Tumors : RECIST) が発表された。その後、この RECIST は広く普及した治療効果判定法となった。2009 年に改訂が行われ、現在の最新版は RECIST 1.1 である。RECIST 1.1 では腫瘍縮小効果の評価のために、腫瘍病変を一次元的に測定する。腫瘍量の測定の精密性を向上させることそのものより、方法論の標準化と単純化が求められていたためだが、腫瘍縮小効果判定の指標とするには精度の上で難点があることは否めない。なぜなら、腫瘍は治療経過中で様々な形態をとりながら縮小することがあるため、評価時点で腫瘍サイズの測定方向が異なることがあり、測定する画像断面も全く異なることが起こり得る。また、腫瘍サイズを人が測定するため測定者間で測定誤差があり、たとえ同じ測定者であっても測定毎に誤差が生じる。現在、CT から得られるボリュームデータを 3 次元的に画像解析することで、より精度高く、より客観的な指標が導出可能となった。近年特に日本においては、ヘリカル CT やマルチスライス CT の普及と高機能化が急速に進み、ボリュームデータの取得が容易となった。また、コンピュータ支援画像診断の進歩により、ボリュームデータを用いた様々な解析が可能となっている。今後、この三次元的ＣＴ体積測定法を WT1 がんワクチンによる肺癌の治療効果判定に応用する。

E. 結論

肺癌の治療効果評価において、三次元的ＣＴ体積測定法は有用である。

F. 研究発表

1. 論文発表

2. 学会発表

（発表誌名巻号・頁・発行年）

Yanagawa M, Tanaka Y, Morii E, Okumura M, Johkoh T, Tomiyama N, et al. Three-dimensional (3-D) quantitative analysis of preoperative CT images in pathological stage I pulmonary adenocarcinomas: Correlation of radiologic CT data with prognostic factors. RSNA 2011, Chicago, USA

H. 知的財産権の出願・登録状況（予定含む）

1. 特許取得
2. 実用新案登録
3. その他

無し

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

肺癌に対する治療に関する研究

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研究要旨
非小細胞肺癌切除例に対する術後補助化学療法の有用性について、カルボプラチン＋パクリタクセル療法とUFT経口投与療法の有効性を比較検討するrandomized phase III studyを行っている

A. 研究目的　我が国における肺癌補助化学療法の標準治療法を探索し、WT-1 免疫療法の効果の評価にあたってのbaseline治療を確立する

B. 研究方法
瀬戸内肺癌研究会と協力して非小細胞性肺癌症例を randomize し、補助療法におけるカルボプラチン＋パクリタクセル群と UFT 群の比較を行う

（倫理面への配慮）
参加各施設のIRBの承認を得て臨床研究を行っている。

C. 研究結果
既に目標症例 401 例が 2010 年末までに集積され、patients' characteristics, compliance などの情報をまとめた段階である

D. 考察　この臨床試験によって、わが国の肺癌補助療法における WT-1 治療の best partner としての chemotherapy を選択することが可能になる。

E. 結論
2016 年の最終生存期間の比較が待たれる

F. 研究発表

1. 論文発表
2011 年アムステルダムにて開催された世界肺癌学会で発表を行った

2. 学会発表
International Association for the Study of Lung Cancer Meeting 2011

H. 知的財産権の出願・登録状況（予定含む）

1. 特許取得　特になし
2. 実用新案登録　特になし
3. その他　なし

別紙 3

厚生労働科学研究費補助金（難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

肺癌に対する WT1 ペプチド免疫療法の開発
分担研究「がんワクチン療法開発臨床研究における生物統計学的手法」

分担研究者　森田　智視　横浜市立大学大学院医学系研究科　教授

研究要旨：がんワクチン療法開発において臨床試験を計画・実施するにあたって重要な点としてデータベース集積と集積したデータの有効活用である。そのための最適な統計的方法の一つがベイズ流統計手法である。そこで、本分担研究ではベイズ流統計手法の利用可能性について検討・評価を行った。

A.　研究目的

がんワクチン療法をはじめとする新治療法開発のためのエビデンスを得るためには前向きに臨床試験を実施するのがもっとも望ましい。しかしながら、現実の臨床現場ではエビデンスが乏しいもとでも様々な治療法が手探り的に使用される側面がある。そのような場合には、日常診療で得られた情報をデータベース化し、その中からデータ解析によりエビデンスの充実化を図ることが要求される。がんワクチン療法など臨床試験のデータが相対的に十分とは言えない分野での新規治療法の評価においては、データベースから得られるヒストリカルコントロールとの比較が有用である。そのための最適な統計的方法はベイズ流統計手法であり、米国で一、二を争う M.D. アンダーソン癌センターにおいても実際の臨床試験において頻用されている[参考文献 1,2]。この方法を用いてヒストリカルコントロールとの比較解析を有効性および安全性に関して実施することで、限られたリソース（人的資源、症例数、研究費など）を“効率的”に利用することが可能となる。がんワクチン療法開発において最適な手法の一つであると考えられる。

ベイズ流”アプローチの特徴は複雑化する様々な臨床的要求に対して柔軟に対応できる点や事前情報を積極的に活用できることから、ヒストリカルコントロールデータを取り込んだデータ解析にとても馴染みやすく、適用事例もますます増えてきている。本研究では、データベース情報を用いた解析手法を検討するための準備として、過去に実施された臨床試験の結果を事前情報として用いるベイズ流統計手法が積極的に用いられているがん第Ⅰ相および第Ⅱ相臨床試験について検討する。

B.　研究方法

主要な臨床統計のジャーナルである、Biometrics、Statistics in Medicine、Biostatistics、Clinical Trials などに出版された論文あるいは著書を対象として、がん第Ⅰ相および第Ⅱ相試験に活用可能な

ベイズ流試験デザインおよびデータ解析方法を調べる。

C. 研究結果

ベイズ流統計とは、まず、有効性や安全性に関して調べたい興味のあるパラメータ（θ）を考える。奏効率（CR: complete response + PR: partial response の割合）と DLT（用量規定毒性：dose limiting toxicity）発現率がそれぞれ代表的なものである。θは１つの値に決まったものとして考えず、ランダムな変数であると考える。すなわち、平均的にどれ位の値をとり、バラツキはどの程度であるかを考える。つぎに、過去の臨床的データをもとにしたθに関する知識あるいは不確からしさを統計的な確率分布を用いて‘事前分布’として表す。新たに実施する研究／試験で観察されたデータを事前情報に加えてθの推定精度を高めていく。このプロセスのことを観察データで事前分布を‘更新する(update)’と呼ぶ。データで更新された後のθに関する情報を‘事後分布’として表す。

第Ⅰ相試験は、第Ⅱ相試験で用いる投与量レベルを決定するため、新試験治療の最大耐用量（MTD: maximum tolerated dose）の推定を目的として実施される。第Ⅰ相試験において用いられるベイズ流試験デザインが “continual reassessment method (CRM)” [3-13]である。CRM は、これまで標準的試験計画として用いられてきた“3＋3”コホートデザインのように直前 3 例のデータだけでなく、その時点までに得られた全てのデータを用いて“用量 - 毒性カーブ”の推定を行う。また、試験開始時までに得られている情報をデータとして解析に取り込むことができるため、事前情報を効果的に活用することができる。また、用量―毒性関係に対して統計的なモデルを仮定するため、ターゲットとする毒性発現率に相当する用量レベルの推定を行うことも可能である。

