保護を厳守する。

4) 臨床試験審査委員会、効果・安全性評価委員会、監査委員会による、臨床試験研究の第三者的監視を実施する。

C. 研究結果

平成１９年度は上記２研究のための研究計画書（プロトコール）を作成した。平成２０年度は、症例登録を行い作成プロトコールに従って治療研究を進める。

D. 考察

予後不良な CD２０陽性びまん性大細胞型リンパ腫とマントル細胞リンパ腫は、従来の治療法での治療成績が不良であり、新たな治療法の開発が急務な分野である。本研究によって同疾患に対する有効な治療法が開発される期待がある。

E. 結論

本研究はこれから登録を開始し症例集積をする段階なので現時点では結論できる結果を得ていないが、予後不良の CD２０陽性びまん性大細胞リンパ腫およびマントル細胞リンパ腫には、自家末梢血幹細胞移植併用大量化学療法の有効性が期待されており、本研究での結果が期待されている。

F. 研究発表

なし

G. 知的財産権の出願・登録状況（予定を含む）

なし

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書

進行期難治性B細胞リンパ腫に対する大量化学療法を併用した効果的治療に関する臨床研究

分担研究者　渡辺隆　国立がんセンター中央病院　特殊病棟部 11B 病棟医長

研究要旨
進行難治悪性リンパ腫に対する大量化学療法併用療法の確立（臨床試験の実施）

A. 研究目的

年齢調節国際予後指標で予後不良群とされるびまん性大細胞型 B 細胞リンパ腫ならびにマントル細胞リンパ腫を対象として、抗 CD20 抗体リツキシマブを併用した導入療法に引続き、up-front での自家移植を併用した大量化学療法を施行し、その治療成績の向上を図る。

1) 微少残存病変量をモニターすることにより、本試験に採用された一連のプロトコール治療各ステップの、治療全体に果たす役割を明らかにする。特に十分な成績が得られなかった場合には、改善の余地のある部分を探索する手段となる。
2) 強力な化学療法と抗CD20抗体リツキシマブを併用すると、化学療法剤による骨髄抑制から既に回復していると思われる時期に、遅発性好中球減少症を合併する例に遭遇することがある。患者によってはこのことによる治療強度が落ちる可能性があるため、特に導入療法に引続いて up-front で大量化学療法を行うような本研究では問題となり得る。したがって、その原因ならびにそのような患者群を推定できる方法を検討する。
3) 本プロトコールにても一定の割合で救済されない患者群が存在することが想定される。そのような個体群のもつ腫瘍の特徴を明らかにするため、リンパ腫細胞に発現されている蛋白質マーカーを化学療法の感受性という観点から検討する。

B. 研究方法

特に PET または PET/CT を新規に採用した効果判定規準を国内で初めて導入するため特別に会議を招集して、意見を交換した。

その他プロトコール作成に際しては、他の臨床試験プロトコール作成の経験を生かして、プロトコールの記載内容について助言をした。

1) 特にマントル細胞リンパ腫においては、第 11 番と第 14 番染色体間相互転座に由来する、cyclin D1 と免疫グロブリン重鎖の融合遺伝子量をリアル・タイム PCR 法で定量する。
2) 「遅発性好中球減少症」を合併した患者の

骨髄クロット標本を用いて、抗顆粒球抗体を用いて、その免疫染色性を検討する。

3) DNA 障害を来す化学療法剤によるアポトーシス誘導に関与する p53 蛋白と結合して、これを不活化するヒト *MDM2* 蛋白、ならびにこの調節蛋白である *MDMX* 蛋白発現の多寡を免疫染色あるいはウェスタン・ブロット法で検討する。

(倫理面への配慮)

付随研究については、まだ患者検体で実施可能性の見込みが立っていないため、現段階ではリンパ腫細胞株を用いて検討し、あくまでも *in vitro* での検討段階であり、患者への検体採取に関する情報提供は保留にしている。

C. 研究結果

マントル細胞リンパ腫に対してのプロトコールは 2 次審査提出に向けて完成した。本年度はまだプロトコール審査中のため、臨床試験が開始されていなかった。*in vitro* の研究結果は今年度はまだ得られず。

D. 考察

上記につき、特になし。

E. 結論

臨床試験が開始されておらず、研究結果が得られていないため、特になし。

G. 研究発表

1. 論文発表

1) Maruyama D, Watanabe T, Beppu Y, Kobayashi Y, Kim SW, Tanimoto K, Makimoto A, Kagami Y, Terauchi T, Matsuno Y, Tobinai K. Primary Bone Lymphoma: a New and detailed characterization of 28 patients in a single-institution study. ***Jpn J Clin Oncol*** 37(3) : 216-223, 2007.

2) Ohara F, Kobayashi Y, Akabane D, Maruyama D, Tanimoto K, Kim SW, Watanabe T, Tobinai K. Abdominal pain and syndrome of inappropriate antidiuretic hormone secretion as a manifestation of visceral varicella zoster virus infection in a patient with non-Hodgkin's lymphoma. ***Am J Hematol*** 82(5) : 416, 2007.

3) Tanimoto K, Kaneko A, Suzuki S, Sekiguchi N, Watanabe T, Kobayashi Y, Kagami Y, Miyagi Maeshima A, Matsuno Y, Tobinai K. Primary ocular adnexal MALT lymphoma: a long-term follow-up study of 114 patients. Jpn J Clin Oncol 37(5) : 337-344, 2007.

4) Kusumoto S, Mori S, Nosaka K, Morita-Hoshi Y, Onishi Y, Kim SW, Watanabe T, Heike Y, Tanosaki R, Takaue Y, Tobinai K. T-cell granular lymphocyte leukemia of donor origin after blood transplantation. ***Clin Lymph & Myeloma*** 7(7) : 475-479, 2007.

2. 学会発表

1) 渡辺隆。効果判定の標準化・その問題点。企画 2、ちからだめし実践講座。悪性リンパ腫の病期判定と効果判定。第 47 回日本リンパ網内系学会。2007 年 5 月 26 日兵庫。

2) 木下朝博、渡辺隆。WHO 分類に基づく T

細胞リンパ腫の治療成績-JCOG study-。シンポジウム発表。第 47 回日本リンパ網内系学会。2007年5月25日兵庫。

3) 菊地我子、堀田知光、渡辺隆、小椋美知測、森島泰雄、石澤賢一、伊藤國明、岡本真一郎、谷脇雅史、塚本憲史、奥村廣和、林正樹、遠藤啓吾、飛内賢正。再発・難治性低悪性度 B 細胞リンパ腫（B-NHL）に対する SHL749（^{90}Y-ibritumomab tiuxetan）の第 II 相試験。Phase II study of SHL749 (^{90}Y-ibritumomab tiuxetan) in relapsed/refractory indolent B-cell lymphoma. 第 69 回日本血液学会・第 49 回日本臨床血液学会合同総会。2007年10月11-13日横浜。

4) 横山洋紀、渡辺隆、丸山大、金成元、小林幸夫、飛内賢正。リツキシマブ併用 CHOP (R-CHOP) 療法施行中に進行性多巣性白質脳症を合併した B 細胞リンパ腫。Progressive multifocal leukoencephalopathy in a patient with B-cell lymphoma during R-CHOP therapy. 第69回日本血液学会・第49回日本臨床血液学会合同総会。2007年10月11-13日横浜。

5) 渡辺隆、小林幸夫、山崎聡、星百合子、横山洋紀、加藤晴美、森島泰雄、西尾和人、荒尾徳三、Stanley R. Frankel、大月哲也、飛内賢正。Phase I trial of vorinostat (suberoylanilide hydroxamic acid, SAHA) in Japanese pts with non-Hodgkin lymphoma (NHL). イングリッシュ・ワークショップ発表。第66回日本癌学会学術総会。2007年10月3-5日横浜。

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

厚生労働科学研究費補助金（がん臨床研究事業）
（総括・分担）研究報告書

進行期難治性Ｂ細胞性リンパ腫に対する大量化学療法を併用した効果的治療に関する臨床研究

分担研究者　小松弘和　　名古屋市立大学病院　化学療法部　部長

研究要旨
本臨床研究の実施治療プロトコールである CHASER 療法の当院（名古屋市立大学病院）での安全性と末梢血幹細胞採取の有効性、さらに LEED 療法を前処置とした自己末梢血幹細胞移植（auto-PBSCT）の安全性を後方視的に検討した。 11 例の患者の臨床的解析の結果、CHASER 療法の安全性と幹細胞採取の有効性、及び LEED を前処置とする auto-PBSCH の安全性を確認した。これにより本施設での当臨床試験実施の妥当性を確認できた。

Ａ．研究目的
当院（名古屋市立大学病院）でのＢ細胞性リンパ腫において CHASER 療法の安全性と末梢血幹細胞採取の有効性、LEED 療法を前処置とした自己末梢血幹細胞移植（auto-PBSCT）の安全性を検討する。

