $CD20^-$, $CD79a^+$ and $CD30^+$. Genotyping with SBH and PCR for *IgH* gene rearrangement defined the presence of B-cell clonality, supporting the clonal B-cell lineage. The *TCRγ* gene was also rearranged based on the PCR result, suggesting abortive processing. This dual rearrangement of antigen receptor genes is well known to be observed in B-acute lymphoblastic leukemia (ALL), which is derived from immature progenitors. Similarly, recent reports have described that about 10% of B-cell lymphomas have genetically dual rearranged antigen receptor genes of *TCRγ* and *IgH.* Interestingly, Wakely et al.[5] have reported two cases with dual rearrangement of the *IgH* and *TCRß* genes among their 5 cases of PEL, suggesting that the dual rearrangement may be common feature in unusual PEL. Unexpectedly, *bcl-6* translocation is not evident in their two cases with the dual rearrangement.

As regards genetic alteration in our case, the *bcl-6* gene was involved in translocation with the immunoglobulin heavy chain locus. *bcl-6* is a zinc finger transcription factor that is highly expressed not only in normal germinal B-cells but also in tumor cells derived from diffuse large cell, Burkitt and follicular lymphomas. This overexpression is considered responsible for the development of lymphoma, because it serves the prevention of differentiation and apoptosis, and the promotion of growth. Indeed, in diffuse large cell lymphoma (DLCL), the translocation of the *bcl-6* gene was exhibited in about 50%, and was reported to correlate with clinical presentation at extra-nodal sites including the body cavities,[14] suggesting that the *bcl-6* translocation is a causative candidate for the pathogenesis of PEL. The prototypic PEL is causatively characterized by the uniform presence of HIV, HHV-8 and EBV. Our case was, however, negative for all three viruses. Although we could not find a causative factor leading to immunodeficiency in our case, the low level of serum Ig and lymphocyte count may indicate the same situation as that of AIDS. Post-transplants also have been reported to develop unusual PEL. Therefore, severe immuno-suppression, related or not to the cause of AIDS, appears to promote the emergence of lymphoproliferative disorders, including PEL.

Of the pathogenesis of HHV-8-negative PEL, Ohshima et al.[15] have suggested, based on the findings of complex genomic abnormalities, mainly chromosome 8, in their CGH study, that multi-step carcinogenesis may be involved in HIV/HHV-8 negative cases. Shimazaki et al.[16] and Fujisawa et al.[17] have presented the pathogenic possibility of c-*myc* abnormalities, such as c-*myc/IgH* rearrangement or 8q24 gains, in an unusual PEL case. They reported that a fraction of PEL harbor the Burkitt type c-*myc* rearrangement, suggesting that lymphomatous effusion is in part one of the extra-nodal symptoms of Burkitt-like lymphoma or DLCL. A half of HHV-8-negative PEL cases had the non-Burkitt type c-*myc* translocation or the germ line configuration of c-*myc* with/without oncogene alteration.

Conclusively, the *bcl-6/IgH* translocation may be deeply associated with the pathogenesis of unusual PEL without HIV or HHV-8, but there remains the possibility that unusual PEL is a spectrum of DLCL with neoplastic cells localized within major body cavities.

Acknowledgment

This study was supported in part by Grant-in-Aid Scientific Research (No.17390165/No.15659136) from the Japan Society for the Promotion of Science.

References

1. Gaidano G, Carbone A. Primary effusion lymphoma: a liquid phase lymphoma of fluid-filed body cavities. *Adv Cancer Res* 80: 115-146, 2001
2. Nador RG, Cesarman E, Chadburn A et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. *Blood* 88: 645-656, 1996
3. Chan AC, Chan AK, Yan KW, Kwong YL. Anaplastic large cell lymphoma presenting as a pleural effusion and mimicking primary effusion lymphoma. A report of 2 cases. *Acta Cytologica* 47: 809-816, 2003
4. Klepfish A, Sarid R, Shtalrid M, Shvidel L, Berrebi A, Schttner A. Primary effusion lymphoma (PEL) in HIV-negative patients—a distinct clinical entity. *Leuk Lymphoma* 41: 439-443, 2001
5. Wakely PE, Menezes G, Nuovo GE. Primary effusion lymphoma: cytopathologic diagnosis using in situ molecular genetic analysis for human herpes virus 8. *Modern Pathol* 15: 944-950, 2002
6. Matsumoto Y, Nomura K, Ueda K et al. Human herpes virus 8-negative malignant effusion lymphoma: a distinct clinical entity and successful treatment with rituximab. *Leuk Lymphoma* 46: 415-419, 2005
7. Uemura A, Sugahara K, Nagai H et al. An ATL cell line with an IgH pseudo-rearranged band pattern by southern blotting: a pitfall of genetic diagnosis. *Lab Hematol* 11: 8-13, 2005
8. Benhattar J, Delacretaz F, Martin P, Chaubert P, Costa J. Improved polymerase chain reaction detection of clonal T-cell lymphoid neoplasms. *Diagn Mol Pathol* 4: 108-112, 1995
9. Mori S, Sugahara K, Uemura A et al. Usefulness of a comprehensive PCR-based assay for human herpes viral DNA in blood mononuclear cell samples. *Lab Hematol* 11: 163-170, 2005
10. Fan H, Gulley ML, Gascoyne RD, Horsman DE, Adomat SA, Cho CG. Molecular methods for detecting t(11;14) translocations in mantle-cell lymphomas. *Diagn Mol Pathol* 7: 209-214, 1998
11. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA. Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the

detection of t(14; 18) in follicular lymphoma. *Am J Clin Pathol* 103: 472-478, 1995
12. Kawamata N, Nakamura Y, Miki T et al. Detection of chimaeric transcripts of the immunoglobulin heavy chain and BCL6 genes by reverse-transcriptase polymerase chain reaction in B-cell non-Hodgkin's lymphomas. *Br J Haematol* 100: 484-489, 1998
13. Venizelos I, Tamiolakis D, Lambropoulou M et al. An unusual case of post-transplant peritoneal primary effusion lymphoma with T-cell phenotype in a HIV-negative female, not associated with HHV-8. *Pathol Oncol Res* 11: 178-181, 2005
14. Aliello A, Tanborini E, Frantoni M et al. Genetic markers in sporadic tumors. In *Principles of Molecular Oncology 2nd ed.* (Bronchud MH, Foote MA, Giaccone G eds.; Human Press, Ottawa, IL) pp. 73-150, 1990
15. Ohshima K, Ishiguro M, Yamasaki S et al. Chromosomal and comparative genomic analyses of HHV-8-negative primary effusion lymphoma in five HIV-negative Japanese patients. *Leuk Lymphoma* 43: 595-601, 2002
16. Shimazaki M, Fujita M, Tsukamoto K et al. An unusual case of primary effusion lymphoma in a HIV-negative patient not pathogenetically associated with HHV-8. *Eur J Haematol* 71: 62-77, 2003
17. Fujisawa S, Tanioka F, Matsuoka T, Ozawa T. CD5+ diffuse large B-cell lymphoma with c-myc/IgH rearrangement presenting as primary effusion lymphoma. *Int J Hematol* 81: 315-318, 2005
18. Chen CY, Shiesh SC, Wu SJ. Rapid detection of K-ras mutations in bile by peptide nucleic acid-mediated PCR clamping and melting curve analysis: comparison with restriction fragment length polymorphism analysis. *Clin Chem* 50: 481-489, 2004