第Ⅰ相試験で設定された用量において当該試験治療法の有効性を評価し、最終的検証ステージである第Ⅲ相試験への移行に値するかどうかを調べるのが第Ⅱ相試験の主目的である。試験治療群一群での評価が標準的試験デザインとなる。第Ⅱ相試験におけるベイズ流デザインの適用事例として、米国 MD アンダーソンがんセンター（MDACC）の Estey ら[2]がある。AML 患者を対象とし、エンドポイントに CR 率を用いて、Liposomal daunorubicin (LD) + ara-C や LD + Topotecan ら 4 つの新治療を既存の標準療法と比較するランダム化第Ⅱ相試験を実施した。MDACC でそれまでに蓄積された 591 例分の標準療法のヒストリカルデータ：CR 率 49%（291／591 例）を比較対照として、試験治療群それぞれの効果を調べた。試験治療群で 40 例の評価が完了した時点で最も成績の良い群でも CR が得られたのは 18 例であった。このデータをもとに、標準治療群より CR 率が 20%、10%以上勝る事後確率を計算したところそれぞれ 0.001 と 0.039 となった。この結果に基づいて標準治療法を上回る十分な効果は期待できないと判断し、その時点で試験を中止した。さらに、有効性と毒性を一つの評価指標にまとめて同時にモニタリングを行うといったデザインも提案されている[14-18]。また、複数の治療法を同時に比較するため、“ランダム化第Ⅱ相試験” [19-21]と呼ばれるデザインが用いられるケースが増えている。ランダム化第Ⅱ相試験は Simon ら(1985)[19]により提案され、ランダム化によりもたらされるバイアスのない比較に基づき、次に行う検証第 III 相試験に組み込む治療群を選択することを目的とする。ランダム化第Ⅱ相

試験においてもベイズ流統計の事前情報を活用する点は大きな魅力となる。Morita and Sakamoto (2006)はランダム化第Ⅱ相試験におけるベイズ流試験デザインの適用事例を報告しており、事前情報を積極的に用いることの有用性を議論している[21]。Neuenschwander ら[22]は、ベイズ流統計手法でもちいる事前情報分布を過去の情報に基づいてどのように構築していくかについて議論している。

D. 考察

事前情報を積極的に活用するベイズ流統計手法を適切に用いるためには、事前情報のもとになるデータの質の高さが鍵となる。事前情報の有効活用方法についてはいまだ研究段階であるといわざるをえず、たとえば、情報収集ルールの統一化など入手した事前情報の質を担保するための方策を厳密に議論することが重要である。

E. 結論

事前情報の活用に際しベイズ流アプローチの適用を考慮することは重要である。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

現時点でなし

2. 学会発表

なし

H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

[参考文献リスト]

1. Thall PF, Lee SJ. (2003) Practical model-based dose-finding in phase I clinical trials: methods based on toxicity. Int J Gynecol Cancer; 13: 251-261.
2. Estey EH, Thall PF (2003). New designs for phase 2 clinical trials. Blood, 102, 442-448.
3. O'Quigley J, Pepe M, Fisher L. (1990) Continual reassessment method: a practical design for phase I clinical trials in cancer. Biometrics;46: 33-48.
4. O'Quigley J. (2005) Phase I and phase I/II dose finding algorithms using continual reassessment method. In: Crowley J eds. Handbook of Statistics in Clinical Oncology, Second Edition. New York: CRC Press.
5. Shen LZ, O'Quigley J. (1996) Consistency of continual reassessment method under model misspecification. Biometrika; 83: 395-405.
6. O'Quigley J, Shen LZ. (1996) Continual reassessment method: a likelihood approach. Biometrics; 52: 673-684.
7. Chevret S. (1993) The continual reassessment method in cancer phase I clinical

trials: a simulation study. Stat Med; 12: 1093-1108.
8. Goodman SN, Zahurak ML, Piantadosi S. (1995) Some practical improvements in the continual reassessment method for phase I studies. Stat Med; 14: 1149-1161.
9. Ishizuka N, Ohashi Y. (2001) The continual reassessment method and its applications: a Bayesian methodology for phase I cancer clinical trials. Stat Med; 20: 2661-2681.
10. Ishizuka N, Morita S. (2005) Practical Implementation of the Continual Reassessment Method. In: Crowley J eds. Handbook of Statistics in Clinical Oncology, Second Edition. New York: CRC Press, 97-116.
11. Morita S, Toi M, Saji S, Iwata H, Ohno S, Ito Y, et al. (2007) Practical application of the Continual Reassessment Method to a phase I dose-finding trial in advanced breast cancer. Drug Information Journal; 41: 691-700.
12. O'Quigley J, Conaway M. (2011) Extended model-based designs for more complex dose-finding studies. Stat Med; 30:2062-9.
13. Morita S. (2011) Application of the continual reassessment method to a phase I dose-finding trial in Japanese patients: East meets West. Stat Med 30: 2090-7.
14. Thall PF, Simon RM, Estey EH (1995). Bayesian sequential monitoring designs for single-arm clinical trials with multiple outcomes. Stat Med, 14, (357-379).
15. Thall PF, Sung HG (1998). Some extensions and applications of a Bayesian strategy for monitoring multiple outcomes in clinical trials. Stat Med, 17, (1563-1580).
16. Thall PF, Cook JD. Dose-finding based on efficacy-toxicity trade-offs. Biometrics 2004;60: 684-693.
17. Houede, N., Thall, P.F., Nguyen, H., Paoletti, X. and Kramar, A. (2010) Utility-based optimization of combination therapy using ordinal toxicity and efficacy in phase I/II trials. Biometrics. 66 532-540.
18. Bekele, B.N. and Shen, Y. (2005) A Bayesian approach to jointly modeling toxicity and biomarker expression in a phase I/II dose-finding trial. Biometrics 60 343-354.
19. Simon R, Wittes RE, Ellenberg SS (1985). Randomized phase II clinical trials. Cancer Treat Rep, 69, 1375-1381.
20. Lee JJ, Feng L (2005). Randomized phase II designs in cancer clinical trials: current status and future directions. J Clin Oncol, 23, 4450-4457
21. Morita S, Sakamoto J (2006). Application of an adaptive design to a randomized phase II selection trial in gastric cancer. Pharmaceutical Statistics, 5, 109-118.
22. Neuenschwander B, Capkun-Niggli G, Branson M, Spiegelhalter DJ. (2010) Summarizing historical information on controls in clinical trials. Clin Trials; 7: 5-1