Ｂ．研究方法
2005 年 4 月より当院で CHASER 療法を施行した 65 歳以下のＢ細胞性リンパ腫患者を対象として研究目的について診療記録を元に後方視的に検討する。

（倫理面への配慮）対象患者の臨床データはあらかじめ解析前に個人情報を記号化し個人の同定ができないように配慮した。

Ｃ．研究結果
【対象患者】11 例（男性 5 例、女性 6 例）、組織型：びまん性大細胞型 7 例、濾胞性 2 例、縦隔原発大細胞型 2 例。年齢中央値 58 歳（33～65 歳）、CHASER 療法実施総回数 33 回、患者 1 人あたりの CHASER 実施回数中央値 2 回(2～4)、初回再発 7 例、第 2 再発 1 例、初回難治 3 例、CHASER 療法施行時骨髄浸潤陽性 7 例。

【CHASER 療法による血液毒性】(n=30)

	中央値	範囲
好中球数最低値（/μl）	18	0～110
好中球数 500 以下の期間（日）	7	4～13
ヘモグロビン最低値（g/dl）	7.4	5. 9～12. 4
血小板数最低値（×10^4/μl）	2.4	0.8～7. 6
血小板輸血単位数/サイクル	20	0～60
赤血球輸血単位/サイクル	0	0～8

【CHASER 療法による非血液毒性】(n=31)

38℃以上の発熱	あり	20
	なし	11
発熱のあったサイクル	中央値	4
のうち発熱持続期間（日）	範囲	1～15
悪心、嘔吐、発熱、感染	あり	0
以外の grade3以上の副作用	なし	33

【auto-PBSCT】

PBSCH施行患者数		8
十分なCD34幹細胞数採取(患者数)	成功	6
	不成功	2※
auto-PBSCT	施行	6#
	施行せず	5
移植前処置	LEED	5
	LEED以外	1

※濾胞性リンパ腫の2例、#全例生着

D．考察

CHASER 療法は、発熱（感染）、血球減少に伴う抗生剤、GCSF，輸血療法を適切に行うことで全11例、計33サイクルを安全に施行できた。poor mobilizingの2例はいずれも濾胞性リンパ腫で、今回、臨床試験の対象であるびまん性大細胞型には全例で移植可能の CD34 陽性細胞が採取できた。LEED を前処置とした auto-PBSCT 施行5例とも生着が得られ、治療関連死を認めなかった。

E．結論

本臨床試験で計画する CHASER 療法の当院での安全性と幹細胞採取の有効性、及び LEED を前処置とする auto-PBSCH の安全性を確認した。これにより本施設での当臨床試験実施の妥当性を後方視的に確認できた。

F．健康危険情報

特になし

G．研究発表

1．論文発表

1. Yano H, Komatsu H, et al. Defucosylated Anti CC Chemokine Receptor 4 Monoclonal Antibody Combined with Immunomodulatory Cytokines: A Novel Immunotherapy for Aggressive/Refractory Mycosis Fungoides and Sezary Syndrome. Clin Cancer Res. 2007 Nov 1;13(21):6494-500.

2. Yano H, Komatsu H, et al. Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. Int J Cancer. ;120(9):2052-7, 2007.

2．学会発表

1．小松弘和　他　B細胞性リンパ腫における CHASER 療法の安全性と末梢血幹細胞採取の有効性について　2008 年日本血液学会総会発表予定

H．知的財産権の出願・登録状況

1．特許取得

該当無し

2．実用新案登録

該当無し

3．その他

該当無し

Ⅲ. 研究成果の刊行に関する一覧表

別紙４

研究成果の刊行に関する一覧表レイアウト

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
Oki Y, Ogura M, Kagami Y, Morishima Y.	Phase II study of a salvage regimen using cyclophosphamide, high-dose cytarabine, dexamethasone, etoposide, and rituximab in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma.	*Cancer Sci.*	98	179-184	2008
Oyama T, Morishima Y, Nakamura S, et al.	Age-related EBV-associated B-cell lymphoproliferative disorders constitute a distinct clinicopathologic group: a study of 96 patients.	*Clin Cancer Res.*	13	5124-5132	2007
Asano N, Morishima Y, Nakamura S, et al.	Prognostic significance of T-cell or cytotoxic molecules phenotype in classical Hodgkin's lymphoma: a clinicopathologic study.	*J Clin Oncol.*	24	4626-4633,	2007
Tsukasaki K, Tomonaga M.,et al.,	VCAP-AMP-VECP Compared With Biweekly CHOP for Adult T-Cell Leukemia-Lymphoma: Japan Clinical Oncology Group Study JCOG9801.	*J Clin Oncol.*	25(34)	1-7	2007
Tobinai K, Kasai M, et al.	Phase II study of chemotherapy and stem cell transplantation for adult acute lymphoblastic leukemia or lymphoblastic lymphoma: Japan Clinical Oncology Group study 9004.	*Cancer Sci.*	98	1350-1357	2007

Ⅳ. 研究成果の刊行物・別刷

Phase II study of a salvage regimen using cyclophosphamide, high-dose cytarabine, dexamethasone, etoposide, and rituximab in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma

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The management of relapsed or refractory B-cell non-Hodgkin's lymphoma (B-NHL) remains challenging. We investigated the efficacy and safety of salvage chemoimmunotherapy (CHASER) in patients with relapsed or refractory B-NHL who had radiographically measurable disease and adequate major organ function. The CHASER treatment consisted of: rituximab 375 mg/m², day 1; cyclophosphamide 1200 mg/m², day 3; cytarabine 2 g/m², days 4 and 5; etoposide 100 mg/m², days 3–5; and dexamethasone 40 mg, days 3–5. The treatment was repeated every 3 weeks up to a total of four courses in the absence of disease progression. Thirty-two patients were enrolled and received a median of four courses of treatment (range 1–4 courses) per patient. Twenty patients (63%) were previously treated with rituximab-containing regimens. The median age was 54 years (range 28–67 years). The treatment was generally well tolerated, with major toxicities being grade 4 neutropenia (n = 32), thrombocytopenia requiring transfusion (n = 28), and grade 3 transaminase elevation (n = 2). Overall response rates in the entire group, and in patients with indolent (n = 17) and aggressive (n = 15) diseases were 84%, 100% and 67%, respectively. Responses were observed similarly in patients with (n = 20) and without (n = 12) previous rituximab exposure (85% and 83%, respectively). Stem cell harvest was successful in 19 of 22 patients. The median time to treatment failure for the entire group was 24.5 months. This promising result of high activity and favorable toxicity profile warrants further investigation in large-scale multicenter trials. (*Cancer Sci* 2008; 99: 179–184)

Although a certain proportion of patients with NHL have an excellent prognosis after initial treatment, many patients with NHL develop relapsed or refractory disease. Management of such conditions remains challenging, and salvage regimens to better control the disease are needed. We previously reported the safety and efficacy of combination salvage chemotherapy called CHASE[1] that consists of cyclophosphamide, high-dose cytarabine, steroid (dexamethasone), and etoposide. CR was observed in 10 of 14 patients (71%) with relapsed or refractory NHL. This regimen was well tolerated, and was associated with no renal toxicities, in contrast to other commonly used cisplatin containing salvage regimens such as DHAP[2] and ESHAP[3] which are associated with irreversible increase in serum creatinine in 4–8% of patients. Although the original report of CHASE included only a small number of patients, CHASE has been widely used as a salvage therapy in Japan given the significant efficacy and tolerability.

Anti-CD20 monoclonal antibody, rituximab, has recently revolutionized the management of B-NHL. Rituximab can contribute to improved disease control and survival when added to initial standard combination chemotherapy[4,5] and the use of rituximab in the salvage setting has also shown significant activity with minimal toxicity.[6,7] However, it remains to be shown whether rituximab containing salvage chemotherapy is still as effective in patients with previous exposure to rituximab. Based on the encouraging clinical data of CHASE chemotherapy as well as rituximab in salvage settings, we carried out an open-label, phase II clinical trial to evaluate the efficacy and safety of combination chemoimmunotherapy using CHASE and rituximab (CHASER) in patients with relapsed or refractory B-NHL.

Materials and Methods

Patient selection. The protocol for the current study was approved by the institutional review board of Aichi Cancer Center Hospital (Aichi, Japan). To be eligible for the study, patients were required to have histologically confirmed relapsed or refractory NHL, with CD20 positivity on tumor cells by immunohistochemistry and bidimensionally measurable disease by computed tomography scan. Patients were also required to: be aged between 15 and 69 years; have an Eastern Cooperative Oncology Group performance status 0–2; have received ≥1 previous treatment regimens; and have adequate bone marrow function (ANC $\geq 1.5 \times 10^9$/L, and platelet count $\geq 100 \times 10^9$/L), liver function (total bilirubin level ≤2 mg/dL, and aspartate aminotransaminase and alanine aminotransaminase levels ≤2.5 times the upper limit of normal), and kidney function (serum creatinine level ≤2 mg/dL).

Patients were ineligible if they had lymphoma involvement in the central nervous system; had serum hepatitis B surface antigen; had serum HIV antibody; had uncontrolled intercurrent illnesses such as active infection, cardiac diseases, active second malignancy, or psychiatric disease. Those who were pregnant or lactating were ineligible. Written informed consent was obtained from all patients before study entry, consistent with national and local requirements. All patients gave written informed consent indicating that they were aware of the investigational nature of the study, in keeping with the policies of Aichi Cancer Center Hospital.

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Abbreviations: ANC, absolute neutrophil count; B-NHL, B-cell non-Hodgkin's lymphoma; CHASE, cyclophosphamide, high-dose cytarabine, dexamethasone, and etoposide; CHASER, cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CI, confidence interval; CR, complete response; CRu, complete response unconfirmed; DHAP, dexamethasone, high-dose cytarabine, and cisplatin; ESHA, etoposide, methylprednisone, and high-dose cytarabine; ESHAP, ESHA plus cisplatin; NHL, non-Hodgkin's lymphoma; ORR, overall response rate; OS, overall survival; R-CHOP, rituximab with CHOP; R-DHAP, rituximab with DHAP; R-ESHAP, rituximab with ESHAP; SCT, stem cell transplantation; TTF, time to treatment failure.

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Treatment schedule. The regimen consisted of rituximab 375 mg/m^2 intravenously on day 1; cyclophosphamide 1200 mg/m^2 intravenously over 3 h on day 3; cytarabine 2 g/m^2 intravenously over 3 h on days 4 and 5; etoposide 100 mg/m^2 intravenously on days 3–5; dexamethasone 40 mg intravenously on days 3–5 and G-CSF (filgrastim, lenograstim, or nartograstim at the primary physician's choice) 2 μg/kg subcutaneously from day 6 till neutrophil recovery. The treatment was to be repeated every 3 weeks up to a total of four courses unless there is disease progression, persistent grade 3/4 toxicity, or delayed recovery of neutrophils ($<1.0 \times 10^9$/L) or platelets ($<75 \times 10^9$/L). Peripheral blood stem cell harvest was carried out after the second and/or third cycle if the patient was a suitable candidate for future SCT, targeting a total CD34 count of 2.0×10^6/kg body weight or higher.

Supportive care during chemotherapy. Patients were to be premedicated with antihistamine and antipyretics prior to rituximab infusion. Effective antiemetics such as 5-HT3 receptor antagonist were given intravenously prior to chemotherapy and as needed. To prevent hemorrhagic cystitis from cyclophosphamide, at least 3000 mL of hydration with bicarbonate-containing fluid was required on day 1. Mesna was not given in this study.

Response and toxicity assessments. To assess response, patients were required to be re-evaluated with a thoracic, abdominal, and pelvic computed tomography scan every two cycles. The International Workshop Response Criteria for NHL were used for evaluating responses[8] except that clearance of tumor cells from bone marrow needed to be confirmed by morphologic as well as flow cytometric assessment in patients who had bone marrow involvement on study entry.

Toxic effects were originally graded according to the National Cancer Center Institute Common Toxicity Criteria (version 2.0), and regarded after data collection and analyses based on version 3.0. Patients were evaluated daily with a complete history and physical examination. The laboratory assessment was carried out at least twice weekly to monitor organ toxicity and electrolyte abnormalities.

Dose modifications. If a patient's ANC was $>1.0 \times 10^9$/L and platelet count was between 75×10^9/L and 100×10^9/L (condition A) at the due date for initiation of the next treatment, doses of cyclophosphamide, cytarabine, and etoposide were reduced by 25% for the next cycle. If a patient's ANC was $<1.0 \times 10^9$/L or platelet count was $<75 \times 10^9$/L, the treatment needed to be postponed until condition A was achieved, and the doses were reduced as in condition A. If this improvement did not occur in a week, that is, at four weeks after the previous cycle, the patient was removed from the study. If persistent arrhythmia, cardiac ischemia, pericarditis, or grade 3/4 heart failure were observed, the patient was removed from the study. If grade 3/4 hepatotoxicity was present at initiation of the next cycle, the treatment was postponed till the toxicity was grade 2 or less. If grade 3 hepatotoxicity was persistent at 4 weeks after initiation of previous treatment, the patient was removed from the study. If serum creatinine was between 1.6 and 1.9 mg/dL, or increased from baseline by 0.5–1.2 mg/dL (condition B), the dose of cytarabine was reduced by 50% (1 g/m^2) because of the risk of severe neurological toxicity. The doses of other agents were not changed, as they were not expected to significantly increase the risks of severe non-hematologic toxicities from this degree of mild renal impairment. If serum creatinine was ≥2 mg/dL, or increased from baseline by 1.3 mg/dL (condition C), the treatment needed to be postponed till condition B was achieved, and the dose of cytarabine was reduced by 50% when initiating treatment. If condition C was persistent at 4 weeks after previous treatment, the patient was removed from the study. If grade 4 non-hematologic toxicity or performance status of 4 without improvement during the treatment course was observed, the patient was removed from the study.

Statistical methods. The primary endpoint of this study was an ORR and the secondary endpoints were TTF, OS, successful stem cell mobilization (in patients planned for SCT, as described earlier), and toxicities. The study used a two-stage design, where 11 patients were recruited in the first stage and if eight or more responses were observed, an additional 21 patients were recruited for the second stage. The two-stage design was based on alpha = 0.05, power = 90%, undesirable response rate = 60%, and desirable response rate = 85%. Statistical analysis for patients' characteristics, response rates, and adverse events was descriptive. Analysis on response rate, TTF, and OS was carried out on an intent-to-treat basis, using the Kaplan–Meier method. The TTF was calculated from the time of registration to the time of disease progression, change of treatment, or disease- or treatment-related death. Those who eventually underwent SCT were censored for TTF at the initiation of conditioning treatment. OS duration was calculated from the time of registration to the time of death of any cause.

Results

Patient characteristics. Thirty-two eligible patients were enrolled between November 2002 and November 2006. All received at least one course of CHASER chemotherapy. Baseline patient and disease characteristics are shown in Table 1. The median age was 54 years (range 28–67 years), and most ($n = 30$, 94%) of the patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. Seventeen had indolent NHL (all follicular grade 1 or 2), and 15 had aggressive NHL (11 diffuse large B-cell lymphoma, two mantle cell lymphoma, one large cell transformation of marginal zone lymphoma, and one large cell transformation of follicular lymphoma grade 2). The majority of patients ($n = 22$, 69%) had stage III or IV disease.

All patients were previously treated with CHOP-based chemotherapy at the time of initial diagnoses, and the majority of patients ($n = 22$, 69%) entered the current study for the first salvage treatment. Twenty patients (63%) were previously treated with rituximab-containing therapy. Four patients (13%) previously received radiation therapy for local disease control. Overall, 10 patients (31%) had diseases refractory to last treatment, and the remaining 22 patients (69%) had relapsed diseases.

Toxicity. A total of 113 courses of CHASER were given, with a median of four courses (range 1–4 courses) per patient. All patients were assessable for adverse events associated with CHASER (Table 2). There was no treatment-related death or toxicity leading to discontinuation of the treatment. All experienced grade 4 neutropenia, but the duration of neutropenia (<500) was short (median 4 days per course, range 0–8). The median time to neutrophil nadir was 13 days (range 10–19 days). Twenty-five patients (78%) experienced neutropenic fever and all but one were managed successfully with a short course of broad-spectrum antibiotics. One patient experienced a prolonged febrile episode and required 10-day intravenous antibiotic treatment. The majority of patients ($n = 28$, 88%) required at least one platelet transfusion during the therapy, with a median of one transfusion per course (range 1–3 transfusions). The median time to platelet nadir was 14 days (range 10–19 days). Most common non-hematologic toxicity was gastrointestinal (nausea/vomiting, diarrhea, and elevated liver enzymes). Transient elevation of serum transaminases (grade 3) was observed in two patients. One patient experienced an episode of syncope (grade 3), which was most likely to be a vasovagal syncope and the association with the study drugs was unclear. There were no grade 4 or other grade 3 toxicities observed.