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Wada N, Ikeda J, Hori Y, Fujita S, Ogawa H, Soma T, Sugiyama H, Fukuhara S, Kanamaru A, Hino M, Kanakura Y, Morii E, Aozasa K.	Epstein-barr virus in diffuse large B-Cell lymphoma in immunocompetent patients in Japan is as low as in Western Countries.	Med Virol.	33	317-21	2011
Takahara A, Koido S, Ito M, Nagasaki E, Sagawa Y, Iwamoto T, Komita H, Ochi T, Fujiwara H, Yasukawa M, Mineno J, Shiku H, Nishida S, Sugiyama H, Tajiri H, Homma S.	Gemcitabine enhances Wilms' tumor gene WT1 expression and sensitizes human pancreatic cancer cells with WT1-specific T-cell-mediated antitumor immune response.	Cancer Immunol Immunother.	60	1289-97	2011
Ohno S, Takano F, Ohta Y, Kyo S, Myojo S, Dohi S, Sugiyama H, Ohta T, Inoue M.	Frequency of myeloid dendritic cells can predict the efficacy of wilms' tumor 1 Peptide vaccination.	Anticancer Res.	31	2447-52	2011
Hashii Y, Sato-Miyashita E, Matsumura R, Kusuki S, Yoshida H, Ohta H, Hosen N, Tsuboi A, Oji Y, Oka Y, Sugiyama H, Ozono K.	WT1 peptide vaccination following allogeneic stem cell transplantation in pediatric leukemic patients with high risk for relapse: successful maintenance of durable remission.	Leukemia	26	530-532	2011
Dohi S, Ohno S, Ohno Y, Takakura M, Kyo S, Soma G, Sugiyama H, Inoue M.	WT1 peptide vaccine stabilized intractable ovarian cancer patient for one year: a case report.	Anticancer Res.	31	2441-5	2011
Nishioka M, Tanemura A, Nishida S, Nakano A, Tsuboi A, Oji Y, Oka Y, Azuma I, Sugiyama H, Katayama I.	Vaccination with WT-1 (Wilms' Tumor gene-1) peptide and BCG-CWS in melanoma.	Eur J Dermatol.	22	258-9	2012
Kijima N, Hosen N, Kagawa N, Hashimoto N, Nakano A, Fujimoto Y, Kinoshita M, Sugiyama H, Yoshimine T.	CD166/Activated leukocyte cell adhesion molecule (ALCAM) is expressed on glioblastoma progenitor cells and involved in the regulation of tumor cell invasion.	Neuro Oncol.	In press		
Morimoto S, Oka Y, Tsuboi A, Tanaka Y, Fujiki F, Nakajima H, Hosen N, Nishida S, Nakata J, Nakae Y, Maruno M, Myoui A, Enomoto T, Izumoto S, Sekimoto M, Kagawa N, Hashimoto N, Yoshimine T, Oji Y, Kumanogoh A,	Biased usage of T cell receptor β-chain variable region genes of Wilms' tumor gene (WT1)-specific CD8(+) T cells in patients with solid tumors and healthy donors.	Cancer Sci.	103	408-414	2012

Sugiyama H.					
Nakajima H, Oka Y, Tsuboi A, Tatsumi N, Yamamoto Y, Fujiki F, Lie Z, Murao A, Morimoto S, Hosen N, Shirakata T, Nishida S, Kawase I, Isaka Y, Oji Y, Sugiyama H.	nhanced tumor immunity of WT1 peptide vaccination by interferon-β administration.	Vaccine.	30	722-9	2012
Chiba Y, Kinoshita M, Okita Y, Tsuboi A, Isohashi K, Kagawa N, Fujimoto Y, Oji Y, Oka Y, Shimosegawa E, Morita S, Hatazawa J, Sugiyama H, Hashimoto N, Yoshimine T.	Use of (11)C-methionine PET parametric response map for monitoring WT1 immunotherapy response in recurrent malignant glioma	J Neurosurg	116	835-42	2012
Shirakata T, Oka Y, Nishida S, Hosen N, Tsuboi A, Oji Y, Murao A, Tanaka H, Nakatsuka S, Inohara H, Sugiyama H.	WT1 Peptide Therapy for a Patient with Chemotherapy-resistant Salivary Gland Cancer.	Anticancer Res.	32	1081-6	2012
Hosen N, Matsuoka Y, Kishida S, Nakata J, Mizutani Y, Hasegawa K, Mugitani A, Ichihara H, Aoyama Y, Nishida S, Tsuboi A, Fujiki F, Tatsumi N, Nakajima H, Hino M, Kimura T, Yata K, Abe M, Oka Y, Oji Y, Kumanogoh A, Sugiyama H.	CD138-negative clonogenic cells are plasma cells but not B cells in some multiple myeloma patients.	Leukemia	10.1038/leu.2012.80.		2012
Hayashi S, Oji Y, Kanai Y, Teramoto T, Kitaichi M, Kawaguchi T, Okada M, Sugiyama H, Matsumura A.	Low Wilms' tumor gene expression in tumor tissues predicts poor prognosis in patients with non-small-cell lung cancer.	Cancer Invest.	30	165-71	2011
Funaki S, et al.	Novel approach for detection of isolated tumor cells in pulmonary vein using negative selection method: morphological classification and clinical implications.	Eur J Cardiothorac Surg.	40	322	2011
Nakagiri T, et al.	Validation of pN2 sub-classification in patients with pathological stage IIIA N2 non-small cell lung cancer.	Interact Cardiovasc Thorac Surg.	12	733	2011
Kanou T, et al.	The transmembrane adaptor Cbp/PAG1 controls the malignant potential of human non-small cell lung cancers that have c-src upregulation.	Mol. Cancer Res.	9	103	2011
Shintani Y, et al.	Epithelial to mesenchymal transition is a determinant of sensitivity to chemoradiotherapy in non-small cell lung cancer.	Ann Thorac Surg.	92	1764	2011

Oneyama C, et al.	MicroRNA-mediated downregulation of mTOR/FGFR3 controls tumor growth induced by Src-related oncogenic pathways.	Oncogene	30	3489	2011
Shintani Y, et al.	Low dihydropyrimidine dehydrogenase correlates with prolonged survival in patients with lung adenocarcinoma treated with 5-Fluorouracil.	Anticancer Res.	31	4665	2011.
Sawabata N, et al.	Clinical implications of the margin cytology findings and margin/tumor size ratio in patients who underwent pulmonary excision for peripheral non-small cell lung cancer.	Surg Today.	42	238	2011
Iwahori K, et al.	Overexpression of SOCS3 exhibits preclinical antitumor activity against malignant pleural mesothelioma.	Int J Cancer.	129	1005	2011
Detterbeck F, et al.	A review of prognostic factors in thymic malignancies.	J Thorac Oncol.	6 (Supple 3)	S1698	2011
Takahashi R, et al.	Early [18F]Fluorodeoxyglucose Positron Emission Tomography at Two Days of Gefitinib Treatment Predicts Clinical Outcome in Patients with Adenocarcinoma of the Lung.	Clin Cancer Res.	18	220	2012
Murakami S, Saito H, Sakuma Y, Kondo T, Oshita F, Ito H, Tsuboi M, Hasegawa C, Yokose T, Kameda Y, Nakayama H, Yamada K.	Prognostic value of preoperative FDG-PET in stage IA lung adenocarcinoma.	Eur J Radiol.	May 3. [Epub ahead of print]		2011
Saji H, Tsuboi M, Yoshida K, Kato Y, Nomura M, Matsubayashi J, Nagao T, Kakihana M, Usuda J, Kajiwara N, Ohira T, Ikeda N.	Prognostic impact of number of resected and involved lymph nodes at complete resection on survival in non-small cell lung cancer.	J Thorac Oncol.	6	1865-71	2011
Van Schil PE, Asamura H, Rusch VW, Mitsudomi T, Tsuboi M, Brambilla E, Travis WD.	Surgical implications of the new IASLC/ATS/ERS adenocarcinoma classification.	Eur Respir J.	39	478-86.	2012
佐治 久，池田徳彦	肺癌の外科治療に関する臨床試験	医学書院・臨床外科	66(6)	618-625	2011
西條天基，長瀬清亮，池田徳彦	アジュバント化学療法を巡る最近の話題	科学評論社・呼吸器内科	19(4)	349-356	2011
前田純一，池田徳彦	肺癌の診断と治療の最前線 新TMN分類（第7版）の臨床的意義	ライフメディコム・Current Therapy	29(11)	12-16	2011
Usuda J, Ichinose S, Ishizumi T, Ohtani K, Inoue	Klotho is a novel biomarker for good survival in resec	Lung Cancer	72(3)	355-359	2011