Three patients (9%) required delay in the treatment schedule due to slow recovery of the platelet count. One experienced a non-life-threatening but prolonged febrile neutropenia after the third cycle as described earlier, and doses of cyclophosphamide, cytarabine, and etoposide for the fourth course were reduced by

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Table 1. Characteristics of patients ($n = 32$) with relapsed or refractory B-cell non-Hodgkin's lymphoma who participated in this study

Characteristic	No.	%
Total	32	100
Median age in years (range)	54 (28–67)	
Male/female	17/15	53/47
Histology		
Indolent	17	53
Follicular grade 1/2	17	53
Aggressive	15	47
DLBCL	11	34
MCL	2	6
Large cell transformation of indolent lymphoma	2	6
ECOG performance status at entry		
0/1	30	94
2	2	6
Stage at entry		
1	5	16
2	5	16
3	5	16
4	17	53
LDH at entry		
Normal	18	56
High	14	44
No. of sites of extranodal involvement		
0	13	41
1	14	44
2 or more	5	16
IPI score at study entry		
1	12	38
2	10	31
3	10	31
No. of prior treatment regimens		
1	22	69
2	4	16
3	3	6
4 or more	3	9
Prior platinum-containing therapy	1	3
Prior rituximab-containing therapy	20	63
Prior radiation therapy	4	13
Prior radioimmunotherapy (^{90}Yttrium-ibritumomab)	1	3
Prior autologous stem cell transplant	2	6
Refractory to last chemotherapy	10	31
Relapsed disease		
Previous remission duration ≤1 year	8	25
Previous remission duration >1 year	14	44

DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma.

25%. One patient who experienced hyperglycemia, which was difficult to control with insulin after the third cycle, received only 12 mg of dexamethasone for the fourth cycle at the discretion of the responsible physician.

Response. The objective response of all 32 evaluable patients is summarized in Table 3. ORR was 84% (95% CI [67–95%]), including CR or CRu in 24 patients (75% [57–89%]), and partial response in three patients (9%). The ORR and CR rates in indolent lymphoma were 100% (84–100%; 17 of 17) and 94% (71–99%; 16 of 17), respectively, and those in aggressive lymphoma were 67% (38–88%; 10 of 15) and 53% (27–79%; 8 of 15), respectively. Response was observed both in patients who previously received rituximab (17 of 20, 85%) and those who did not (10 of 12, 83%) ($P = 0.37$). In patients with aggressive B-NHL with ($n = 8$) or without ($n = 7$) prior rituximab exposure, ORR was 63% and 71%, respectively ($P = 0.39$), and CR rate was 57% and 50%, respectively ($P = 0.38$). The CR rate was higher in patients with longer than 1 year of response duration after last treatment (93%, 13 of 14) than in patients with 1 year or shorter of response duration or refractory disease after last treatment (61%, 11 of 18) ($P = 0.047$). Other factors such as International Prognostic Index (IPI) score at study entry, response to the first treatment were not significantly associated with overall or complete response to CHASER (data not shown). Two patients who had previously undergone autologous SCT also experienced responses (CR and partial response, respectively). Three patients achieved only stable disease and proceeded to different salvage regimens after two, two and four cycles, respectively. Two patients had rapidly progressive disease after one and two cycles, respectively, and eventually received different salvage regimens.

Stem cell collection and SCT. Although not required to enter the study, all patients aged 65 years ($n = 30$) were offered at study entry an option of peripheral blood stem cell harvesting, to be carried out after the second (and third, if necessary) course of CHASER for future SCT. Out of 30 patients, stem cell collection was not attempted in eight patients: three patients with follicular

Table 2. Toxicity observed in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma during salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab ($n = 32$)

	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Neutropenia (%)	0 (0)	0 (0)	0 (0)	32 (100)
Thrombocytopenia (%)	0 (0)	0 (0)	4 (13)	28 (88)
Febrile neutropenia (%)	0 (0)	0 (0)	25 (78)	0 (0)
Gastrointestinal				
Nausea/vomiting (%)	9 (28)	4 (13)	0 (0)	0 (0)
Diarrhea (%)	6 (19)	1 (3)	0 (0)	0 (0)
Elevated liver enzymes (%)	14 (44)	4 (13)	2 (6)	0 (0)
Neurological				
Peripheral neuropathy (%)	2 (6)	0 (0)	0 (0)	0 (0)
Syncope (%)	0 (0)	0 (0)	1 (3)	0 (0)
Pain (%)	1 (3)	4 (13)	0 (0)	0 (0)
Edema (%)	4 (13)	0 (0)	0 (0)	0 (0)

Table 3. Responses observed in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma (B-NHL) after treatment with salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab

Type of B-NHL	Prior treatment	Total no.	CR or Cru	Overall response
Indolent B-NHL	All	$n = 17$	$n = 16$ 94% (71–99%)	$n = 17$ 100% (84–100%)
	Previous rituximab	$n = 12$	$n = 11$ 92% (62–99%)	$n = 12$ 100% (78–100%)
	Rituximab-naive	$n = 5$	$n = 5$ 100% (55–100%)	$n = 5$ 100% (55–100%)
Aggressive B-NHL	All	$n = 15$	$n = 8$ 53% (27–79%)	$n = 10$ 67% (38–88%)
	Previous rituximab	$n = 8$	$n = 4$ 50% (16–84%)	$n = 5$ 63% (24–91%)
	Rituximab-naive	$n = 7$	$n = 4$ 57% (18–90%)	$n = 5$ 71% (29–96%)
Total	All	$n = 32$	$n = 24$ 75% (57–89%)	$n = 27$ 84% (67–95%)
	Previous rituximab	$n = 20$	$n = 15$ 75% (51–91%)	$n = 17$ 85% (62–97%)
	Rituximab-naive	$n = 12$	$n = 9$ 75% (43–95%)	$n = 10$ 83% (52–98%)

Ranges in parentheses indicate 95% confidence interval. CR, complete response; CRu, complete response unconfirmed.

lymphoma declined this option; two patients had undergone autologous SCT prior to CHASER; and three patients had poor control of disease during CHASER (two progressive disease and one stable disease). As a result, stem cell collection was attempted in 22 patients. Three had insufficient mobilization of CD34 positive cells in peripheral blood; one of these patients had had three prior regimens including one cladribine-containing regimen. The remaining 19 patients successfully completed stem cell collection, with a median CD34 count of 4.0×10^6/kg body weight (range 1.9–23.4×10^6) by a median of two rounds of apheresis (range 1–3 rounds). All collected stem cell sources were free of malignant B cells, determined by flow cytometric analyses. In six patients with follicular lymphoma with MBR/JH rearrangement detected by seminested polymerase chain reaction (using primer sets LJH-P, TGAGGAGACGGTGACC and MBR-P, CCAAGTCATGTGCATTTCCACGTC for the first step, and VLJH-P, GTGACCAGGGTNCCTTGGCCCCAG and MBR-P for the second step). Negativity of tumor cell contamination in the stem cell sources was confirmed by the same method (data not shown). Two of 19 patients with aggressive NHL had suboptimal response (stable disease) on imaging studies after CHASER, thus proceeded to other salvage regimens. One patient who had adequate stem cell collection refused to undergo SCT. As a result, a total of 16 patients (50%) underwent autologous SCT as an immediate next treatment after CHASER treatment. One patient who had undergone autologous SCT prior to CHASER underwent allogeneic SCT as an immediate next treatment after CHASER.

TTF and OS. The Kaplan–Meier estimates of TTF and OS are shown in Fig. 1. The median TTF and OS durations for the entire group were 24.5 months and not reached, respectively. The median TTF in patients with indolent and aggressive lymphoma was 24.5 months and not reached, respectively. The median OS duration in patients with indolent and aggressive lymphoma was not reached and 39.3 months, respectively. Neither TTF nor OS duration was significantly different by IPI score at study entry, response duration after last chemotherapy (refractory or ≤1 year *vs* >1 year), previous rituximab exposure, or response to the first treatment (log–rank test, data not shown).

Discussion

Patients with relapsed or refractory NHL have limited options and poor prognosis. Even in patients who might be candidates for autologous SCT, it is critical to reduce the tumor size with

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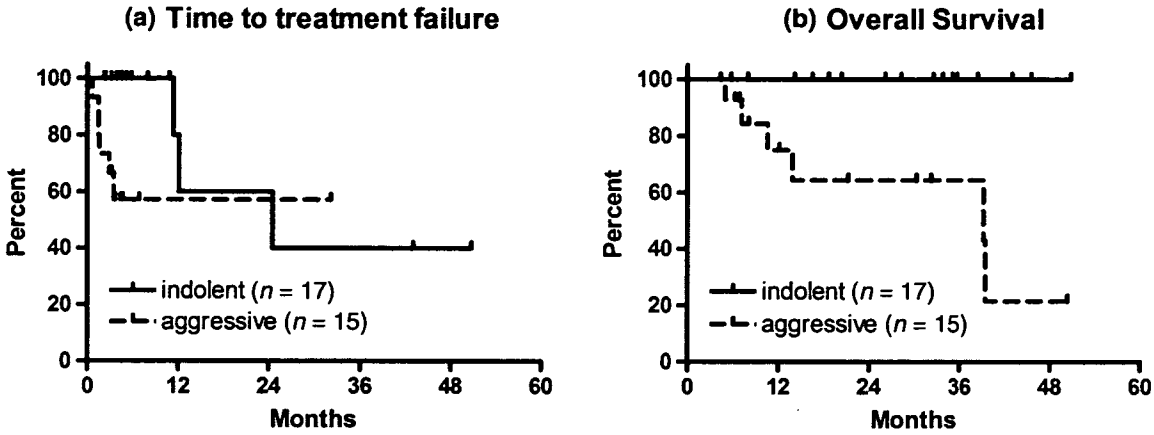

Fig. 1. Overall survival (OS) and time to treatment failure (TTF) in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma undergoing salvage chemoimmunotherapy incorporating cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab. Solid lines indicate survival curves of patients with indolent lymphoma ($n = 17$). Dashed lines indicate those of patients with aggressive lymphoma ($n = 15$). (a) The median TTF in patients with indolent and aggressive lymphoma was 24.5 months and not reached, respectively. Those who had stem cell transplant were censored for TTF at the initiation of conditioning regimen. (b) The median OS duration in patients with indolent and aggressive lymphoma was not reached and 39.3 months, respectively.

Table 4. Comparison of CHASER, R-DHAP, R-ESHAP and R-ICE in relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma (doses are per course)

	CHASER	R-DHAP[9]	R-ESHAP[7]	R-ICE[6]
Rituximab	375 mg/m² × 1	375 mg/m² × 1	375 mg/m² weekly × 8	375 mg/m² × 1
Cytarabine	2 g/m² × 2	2 g/m² × 2	2 g/m² × 1	–
Etoposide	100 mg/m² × 3	–	40 mg/m² × 4	100 mg/m² × 3
Steroid	Dexamethasone 40 mg × 3	Dexamethasone 40 mg × 4	Methylprednisolone 500 mg × 5	–
Platinum agent	–	Cisplatin 25 mg/m² × 4	Cisplatin 25 mg/m² × 4	Carboplatin AUC 5 × 1
Non-platinum alkylator	Cyclophosphamide 1200 mg/m² × 1	–	–	Ifosfamide 5 g/m² × 1
No. of patients	15	53	26	36
Prior rituximab exposure (%)	53	4	19†	0
CR rate % (95% CI)	53 (27–79)	32 (20–46)	46 (27–65)	53 (36–69)
OR rate % (95% CI)	67 (38–88)	62 (48–75)	92 (82–100)	78 (61–90)

†L. Hicks *et al.*, 2007, personal communication; –, not included in treatment; AUC, area under the curve; CI, confidence interval; CR, complete response; OR, overall survival.

an effective salvage regimen prior to SCT. For those who are not candidates for transplant, a treatment regimen to induce a durable response is the sole key for long-term survival. The present study showed the significant activity of the new combination salvage regimen CHASER in patients with relapsed or refractory B-NHL who may or may not have undergone prior rituximab-containing treatment such as R-CHOP.

Although rituximab has been studied in salvage settings as an additional drug to commonly used combination chemotherapy, such as ESHAP[7] DHAP[9,10], and ICE (ifosfamide, carboplatin and etoposide)[6,10], currently available data are from studies recruiting mostly rituximab-naive patients (Table 4). Therefore, it remains to be shown whether R-ICE (rituximab with ICE) or R-DHAP is still as effective in patients who were previously treated with a rituximab-containing regimen.[10] It is noteworthy in our study that CHASER produced high CR rates in relapsed or refractory B-NHL after rituximab-containing chemotherapy, and that the activity seems comparable to those of other platinum-containing regimens in patients with aggressive B-NHL (Table 4). Randomized trials would be needed to further compare the efficacy of CHASER with other regimens. Also, careful long-term follow-up is needed to assess the potential late effect of rituximab, such as delayed neutropenia as has recently been recognized.[11–14]

Both CHASER and R-ESHAP contain high-dose cytarabine, etoposide, steroid, and rituximab in common. In the original study of ESHAP, Velasquez *et al.* initially compared ESHA with ESHAP[3], revealing that the addition of cisplatin significantly improved the response rate (33% *vs* 75% at initial phase of the study, but the response rate of ESHAP at the end of the study was 64%), despite only moderate activity of single agent cisplatin against NHL (response rate 26%[15]). Further addition of rituximab to ESHAP seems even more active, and in a phase II study of R-ESHAP in patients with aggressive B-NHL ($n = 26$, 21 were rituximab-naive), a response rate of 92% (95% [CI 84–100%]) including a CR rate of 46% (95% [27–65%]) was observed. CHASER contains 1200 mg/m² of cyclophosphamide instead of cisplatin, producing comparable response rates to R-ESHAP. Virtually all patients with relapsed or refractory B-NHL were exposed to cyclophosphamide at 750 mg/m² as a part of CHOP therapy, however, a higher dose of cyclophosphamide seems to play a significant role in overcoming resistance in this setting. Furthermore, one major benefit of using cyclophosphamide instead of cisplatin is absence of renal toxicity.

One important aspect of salvage regimens for relapsed or refractory NHL is their stem cell mobilizing effect. In our study, 19 of 22 attempts at stem cell collection were successful, but it should be noted that one of three who experienced poor stem cell mobilization had been heavily pretreated. Furthermore, addition of rituximab to the CHASE regimen might add an *in vivo* purging effect and allow tumor-free stem cell collection. Further studies are necessary to determine whether *in vivo* purged autologous SCT will improve outcomes compared to non-purged SCT.

In conclusion, CHASER showed favorable tolerability, significant antitumor activity, and stem cell mobilizing effects in patients with relapsed or refractory B-NHL with or without prior rituximab-containing treatment such as R-CHOP. This promising result warrants the further investigation of CHASER in large-scale multicenter trials and comparison to other salvage regimens.

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References

1 Ogura M, Kagami Y, Taji H *et al.* Pilot phase I/II study of new salvage therapy (CHASE) for refractory or relapsed malignant lymphoma. *Int J Hematol* 2003; **77**: 503–11.
2 Velasquez WS, Cabanillas F, Salvador P *et al.* Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). *Blood* 1988; **71**: 117–22.
3 Velasquez WS, McLaughlin P, Tucker S *et al.* ESHAP – an effective chemotherapy regimen in refractory and relapsing lymphoma: a 4-year follow-up study. *J Clin Oncol* 1994; **12**: 1169–76.
4 Coiffier B, Lepage E, Briere J *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 235–42.
5 Pfreundschuh M, Trumper L, Osterborg A *et al.* CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol* 2006; **7**: 379–91.
6 Kewalramani T, Zelenetz AD, Nimer SD *et al.* Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. *Blood* 2004; **103**: 3684–8.
7 Hicks L, Buckstein R, Mangel J *et al.* Rituximab increases response to ESHAP in relapsed, refractory, and transformed aggressive B-cell lymphoma (Abstract). *Blood* 2006; **108**: 3067.
8 Cheson BD, Horning SJ, Coiffier B *et al.* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol* 1999; **17**: 1244.
9 Mey UJ, Olivieri A, Orlopp KS *et al.* DHAP in combination with rituximab vs DHAP alone as salvage treatment for patients with relapsed or refractory diffuse large B-cell lymphoma: a matched-pair analysis. *Leuk Lymphoma* 2006; **47**: 2558–66.
10 Hagberg H, Gisselbrecht C: CORAL Study Group. Randomised phase III study of R-ICE versus R-DHAP in relapsed patients with CD20 diffuse large B-cell lymphoma (DLBCL) followed by high-dose therapy and a second randomisation to maintenance treatment with rituximab or not: an update of the CORAL study. *Ann Oncol* 2006; **17** (Suppl 4): iv31–2.
11 Chaiwatanatorn K, Lee N, Grigg A, Filshie R, Firkin F. Delayed-onset neutropenia associated with rituximab therapy. *Br J Haematol* 2003; **121**: 913–8.
12 Lemieux B, Tartas S, Traulle C *et al.* Rituximab-related late-onset neutropenia after autologous stem cell transplantation for aggressive non-Hodgkin's lymphoma. *Bone Marrow Transplant* 2004; **33**: 921–3.
13 Nitta E, Izutsu K, Sato T *et al.* A high incidence of late-onset neutropenia following rituximab-containing chemotherapy as a primary treatment of CD20-positive B-cell lymphoma: a single-institution study. *Ann Oncol* 2007; **18**: 364–9.
14 Voog E, Morschhauser F, Solal-Celigny P. Neutropenia in patients treated with rituximab. *N Engl J Med* 2003; **348**: 2691–4.
15 Cavalli F, Jungi WF, Nissen NI, Pajak TF, Coleman M, Holland JF. Phase II trial of cis-dichlorodiammineplatinum (II) in advanced malignant lymphoma: a study of the cancer and acute leukemia group B. *Cancer* 1981; **48**: 1927–30.