T, Saji H, Kakihana M, Kajiwara N, Uchida O, Nomura M, Tsutsui H, Ohira T, Ikeda N	ted large cell neuroendocrine carcinoma of the lung.				
Imamura F, Nishio M, Noro R, Tsuboi M, Ikeda N, Inoue A, Ohsaki Y, Kimura Y, Nishino K, Uchida J, Horai T	Randomized Phase II Study of Two Schedules of Carboplatin and Gemcitabine for Stage IIIB and IV Advanced Non-Small Cell Lung Cancer (JACCRO LC-01 Study).	Chemotherapy	57(4)	357-362	2011
Nomura M, Fukuda T, Fujii K, Kawamura T, Tojo H, Kihara M, Bando Y, Gazdar AF, Tsuboi M, Oshiro H, Nagao T, Ohira T, Ikeda N, Gotoh N, Kato H, Marko-Varga G, Nishimura T	Preferential expression of potential markers for cancer stem cells in large cell neuroendocrine carcinoma of the lung. An FFPE proteomic study.	J Clin Bioinforma	1(1)	23	2011
Saji H, Tsuboi M, Yoshida K, Kato Y, Nomura M, Matsubayashi J, Nagao T, Kakihana M, Usuda J, Kajiwara N, Ohira T, Ikeda N	Prognostic Impact of Number of Resected and Involved Lymph Nodes at Complete Resection on Survival in Non-small Cell Lung Cancer.	J Thorac Oncol	6(11)	1865-1871	2011
Usuda J, Ichinose S, Ishizumi T, Ohtani K, Inoue T, Saji H, Kakihana M, Kajiwara N, Uchida O, Nomura M, Ohira T, Ikeda N	Klotho predicts good clinical outcome in patients with limited-disease small cell lung cancer who received surgery.	Lung Cancer	74(2)	332-337	2011
Ikeda N, Usuda J, Kato H, Ishizumi T, Ichinose S, Otani K, Honda H, Furukawa K, Okunaka T, Tsutsui H	New Aspects of Photodynamic Therapy for Central Type Early Stage Lung Cancer.	Lasers in Surgery and Medicine	43(7)	749-754	2011
Suzuki, K., Koike, T., Asakawa, T., Kusumoto, M., Asamura, H., Nagai, K., Tada, H., Mitsudomi, T., Tsuboi, M., Shibata, T., Fukuda, H., Kato, H.	A prospective radiological study of thin-section computed tomography to predict pathological non-invasiveness in peripheral clinical IA lung cancer (JCOG0201).	J Thorac Oncol	6	751-756	2011
Miyasaka, Y., Oh, S., Takahashi, N., Takamochi, K., Suzuki, K.	Postoperative complications and respiratory function following segmentectomy of the lung - comparison of the methods of making an inter-segmental plane.	Interact Cardiovasc Thorac Sur	12(3)	426-429	2011
Takamochi, K., Oh, S., Matsuoka, J., Suzuki, K.	Clonality status of multifocal lung adenocarcinomas based on the mutation patterns of *EGFR* and *K-ras*.	Lung Cancer	75(3)	313-20	2012
Takahashi, N., Suzuki, K., Takamochi, K., Oh, S.	Prognosis of surgically resected lung cancer with extremely high preoperative serum carcinoembryonic antigen level.	Gen Thorac Cardiovasc Surg	59(10)	699-704	2011

Takamochi, K., Oh, S., Matsuoka, J., Suzuki, K.	Risk factors for morbidity after pulmonary resection for lung cancer in younger and elderly patients.	Interact Cardiovasc Thorac Surg	12(5)	739-743	2011
Kozu, Y., Tsuta, K., Kohno, T., Sekine, I., Yoshida, A., Watanabe, S., Tamura, T., Yokota, J., Suzuki, K, Asamura H, Furuta K, Tsuda H.	The usefulness of mutation-specific antibodies in detecting epidermal growth factor receptor mutations and in predicting response to tyrosine kinase inhibitor therapy in lung adenocarcinoma.	Lung Cancer	73(1)	45-50	2011
Ichinokawa, H., Ishii, G., Nagai, K., Yoshida, J., Nishimura, M., Hishida, T., Suzuki, K., Ochiai, A.	Clinicopathological characteristics of primary lung adenocarcinoma predominantly composed of goblet cells in surgically resected cases.	Pathol Int	61(7)	423-429	2011
K. Suda	Reciprocal and Complementary Role of MET Amplification and EGFR T790M Mutation in Acquired Resistance to Kinase Inhibitors in Lung Cancer. Clin. Cancer Res. 16(22): 5489-98, 2010.	Clin. Cancer Res.	16 (22)	5489	2010
K. Suda	Biological and clinical significance of KRAS mutations in lung cancer	Cancer Metastasis Rev. 29:49–60, 2010	29	49	2010
Tomizawa K	Analysis of *ERBB4* Mutations and Expression in Japanese Patients with Lung Cancer. Journal of Thracic Oncology, 5(11):1859-61, 2010	Journal of Thracic Oncology, 5(11):1859-61, 2010	5(11)	1859	2010
多田　弘人	EGFR遺伝子変異陽性非小細胞胞がんに対するEGFR-TKIによる術後補助療法の臨床試験－Pro－	腫瘍内科	7(6)	537-540	2011年
高濱　誠，山本　良二，月岡　卓馬，多田　弘人	呼吸困難で発症した中枢気道の腫瘍性病変に対する二期的手術－硬性気管支鏡下core outの有用性－	肺癌	51(4)	233－236	2011年
月岡　卓馬，山本　良二，高濱　誠，中嶋　隆，多田　弘人	左B^{1+2}分岐異常領域に発生した肺癌の1切除例	肺癌	25(4)	110－114	2011年
Kanzaki R, Higashiyama M, Fujiwara A, Tokunaga T, Maeda J, Okami J, Nishimura K, Kodama K.	Long-term results of surgical resection for pulmonary metastasis from renal cell carcinoma: a 25-year single-institution experience.	Eur J Cardiothorac Surg.	39	167-72	2011
Sakai M, Kanzaki R, Kozuka T, Higashiyama M, Tomita Y, Kodama K, Nakanishi K.	Left innominate venous aneurysm presenting as an anterior mediastinal mass.	Ann Thorac Surg	91	1995	2011
Kanzaki R, Higashiyama M, Fujiwara A, Tokunaga T, Maeda J, Okami J, Kozuka T, Hosoki T, Hasegawa Y, Takami M, Tomita Y, Kodama K.	Occult mediastinal lymph node metastasis in NSCLC patients diagnosed as clinical N0-1 by preoperative integrated FDG-PET/CT and CT: Risk factor	Lung Cancer	71	333-7	2011

	s, pattern, and histopathological study.				
Lococo F, Okami J, Higashiyama M, Kodama K	Indications and clinical benefits of min i-invasive parenchymal-sparing bronch oplastic procedures.	Inter CardiVacular T horac Surg	12: 538-9, 2011	589-9	2011
Higashiyama M, Okami J, Maeda J, Tokunaga T, Fujiwara A, Kodama K, Imamura F, Kobayashi H.	Differences in chemosensitivity betwee n primary and paired metastatic lung cancer tissues: *In vitro* analysis based on the collagen gel droplet embedde d culture drug test (CD-DST)	J Thorac Dis	4	40-7	2011
Kanzaki R, Higashiyama M, Oda K, Fujiwara A, Tokunaga T, Maeda J, Okami J, Tanaka T, Shingai T, No ura S, Ohue M; Kodama K.	Outcome of surgical resection for recu rrent pulmonary metastasis from color ectal carcinoma.	Am J Surg,	202	419-26	2011
Kanzaki R, Higashiyama M, Fujiwar a A, Tokunaga T, Maeda J, Okami J, Tomita Y; Kodama K	Surgical resection of pulmonary metas tases from meningioma: Report of a Case.	Surgery Today	41	995-8	2011
Nakamura H, Hori K, Tanaka-Okam oto M, Higashiyama M, Itoh Y, Inou e M, Morinaka S, Miyoshi J.	Decreased expression of LMO7 and it s clinicopathological significance in hu man lung adenocarcinoma.	Exp Therapeutic Me d	2	1053-7	2011
Miyagi Y, Higashiyama M, Gochi A, Akaike M, IshikawaT, Miura T, Sar uki N, Bando E, Kimura H, Imamur a F, Moriyama M, Ikeda I, Chiba A, Oshita F, Imaizumi A, Yamamoto H, Miyano H, Horimoto K, Tochikub o O, Mitsushima T, Yamakado M, Okamoto N.	Plasma free amino acid profiling of fiv e types of cancer patients and its ap plication for early detection.	PLos ONE,	6	e24143,	2011
Taniguchi K, Uchida J, Nishino K, Kumagai T, Okuyama T, Okami J, Higashiyama M, Kodama K, Imamur a F, Kato K	Quantitative detection of EGFR mutati ons in circulating tumor DNA derived from lung adenocarcinomas	Clin Cancer Res	17	7808-15	2011
Tokunaga T, Higashiyama M, Okam i J, Maeda J, Fujiwara A, Kodama K.	Intrathoracic chemo-thermotherapy wit h radiofrequency waves after extraple ural pneumonectomy for malignant ple ural mesothelioma.	Interact Cardiovasc Thorac Surg	13	267-70	2011
Yano S, Yamada T, Takeuchi S, Ta chibana K, Minami Y, Yatabe Y, Mit sudomi T, Tanaka H, Kimura T, Ku doh S, Nokihara H, Ohe Y, Yokota J, Uramoto H, Yasumoto K, Kiura K, Higashiyama M, Oda M, Saito H, Yoshida J, Kondoh K, Noguchi M.	Hepatocyte Growth Factor Expression in EGFR Mutant Lung Cancer with I ntrinsic and Acquired Resistance to T yrosine Kinase Inhibitors in a Japanes e Cohort.	J Thorac Oncol,	6	2011-7	2011
Fujiwara A, Okami J, Tokunaga T, Maeda J, Higashiyama M, Kodama K.	Surgical treatment for gastrointestinal metastasis of non-small-cell lung canc er after pulmonary resection.	Gen Thorac Cardio vasc Surg	59	748-52	2011
東山聖彦、堀　正二	「がん診療と地域連携」地域におけるがん診療連携（大阪府の地域連携パス実例報告）	日本癌治療学会誌	46	1468-72	2011
Kawaguchi T, Takada M, Ando M, Okishio K,Atagi S, Fujita Y, Tomizawa Y, Hayashibara K,Okano Y,Takahashi F,Saito R,Ma tsumura A,Tamura A,	A multi-institutional phase II trial of consolidation S-1 after concurrent chemora diotherapy with cisplatin a nd vinorelbine for locally n on-small cell lung cancer	European Jour nal of cancer	48	672-677	2012

Hayashi S,Oji Y,Kanai Y, Teramoto T,Kitaichi M,Kawaguchi T,Okada M,Sugiyama H,Matsumura A.	Low Wilms' tumor gene expression in tumor tissues predicts poor prognosis in patients with non-small-cell lung cancer	Cancer Investigation	30	165-171	2012
Kanzaki R, Ikeda N, Okura E, Kitahara No, Shintani Y, Okimura A, Kawahara K and Ohta M.	Surgical results and staging of non-small cell lung cancer with interlobar pleural invasion.	Interactive CardioVascular and Thoracic Surgery	0	1－4	2012
SHINTANI Y, INOUE M, FUNAKOSHI Y, MATSUMURA A,OHTA M, MAEDA H and OKUMURA M	Low Dihydropyrimidine Dehydrogenase Correlates with Prolonged Survival in Patients with Lung Adenocarcinoma Treated with 5-Fluorouracil	ANTICANCER RESEARCH	31	4665-72	2011
Maeda H. Matsumura A. Kawabata T. Suito T. Kawashima O. Watanabe T. Okabayashi K. Kubota I.	Adenosquamous carcinoma of the lung: surgical results as compared with squamous cell and adenocarcinoma cases.	Eur J Cardiothorac Surg	41	357-61	2012
Nojiri T. Yamamoto K. Maeda H. Takeuchi Y. Funakoshi Y. Okumura M.	Effect of low-dose human atrial natriuretic peptide on postoperative atrial fibrillation in patients undergoing pulmonary resection for lung cancer: A double-blind, placebo controlled study.	J Thorac Cardiovasc Surg	143	488-94	2012
Funakoshi Y. Sawabata N. Takeuchi Y. Kusumoto H. Kimura T. Maeda H.	Clinical implication of pulmonary excision for undiagnosed peripheral lung cancer.	Int Cardiovasc Thorac Surg	13	485-9	2011
Nojiri T. Yamamoto K. Maeda H. Takeuchi Y. Funakaoshi Y. Maekura R. Okumura M.	Efficacy of low-dose landiolol, an ultrashort-acting β-blocker, on postoperative atrial fibrillation in patients undergoing pulmonary resection for lung cancer.	Gen Thorac Cardiovasc Surg	59	799-805	2011
Nojiri T. Inoue M. Yamamoto K. Maeda H. Takeuchi Y. Funakoshi Y. Okumuura M.	B-type natriuretic peptide as a predictor of postoperative cardiopulmonary complications in elderly patients undergoing pulmonary resection for lung cancer.	Ann Thorac Surg	92	1051-5	2011
Shintani Y. Inoue M. Funakoshi Y. Matsumura A. Ohta M. Maeda H. Okumura M.	Low dihydropyrimidine dehydrogenase correlates with prolonged survival in patients with lung adenocarcinoma treated with 5-fluorouracil.	Anticancer Res	31	4665-72	2011
Sakai Y, Ohbayashi C, Kanomata N, Kajimoto K, Sakuma T, Maniwa Y, Nishio W, Tauchi S, Uchino K, Yoshimura M	Significance of microscopic invasion into hilar peribronchovascular soft tissue in resection specimens of primary non-small cell lung cancer	Lung Cancer	73(1)	89-95	2011
Doi T., Maniwa Y., Tanaka Y., Tane S., Hashimoto S., Ohno Y., Nishio W., Nishimura N., Ohbayashi C., Okita Y., Hayashi Y., Yoshimura M	MT1-MMP plays an important role in an invasive activity of malignant pleural mesothelioma cell	Exp Mol Pathol	90(1)	91-6	2011
Satoh N, Maniwa Y, Bermudez VP, Nishimura K, Nishio W, Yoshimura M, Okita Y, Ohbayashi C, Hurwitz J, Hayashi Y	Oncogenic phosphatase Wip1 is a novel prognostic marker for lung adenocarcinoma patient survival	Cancer Sci	102(5)	1101-6	2011
Ohno Y, Koyama H, Nogami M, Takenaka D,Onishi Y, Matsumoto	State-of-the-art radiological techniqu	Eur J Radiol	77(1)	97-104	2011