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Age-Related EBV-Associated B-Cell Lymphoproliferative Disorders Constitute a Distinct Clinicopathologic Group: A Study of 96 Patients

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Abstract **Purpose:** We have recently reported EBV+ B-cell lymphoproliferative disorders (LPD) occurring predominantly in elderly patients, which shared features of EBV+ B-cell neoplasms arising in the immunologically deteriorated patients despite no predisposing immunodeficiency and were named as senile or age-related EBV+ B-cell LPDs. To further characterize this disease, age-related EBV+ B-cell LPDs were compared with EBV-negative diffuse large B-cell lymphomas (DLBCL).

Experimental Design: Among 1,792 large B-cell LPD cases, 96 EBV+ cases with available clinical data set were enrolled for the present study. For the control group, 107 patients aged over 40 years with EBV-negative DLBCL were selected. We compared clinicopathologic data between two groups and determined prognostic factors by univariate and multivariate analysis.

Results: Patients with age-related EBV+ B-cell LPDs showed a higher age distribution and aggressive clinical features or parameters than EBV-negative DLBCLs: 44% with performance status >1, 58% with serum lactate dehydrogenase level higher than normal, 49% with B symptoms, and higher involvement of skin and lung. Overall survival was thus significantly inferior in age-related EBV+ group than in DLBCLs. Univariate and multivariate analyses further identified two factors, B symptoms and age older than 70 years, independently predictive for survival. A prognostic model using these two variables well defined three risk groups: low risk (no adverse factors), intermediate risk (one factor), and high risk (two factors).

Conclusions: These findings suggest that age-related EBV+ B-cell LPDs constitute a distinct group, and innovative therapeutic strategies such as EBV-targeted T-cell therapy should be developed for this uncommon disease.

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Diffuse large B-cell lymphoma (DLBCL) is the largest category of aggressive lymphomas and regarded as a heterogeneous group of lymphomas in terms of clinicopathologic profiles and biological properties (1). Recent advance in the lymphoma research shed the light on the distinct subgroups such as *de novo* CD5+ DLBCL (2), intravascular large B-cell lymphoma (Asian variant; ref. 3), primary effusion lymphoma (4), and pyothorax-associated lymphoma (5) under the nosologic term of DLBCL. In addition, we have recently identified a series of elderly patients of EBV+ B-cell lymphoproliferative disorders (LPD) and/or large-cell lymphomas without predisposing immunodeficiencies and named those senile or age-related EBV+ B-LPDs (6).

EBV is a ubiquitous γ-herpesvirus that infects more than 90% of worldwide adult population (7, 8). In contrast to its high prevalence, EBV is also well recognized as an apparent oncogenic agent (9). It transforms B cells into lymphoblastoid cell lines *in vitro*, and many human cancers, including Burkitt lymphoma (BL) (10), Hodgkin lymphoma (7), immunodeficiency-associated LPDs (11), and a part of diffuse large B-cell lymphoma (12), have close relation with EBV. Although the precise mechanism is not fully clarified, it is widely accepted that the T cell plays a crucial role for the suppression of EBV-associated oncogenesis (7). In fact, the use of strong

immunosuppressive agents in organ transplantation settings such as tacrolimus or cyclosporin A, or HIV infection, sometimes causes EBV-positive B-cell LPDs (13, 14). Four clinical settings of immunodeficiency associated with an increased incidence of lymphoma and other LPDs are recognized by the WHO classification: (*a*) primary immunodeficiency syndromes and other primary immune disorders; (*b*) infection by the HIV; (*c*) iatrogenic immunosuppression in patients who have received solid organ or bone marrow allografts; and (*d*) iatrogenic immunosuppression associated with methotrexate treatment, most commonly in patients with autoimmune disease (15).

We have highlighted the over-profile of senile EBV+ B-cell LPDs appearing analogous in many respects to that of immunodeficiency-associated B-cell LPDs, which were exemplified by a marked propensity to involve extranodal sites and a morphologic spectrum ranging from the precursor polymorphous proliferation of lymphoid cells to diverse types of lymphomas, although no evidence of underlying immunodeficiency was found (6). Therefore, it is speculated that this disease is related to an immunologic deterioration derived from the aging process, i.e., senescence in immunity. However, the detailed clinicopathlogic features and follow-up information of age-related EBV+ B-cell LPDs remain limited because of an inclusion of a small number of patients and the lack of the comparison with EBV-negative DLBCL. To address these issues further, we retrospectively assessed the clinicopathologic features of 96 cases with age-related EBV+ B-cell LPDs as a collaborative study.

Materials and Methods

Diagnosis. The diagnosis of age-related EBV-associated B-cell LPDs was made when more than 50% of the proliferating, often neoplastic-appearing cells showed both of the expression of one or more pan-B-cell antigens (CD20/CD79a) and/or light-chain restriction and positive signal for *in situ* hybridization using EBV-encoded small nuclear early region (EBER) oligonucleotides on paraffin section (Fig. 1) for patients more than 40 years of age without predisposing immunodeficiency such as HIV infection or past history of immunosuppressive agents (6) The cases <40 years old were excluded because we could not deny the possibility that they may be associated with any primary immune disorder or chronic active EBV infection (16, 17). In addition, pyothorax-associated lymphoma and EBV-associated lymphomas of T- or natural killer-cell phenotype were excluded from the present series because they were considered to constitute distinct clinicopathologic groups (5, 18). In particular, attention was given to the differential diagnoses of peripheral T-cell lymphoma with Reed-Sternberg-like cells of B-cell or angioimmunoblastic T-cell lymphoma with proliferation of large B cells (19). Only well-documented cases that had paraffin sections available for immunohistochemistry were included in this study. Each case was reviewed by five pathologists (authors K.T., K.O., N.N. T.Y., and S.N.) to confirm the diagnosis and immunophenotype. Among 149 cases fulfilling these criteria (Supplementary Table S1), 96 cases with available clinical data set were enrolled for the present study, including the 22 cases of senile EBV+ B-cell LPDs previously reported by us (6). For the control group, 107 patients aged over 40 years with EBV-negative DLBCL were selected from malignant lymphoma cases treated consecutively at Aichi Cancer Center between 1993 and 2000. This study was done by following the Ethical Guidelines for Clinical Studies and the Ethical Guideline for Epidemiological Research in Japan. The Institutional Review Board of the Aichi Cancer Center and the other institutes involved approved this study.

Histopathology. Tissue samples were fixed in 10% formalin and embedded in paraffin. Sections (5 μm thick) were stained with H&E, Elastica-van Gieson, silver impregnation, periodic acid-Schiff, May-Gruenwald-Giemsa, and methyl green-pyronine staining.

Immunohistochemistry. Immunoperoxidase studies were done on formalin-fixed paraffin sections with the avidin-biotin peroxidase complex method. A panel of monoclonal antibodies against human immunoglobulin light and heavy chains, CD3, CD8, UCHL-1/CD45RO, L26/CD20, Ber-H2/CD30, CD79a, latent membrane protein-1 (LMP-1), EBV-encoded nuclear antigen-2 (EBNA2; DAKO); CD2, CD4, CD5, CD56 (Novocastra Laboratories); LeuM1/CD15, Leu7/CD57