K, Matsumoto S, Maniwa Y, Yoshimura M, Nishimura Y, Sugimura K	es improve the assessment of postop erative lung function in patients wit h non-small cell lung cancer				
Okada M, Nakayama H, Okumura S, Daisaki H, Adachi S, Yoshimu ra M, Miyata Y.	Multicenter analysis of high-resolutio n computed tomography and positro n emission tomography/computed to mography findings to choose therape utic strategies for clinical stage IA l ung adenocarcinoma	J Thorac Cardiovas c Surg	141(6)	1384-91	2011
Mimae T, Okada M, Hagiya ma M, Miyata Y, Tsutani Y, Inoue T, Murakami Y, Ito	Upregulation of Notch2 and Si x1 Is Associated with Progressi on of Early-Stage Lung Adeno carcinoma and a More Aggress ive Phenotype at Advanced Sta ges.	Clin Cancer Res	18	945-955	2012
Ohba T, Sugio K, Kometa ni T, Yamaguchi M, Hama take M, Nosaki K, Takeok a H, Kitajima H, Hirai F, Seto T, Taguchi K, Nishiy ama K, Shida Y, Ichinose Y	Signet ring cell adenocarcin oma of the lung with an *E ML4–ALK* fusion gene mim icking mucinous (colloid) a denocarcinoma	A case report. Lung Cancer	73	375-378	2011
Maruyama R, Hirai F, Ond o K, Kometani T, Hamatak e M, Seto T, Sugio K, Ich inose Y.	Concurrent chemoradiothera py using cisplatin plus S-1, an oral fluoropyrimidine, f ollowed by surgery for sele cted patients with stage III non-small cell lung cancer	a single center feasibility stu dy. Surgery T oday	41 (11)	1492-149 7	2011
Osoegawa A, Kometani T, Nosaki K, Ondo K, Hama take M, Hirai F, Seto T, S ugio K, Ichinose Y	LKB1 mutations frequently detected in mucinous bronc hioloalveolar carcinoma.	Jpn J Clin On col	41(9)	1132-113 7	2011
Maeda, R., Yoshida, J., Ishii, G., Hishida, T., Nishimura, M., Nagai, K.	Risk factors for tumor recurrence in patients with early-stage (stage I and II) non-small cell lung cancer: patient selection criteria for adjuvant chemotherapy according to the seventh edition TNM classification.	Chest	140(6)	1494-502	2011
Takahashi, Y., Ishii, G., Taira, T., Fujii, S., Yanagi, S., Hishida, T., Yoshida, J., Nishimura, M., Nomori, H., Nagai, K., Ochiai, A.	Fibrous stroma is associated with poorer prognosis in lung squamous cell carcinoma patients.	J Thorac Oncol	6(9)	1460-7	2011
Maeda, R., Yoshida, J.,Ishii, G., Hishida, T., Nishimura, M., Nagai, K.	Prognostic impact of histology on early-stage non-small cell lung cancer.	Chest	140(1)	135-45	2011
Ohtaki, Y., Yoshida, J., Ishii, G., Aokage, K., Hishida, T., Nishimura, M., Takeyoshi, I., Nagai, K.	Prognostic significance of a solid component in pulmonary adenocarcinoma.	Ann Thorac Surg	91(4)	1051-7	2011
Maeda, R., Ishii, G., Yoshida, J., Hishida, T., Nishimura, M., Nagai, K.	Influence of cigarette smoking on histological subtypes of stage I lung adenocarcinoma.	J Thorac Oncol	6(4)	743-50	2011

Maeda, R., Yoshida, J., Ishii, G., Hishida, T., Nishimura, M., Nagai, K.	The prognostic impact of cigarette smoking on patients with non-small cell lung cancer.	J Thorac Oncol	6(4)	735-42	2011
Kawase,A.,Yoshida,J., Ishii,G.,Nakao,M., Aokage,K.,Hishida,T., Nishimura,M.,Nagai,K.	Differences between squamous cell carcinoma and adenocarcinoma of the lung:are adenocarcinoma and squamous cell carcinoma prognostically equal?	J Clin Oncol	42(3)	189-95	2011
Paul Mathew,Sijin Wen,Satoshi Morita,and Peter F. Thall	Placental Growth Factor and Soluble c-Kit Receptor Dynamics Characterize the Cytokine Signature of Imatinib in Prostate Cancer and Bone Metastases	J Interferon Cytokine Res	31	539-544	2011
Satoshi Morita	Application of the continual reassessment method to a phase I dose-finding trial in Japanese patients: East meets West	Stat Med	30	2090-2097	2011
Morimoto M, Numata K, Kondo M, Hidaka H, Takada J, Shibuya A, Kobayashi S, Ohkawa S, Okuse C, Morita S, Taguri M, Tanaka K	Higher discontinuation and lower survival rates are likely in elderly Japanese patients with advanced hepatocellular carcinoma receiving sorafenib	Hepatol Res	41	296-302	2011
Morizane C, Okusaka T, Morita S, Tanaka K, Ueno H, Kondo S, Ikeda M, Nakachi K, Mitsunaga S	Construction and validation of a prognostic index for patients with metastatic pancreatic adenocarcinoma	Pancreas Journal	40	415-421	2011

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
多田弘人	肺癌とは	西條　長宏 加藤　治文	インフォームドコンセントのための図説シリーズ　肺癌	医薬ジャーナル社	大阪	２０１１年	１４－１７
多田弘人	胸膜中皮腫に対する集学的治療は見通しがあるのか？	永井　厚志 大田　健 江口　研二 橋本　修	EBM呼吸器疾患の治療	中外医学社	東京	２０１１年	３８１－３８４
中嶋　隆、多田弘人	術前・術後の化学療法	西條　長宏	インフォームドコンセントのための図説シリーズ　肺がん抗悪性腫瘍薬	医薬ジャーナル社	大阪	２０１１年	１１０－１１４

Ⅳ．研究成果の刊行物・別刷

Journal of Medical Virology 83:317–321 (2011)

Epstein–Barr Virus in Diffuse Large B-Cell Lymphoma in Immunocompetent Patients in Japan is as Low as in Western Countries

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According to previous reports, the frequency of Epstein–Barr virus (EBV) positivity in diffuse large B-cell lymphoma is higher in East Asia (approximately 9%) than in Western countries. The presence of the EBV genome was examined in diffuse large B-cell lymphoma patients registered with the Osaka Lymphoma Study Group (OLSG) in Osaka, Japan, situated in East Asia. The EBV-positive rate was examined with in situ hybridization (ISH) in 484 immunocompetent diffuse large B-cell lymphoma patients registered with OLSG. The male-to-female ratio was 1.29, with ages ranging from 16 to 95 (median, 68) years. ISH with EBV-encoded small RNAs (EBER) probes revealed positive signals in the nuclei of tumor cells: the frequency of positively stained cells among all tumor cells was almost none in 458 cases, 5–10% in 5, 10–20% in 5, 20–50% in 11, and >50% in 5. When the frequency was >20% or >50%, the EBV-positive rate in the present series (3.3% or 1.0%) was rather similar to that reported in Western cases. Careful evaluation of patient backgrounds, including age distribution, type of lymphomas, exclusion of immunocompromised patients, and establishment of definite criteria for EBV positivity (>20%, >50%, or almost all tumor cells) are essential in comparing geographical differences. ***J. Med. Virol. 83:317–321, 2011.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: Epstein–Barr virus; diffuse large B-cell lymphoma; in situ hybridization; immunocompetence; geographical differences

INTRODUCTION

The Epstein–Barr virus (EBV), a γ-herpesvirus, is transmitted by mucosal secretions among the human population. An association between EBV and human lymphomas, including endemic Burkitt's lymphoma, Hodgkin lymphoma, and non-Hodgkin lymphomas of B-, T-, or NK-cell immunophenotypes, has been reported [Shah and Young, 2009]. In the recent WHO classification (2008), the chapter for EBV-positive diffuse large B-cell lymphoma of the elderly states that the EBV-positive rate in diffuse large B-cell lymphoma in Asian countries is 8–10% [Nakamura et al., 2008]. Hematologists and hematopathologists in the Osaka Lymphoma Study Group (OLSG) in Osaka, Japan, situated in the East Asia, felt a sense of incongruity for this figure, although they had no data on this.

Abbreviations: EBV, Epstein–Barr virus; OLSG, Osaka Lymphoma Study Group; LMP, latent membrane protein; ISH, in situ hybridization, EBER, EBV-encoded small RNAs; MTX, methotrexate; PNA, peptide nucleic acid; FITC, fluorescein isothiocyanate; DAB, 3,3′-diaminobenzidine tetrahydrochloride; CTL, cytotoxic T-lymphocytes.

We declare that we have no conflict of interest.

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There have been two large-scale studies on the EBV positivity in diffuse large B-cell lymphoma from East Asian countries, one in Korea and one in Japan. Both studies showed a similar frequency of EBV positivity among diffuse large B-cell lymphoma, that is, 9% [Oyama et al., 2007; Park et al., 2007]. However, two points regarding these reports should be addressed. First, the age distribution was quite different between the Korean and Japanese cases: the percentage of cases older than 60 years was 36.6% in Korean and 60.6% in Japanese cases. It seems that the EBV-positive rate is higher in elder patients; thus, the difference in age distribution might affect EBV positivity. Second, the criteria for EBV positivity of diffuse large B-cell lymphoma was different: >20% of tumor cells with positive signals by in situ hybridization (ISH) in one report [Park et al., 2007] and >50% in another [Oyama et al., 2007]. Some Japanese investigators, adopting the criteria of almost all tumor cells with positive signals as EBV-positive, reported that 11.4% of their diffuse large B-cell lymphoma cases were EBV-positive [Kuze et al., 2000]. However, reports from Western countries apparently showed a much lower EBV-positive rate than did the East Asian ones [Gibson and Hsi, 2009; Hoeller et al., 2010]. Hoeller et al. [2010] reported that 10 (3.9%) of their 258 diffuse large B-cell lymphoma cases were EBV-positive: the percentage of positive cells was <20% in two cases, <50% in four, and >50% in four. Gibson and Hsi [2009] reported that none of their 90 diffuse large B-cell lymphoma, all older than 60 years, was EBV-positive. They stated that EBV-positive tumor cells were not identified in any of the 90 cases, and only three demonstrated very rare EBV-positive signals in small bystander lymphocytes (<1% of non-tumor cells).

In this study, we examined the presence of the EBV genome with ISH in the tumor cells in cases of diffuse large B-cell lymphoma registered with OLSG. The EBV-positive rate was then evaluated according to the criteria used in the previous and present studies, and the data were then compared with those reported from East Asia and Western countries in the light of clinical findings such as age, gender, and presence of immunodeficiencies.

MATERIALS AND METHODS

Patients

Between November 1999 and October 2009, in total, 4,490 cases were registered with the OLSG, Japan, in which 63 hospitals participate and register cases of malignant lymphomas and related conditions. All of the hematoxylin and eosin- and immunoperoxidase-stained sections were reviewed by one of the authors (K.A.) and classified according to the WHO classification. A diagnosis of malignant lymphoma was confirmed in 3,571 (79.5%) of 4,490 cases. Of these 3,571 cases, 3,273 (91.7%) were non-Hodgkin lymphoma and 298 (8.3%) were Hodgkin lymphoma. The number of diffuse large B-cell lymphoma cases was 1,590, which constituted 48.6% of all non-Hodgkin lymphoma. The latest 500 cases with diffuse large B-cell lymphoma registered during February 2007 to October 2009 were studied for the presence of the EBV genome. Most patients received anthracyclin-based chemotherapy and rituximab after the diagnosis. The numbers of patients who received excisional biopsy or surgical resection, punch biopsy, or needle biopsy were 384, 76, and 40, respectively. In cases receiving punch or needle biopsy, an adequate amount of material for histological analyses was obtained. The primary site was lymph node in 165 cases, extranodal organs in 204, and unknown in 131. Among them, two patients suffered from rheumatoid arthritis, one each from idiopathic thrombocytopenic purpura, interstitial pneumonia, Sjögren's syndrome, and uveitis, and were treated with prednisone, together with cyclosporine in one. These six cases were excluded for further analysis because of possible underlying immune abnormalities. During the same period, 14 cases of polymorphous lymphoproliferative disorders, 14 methotrexate (MTX)-associated lymphoproliferative disorders, five post-transplant lymphoproliferative disorders (liver, kidney, and cord blood in two, two, and one case, respectively), and one Hodgkin-like lymphoproliferative disorder were registered with the OLSG. Polymorphous lymphoproliferative disorders are defined as lymphoproliferative disorders showing a polymorphous pattern of proliferation, consisting of large lymphoid cells and various numbers of inflammatory cells [Nakamichi et al., 2010]. Polymorphous lymphoproliferative disorders usually affect individuals with a background of immunodeficiency. Clinical findings entered on the registration card for the OLSG included the following: name of hospital, age, gender, occupation, site of disease, clinical diagnosis, present illness, and peripheral blood and bone marrow findings, including differential counts, serum data, and chromosomal abnormalities of the tumor cells. History of medication (those receiving immunosuppressants) was also checked.