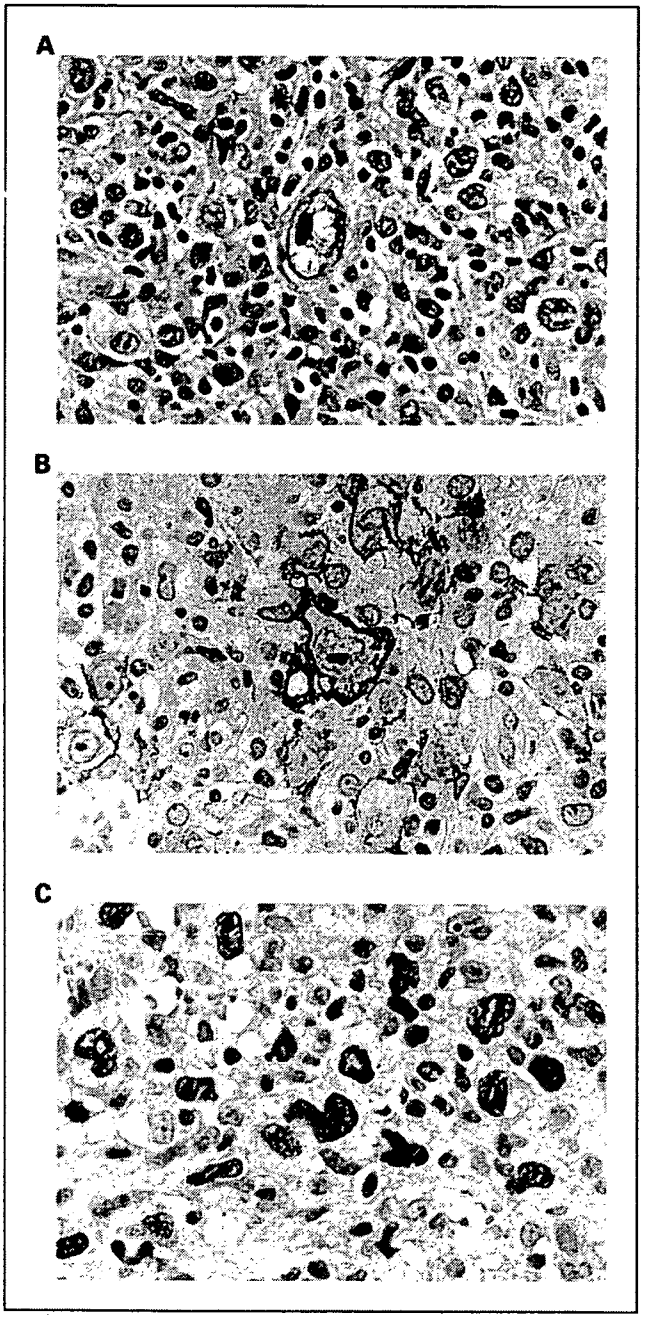

Fig. 1. Senile EBV-associated B-cell LPD, polymorphic subtype, arising in a 62-year-old male. The lesion reveals scattered distribution of Hodgkin and Reed-Sternberg–like giant cells (*A*, ×150), which are positive for CD20 (*B*, ×125). These large cells showed the expression of EBNA2 (*C*, ×125) in addition to the positive signals for EBV-encoded small RNAs (EBERs) *in situ* hybridization, indicating latency III status.

Table 1. Patient characteristics at diagnosis of age-related EBV-positive B-LPDs and EBV-negative DLBCL

Variable	Age-related EBV-positive LPD (n = 96)	EBV-negative DLBCL (n = 107)	P
Sex (male/female)	56/40 (1.4)	54/53 (1.02)	0.26
Age, median (range), y	71 (45-92)	62 (41-85)	<0.0001
	Number of cases (%)	**Number of cases (%)**	
Older than 60	79 (82%)	56 (52%)	<0.0001
ECOG PS 2-4	36 (44%)	18 (17%)	<0.0001
B-symptoms, presence	38 (49%)	18 (20%)	<0.0001
LDH level high	47 (58%)	46 (43%)	0.041
Ann Arbor stage III/IV	48 (58%)	49 (46%)	0.10
Extranodal involvement (>1 site)	28 (33%)	30 (28%)	0.43
Extranodal sites	n = 93	n = 107	
Skin	12 (13%)	5 (5%)	0.037
Lung	8 (9%)	3 (3%)	0.073
Pleural effusion	8 (9%)	5 (5%)	0.26
Stomach	8 (9%)	14 (13%)	0.31
Tonsil	7 (8%)	20 (19%)	0.021
Breast	0 (0%)	7 (7%)	0.012
IPI, High intermediate/high	43 (54%)	39 (37%)	0.017
Anti-EBV antibody titer category,*	18 (67%)	23 (24%)	<0.0001
Treatment			<0.0001
None or radiation only	9 (12%)	1 (1%)	
Ctx without anthracycline	7 (9%)	2 (2%)	
Ctx with anthracycline	62 (79%)	104 (97%)	
Response, in cases underwent Ctx with anthracycline			<0.0001
CR	37 (66%)	93 (91%)	
PR	8 (14%)	8 (8%)	
SD or PD	11 (20%)	1 (1%)	

Abbreviations: PS, performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; Ctx, chemotherapy; CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Cases were determined as having abnormal serum anti-EBV antibody titer if anti-EBV viral capsid antigen antibody was 640-fold or higher, or anti-EBV nuclear antigen antibody was negative.

(Becton Dickinson); TIA-1 (Coulter Immunology); and granzyme B (Monosan) were used. All antibodies were applied after antigen retrieval following microwave oven heating treatment.

In situ *hybridization study.* The presence or absence of EBV small RNAs was assessed by means of *in situ* hybridization using EBER oligonucleotides and done on formalin-fixed paraffin embedded sections. Briefly, a DAKO hybridization kit was used with a cocktail of FITC-labeled EBER oligonucleotides (one oligonucleotide corresponding to EBER1 and one to EBER2, both 30 bases long; DAKO A/S code Y 017). Hybridization products were detected with mouse monoclonal anti-FITC (DAKO M878) and a Vectastain ABC Kit (Vector). RNase A or DNase I pretreatment was used for the negative controls and EBER-positive Hodgkin's disease specimens for positive controls.

Statistical analysis. Variables related to patients, treatment, and disease were compared among the two groups with the use of the χ^2 test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The probability of survival was calculated with the use of the Kaplan-Meier estimator, and the log-rank test was used for comparisons. Univariate and multivariate analyses were done with the Cox proportional hazard regression model. All P values are two sided, with a type I error rate fixed at 0.05. Statistical analyses were done with the STATA version 9.

Results

Case selection. From the files of six collaborating institutions, during the period from January 1990 to December 2004, the positive signals for B-cell [pan-B-cell antigens (CD20/CD79a) and/or light-chain restriction] and EBV were detected on more than 50% of cells in 243 (14%) of 1,792 large B-cell LPD cases, mainly consisting of DLBCL, by EBERs *in situ* hybridization. They contained HIV-associated lymphomas (n = 17), autoimmune disease-associated LPDs (n = 10), secondary lymphoma with prior chemotherapy (n = 7), post-transplant LPDs (n = 10), pyothorax-associated lymphoma (n = 30), BL (n = 13), and cases without any documentation for predisposing immunodeficiency (n = 156; Supplementary Table S1). EBV was detected in 10% of HIV-negative patients with BL in this study, which was comparable to the reported frequency in nonendemic BL (20). A bimodal age distribution with an incidence peak in the 10- to 19-year range and a second peak in older adult aged 70 to 79 was evident for EBV-positive B-cell LPD patients without predisposing immunodeficiency (Supplementary Fig. S1*A*). The positive percentages of this group for all cases examined became higher in parallel with the elder patient populations (≥40 years), showing the highest peak at ages >90 years (Supplementary Fig. S1*B*).

Patient characteristics for age-related EBV-positive B-cell LPDs and EBV-negative DLBCL. In comparison with EBV-negative DLBCL, patients with age-related EBV-positive B-cell LPDs showed higher age distribution (median, 71 versus 62 years: $P < 0.0001$) and a closer association with aggressive clinical features or parameters: 79 patients older than 60 (82%,

$P < 0.0001$), 36 with performance status (PS) >1 (44%, $P < 0.0001$), 47 with serum lactate dehydrogenase (LDH) level higher than normal (58%, $P = 0.041$), 48 with stage III/IV disease at diagnosis (58%, $P = 0.10$), and 38 with B symptoms (49%, $P < 0.0001$; Table 1). As a result, the International Prognostic Index (IPI) score for patients with age-related EBV-positive B-cell LPDs was significantly higher than that for patients with EBV-negative DLBCL ($P = 0.0017$), with 43 (54%) of the EBV-positive group categorized in the IPI high or high intermediate-risk group. There was no statistical difference between two groups in the incidence of having more than one extranodal site.

At diagnosis, 67% of the cases with age-related EBV-positive B-cell LPDs showed abnormal anti-EBV antibody titer, which was defined if anti-EBV VCA immunoglobulin G (IgG) antibody was 640-fold or higher, or anti-EBNA antibody was negative, as compared with only 24% of cases with DLBCL that showed abnormality ($P < 0.0001$).

Sites of extranodal involvement. In 17 patients (20%) of the current EBV-positive series, the disease was limited to extranodal sites. Twenty-seven patients (31%) had only lymphadenopathies without extranodal involvement, and the remaining 43 (49%) had lymphadenopathies with extranodal involvement. The total incidence of extranodal involvement was similar between age-related EBV-positive B-cell LPDs and EBV-negative DLBCL (69% and 72%, respectively).

The main sites of extranodal involvement in age-related EBV-positive B-cell LPDs was skin ($n = 12$; 13%), lung ($n = 8$; 9%), pleural effusion ($n = 8$; 9%), stomach ($n = 8$; 9%), and tonsil ($n = 7$; 8%) in an order of the incidence (Table 1). A comparison with EBV-positive and EBV-negative groups showed that the incidence of cutaneous involvement was significantly higher in age-related EBV-positive B-cell LPDs than those of EBV-negative DLBCLs ($P = 0.027$, respectively). There is a tendency of difference in lung involvement, but no statistical significance (9% versus 3%, $P = 0.073$). Involvement of breast and tonsil occurred less frequently in age-related EBV-positive B-cell LPDs than in EBV-negative DLBCL ($P = 0.012$ and.021, respectively). There were no significant differences between these two groups in the incidence of involvement in the other extranodal sites (Supplementary Table S2).