The Institutional Review Board for Clinical Research at Osaka University Hospital approved this study.

Immunohistochemistry

Antibodies used for immunohistochemistry were CD20, CD79a, CD3, CD30, and anti-Epstein–Barr virus/LMP/Clones CS. 1–4 (DakoCytomation, Glostrup, Denmark, diluted at 1:400, 1:100, 1:50, 1:25, and 1:100, respectively). Immunohistochemistry was performed using an automated staining system (Dako Autostainer, DakoCytomation).

In Situ Hybridization

ISH using the EBV-encoded small RNAs (EBER) probe was performed to examine the presence of the EBV genome in the formalin-fixed paraffin-embedded sections with the EBER DAB application kit (DakoCytomation). Briefly, sections were treated with proteinase K, diluted at 1:10 with TBS (50 mmol/L Tris–HCl buffered saline containing 150 mmol/L NaCl, pH 7.6), then hybridized with EBER PNA (peptide nucleic acid)

probe/fluorescein (DakoCytomation) at 55°C for 90 min. After blocking endogenous peroxidase activity, sections were incubated with rabbit anti-FITC (fluorescein isothiocyanate) antibody (1:50; Invitrogen, Carlsbad, CA) at room temperature for 30 min, incubated with ChemMate ENVISION/HRP polymer (DakoCytomation) at room temperature for 30 min, and colored with DAB (3,3′-diaminobenzidine tetrahydrochloride). This ISH method using an EBER probe was recently used in the author's laboratory. The EBV-positive rate in NK/T cell lymphoma by this method on paraffin-embedded sections was similar (80%) to that with the previously used method using an EBER-1 probe [Li et al., 2000].

The frequency of EBV positivity in the malignant large lymphoid cells was determined by averaging the number of positive cells from three high-power fields where the positive cells were larger in number. The tumors were then separated into five groups: tumors with almost no EBV positivity, tumors with 5–10%, 10–20%, 20–50%, and >50% of cells showing EBV positivity. According to the previous study, we adopted the criterion of >20% as EBV-positive.

Follow-Up

Adequate follow-up data was available in 16 EBV-positive and 204 EBV-negative diffuse large B-cell lymphoma cases. Follow-up periods for survivors in EBV-positive and -negative cases ranged from 5.2 to 38.5 (average 22.6) and 16.4–38.1 (average 25.5) months, respectively. Kaplan–Meier estimated survival rates at 2 and 3 years were calculated, and overall survival rates were compared by a log-rank test.

RESULTS

EBV-ISH was performed in 494 cases; however, informative data was not obtained in 10 cases because of specimen loss during staining procedures or severe non-specific staining of background/overstaining. As a result, staining results could be evaluated in only 484 cases. Positive signals were found in the nuclei of tumor cells: the frequency of positively stained cells among all tumor cells was almost none in 458 cases, 5–10% in 5, 10–20% in 5, 20–50% in 11, and >50% in 5 (Fig. 1).

Of 16 cases with >20% EBV-positive tumor cells (EBV-positive cases), large geographical necrosis was found in five cases. Angiotropism was not found. CD30 positivity was found in 6 of 15 EBV-positive cases. Tumor cells expressed latent membrane protein (LMP)-1 in 8 of 14 EBV-positive cases (Fig. 1).

In total, 40 cases with diffuse large B-cell lymphoma were excluded, due to the putative presence of immunocompromised conditions, including autoimmune diseases, receiving prednisone and cyclosporine, polymorphous lymphoproliferative disorders, MTX-associated lymphoproliferative disorders, post-transplant lymphoproliferative disorders, and Hodgkin-like lymphoproliferative disorders. The frequency of positively stained cells among all tumor cells was 20–50% in six cases and >50% in five.

The estimated survival rates at 2 and 3 years for the EBV-positive and -negative cases were 58.9% and 71.9% at 2 years and 58.9% and 69.0% at 3 years, respectively. The overall survival rates of the two groups were not statistically different (log-rank test; $P > 0.1$).

DISCUSSION

According to previous reports, frequency of EBV positivity in diffuse large B-cell lymphoma was higher in East Asia than in Western countries (Table I) [Kuze et al., 2000; Oyama et al., 2007; Park et al., 2007; Gibson and Hsi, 2009; Hoeller et al., 2010]. However, before accepting this, a careful evaluation of the data presented in the previous studies is necessary. Regarding clinical findings, the age distribution and gender ratio varied among these reports [Kuze et al., 2000; Oyama et al., 2007; Park et al., 2007; Gibson and Hsi, 2009; Hoeller et al., 2010]. A study referenced in the WHO classification adopting >50% of the criteria included cases of immunodeficiency-associated lymphomas, although the exact number was not shown. Lymphomas known to be closely associated with EBV infection such as pyothorax-associated lymphoma and Burkitt's lymphoma were also included [Oyama et al., 2007]. These procedures inevitably increased the EBV-positive rate of the study (13.6%). The routinely registered cases with the OLSG constitute the present series; thus, collection bias in the cases could be avoided. The age distribution and gender ratio in the present series are within the spectrum of the previously reported diffuse large B-cell lymphoma cases; thus, the present findings could be adopted as standard data.

The criteria for defining EBV-positive cases vary among the previous studies; >20% of the tumor cells in one study [Park et al., 2007] and >50% in another [Oyama et al., 2007]. In particular, a study from Japan adopting the criteria of almost all EBV-positive cells reported the EBV-positive rate to be 11.4% [Kuze et al., 2000]. When adopting >20% or >50% as EBV-positive, the EBV-positive rates among diffuse large B-cell lymphoma in immunocompetent hosts in the OLSG (3.3% or 1.0%) were rather similar to those in Western series [0–2.7%, 0–1.9%; Gibson and Hsi, 2009; Hoeller et al., 2010], but much lower than that in Korean (8.9%) [Park et al., 2007] and Japanese series (11.4–13.6%) [Kuze et al., 2000; Oyama et al., 2007].

The present and previous [Park et al., 2007; Gibson and Hsi, 2009; Hoeller et al., 2010] studies have analyzed the presence of the EBV genome in diffuse large B-cell lymphoma in immunocompetent patients. When a total of 40 cases with diffuse large B-cell lymphoma, which had been excluded from the calculation of the EBV-positive rate due to the putative presence of immunocompromised conditions (such as autoimmune disease, receiving prednisone and cyclosporine, polymorphous lymphoproliferative disorders, MTX-associated lymphoproliferative disorders, post-transplant lymphoproliferative disorders, and