Histologic features. Age-related EBV-positive LPDs generally showed a diffuse and polymorphic proliferation of large lymphoid cells with a varing degree of reactive components such as small lymphocytes, plasma cells, histocytes, and epithelioid cells and were sometimes accompanied by necrosis and an angiocentric pattern. These tumor cells were often featured by a broad range of B-cell maturation, containing morphologic centroblasts, immunoblasts, and Hodgkin and Reed-Sternberg (HRS)-like giant cells with distinct nucleoli (Fig. 1*A*). According to the previous report (6), the present series were morphologically divided into two subtypes: large-cell lymphoma (LCL) and polymorphic LPD subtypes. The former ($n = 34$) was characterized by having areas where large lymphoid cells with relatively monomorphic appearance were notably dominant. The remaining 62 cases were simply categorized as polymorphous subtype with the scattered distribution of large cells in the polymorphous composition. The histology was frequently varied from area to area, indicating a continuous spectrum between these two subgroups because several LCL cases had areas of polymorphic LPD in the same or other tissues. In contrast to morphologic divergence, there was no significant difference in any clinical characteristics and immunophenotype between these two groups (Supplementary Table S3).

We detected clonal B-cell population in 10 cases out of 12 cases tested: eight cases by PCR analysis, one case by Southern blot analysis, and one by lambda light-chain restriction. Polyclonal pattern was observed in one case, and no band was detected in the other. As to polymorphic LPDs, the presence of clonal B-cell population was identified in five cases out of seven samples.

Phenotypic features. According to the definition adopted for this study, all patients with age-related EBV-positive B-cell LPDs were positive for EBV and B-cell markers (CD20 and/or CD79a; Fig. 1*B*). Immunohistologic studies for the EBV-latent gene products on paraffin sections showed that LMP1 was positive on the large atypical cells in 67 (94%) out of 71 tested cases. EBNA2 was also detected in the nuclei of 16 (28%) of 57 tested cases (Fig. 1*C*, Supplementary Table S4), indicating latency type III. CD30 was stained more common in age-related EBV-positive B-cell LPDs than in EBV-negative DLBCL (75% versus 13%, $P < 0.0001$). In addition, a comparison of adjacent sections often disclosed an overlapping staining pattern of LMP1 and CD30. There was also a statistically significant difference in the incidence of CD10 expression (18% and 38%, respectively. $P = 0.015$), but not others (CD19, CD20, or CD79a) between age-related EBV-positive B-cell LPDs and DLBCLs (Supplementary Table S4).

Response to treatment and Kaplan-Meier survival estimates. Treatment of age-related EBV-positive B-cell LPDs consisted of chemotherapeutic regimens containing anthracycline for 62 patients (79%) and without anthracycline for 7 patients (9%; Table 1). A total of 40 (63%) of 64 evaluable patients with age-related EBV-positive B-cell LPDs achieved a complete remission (CR) with initial therapy, and the rest of the 24 cases (38%) failed to have a CR with initial chemotherapy. On the other hand, 95 (91%) of 104 evaluable cases with DLBCL achieved a CR, and only 9 cases (9%) were refractory (PR, SD, or PD) to initial chemotherapy ($P < 0.0001$). This difference, in response to treatment, was still in a significant level when compared in cases who received chemotherapy with anthracycline ($P < 0.0001$, Table 1).

In this study, we observed 57 deaths in 96 cases of age-related EBV-positive B-cell LPDs and 34 deaths in 107 cases of DLBCL. The data on the causes of death were available for 47 cases for age-related EBV-positive B-cell LPDs and 29 for DLBCL. Deaths due to disease progression and complications such as infections were observed in 38 and 9 cases, respectively, in age-related EBV-positive B-cell LPDs, whereas 23 and 6 cases in EBV-DLBCL. The observed differences between two disease groups were not significant ($P = 0.870$). As to the cases of more than 70 years of age, 24 and 5 cases were dead due to disease progression, and seven and one were from complications in age-related EBV-positive B-cell LPDs and in DLBCL, respectively. Even in cases more than 70 years old, the observed differences were not significant ($P = 0.747$).

Unadjusted overall survival curves of both groups were shown in Fig. 2*A*. Age-related EBV-positive B-cell LPDs showed strikingly inferior survival to DLBCLs (median survival time, 24 months versus not reached, respectively;

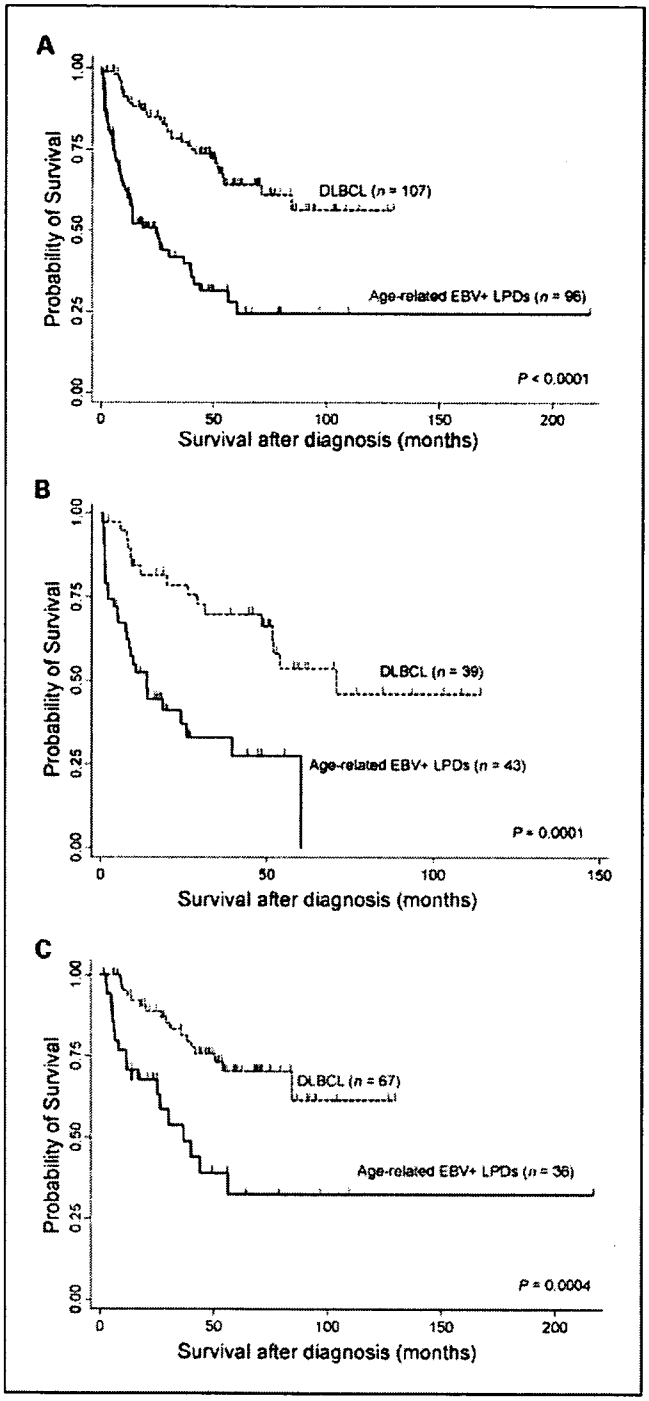

Fig. 2. Overall survival for patients with age-related EBV+ B-cell LPDs and EBV-negative DLBCLs. Age-related EBV+ B-cell LPDs (n = 96) show significantly worse survival than DLBCLs (n = 107) in all patients (*A*), patients with high-intermediate and high IPI risk (n = 43 and n = 39, respectively; *B*), and patients with low and low-intermediate IPI risk group (n = 36 and n = 67, respectively; *C*).

$P < 0.0001$). A significant difference was still found even when accounting for age (age ≤ 60, 60 < age ≤ 75, or age > 75) by performing the stratified log-rank test ($P < 0.0001$). Overall survival curves according to IPI are shown in Fig. 2*B* and *C*. Survival for age-related EBV-positive B-cell LPDs was significantly inferior to that for DLBCLs in both IPI subgroups. In this series, the IPI failed to separate age-related EBV+ B-cell LPD patients into groups with significantly different survivals (P = 0.1; Fig. 3*A*).

Univariate and multivariate analysis for survival. Among a total of 203 patients with EBER-positive (age-related EBV-positive B-cell LPDs) and EBV-negative diseases (DLBCLs), univariate Cox analysis identified the following as prognostic factors: age > 60 years, clinical stage, PS, extranodal involvement of more than one site, LDH, IPI, B symptoms, and EBV association (Table 2). Multivariate analysis, including five IPI factors, B symptoms, and EBV association, showed high LDH level, the presence of B-symptoms, and EBV association to be significant factors (Table 2). When multivariate analysis was done for EBV association and IPI categories, both of them were recognized as independent significant prognostic factors (Table 2).

Among patients with age-related EBV+ B-cell LPDs, the clinical parameters associated with reduced survival in univariate analysis are listed in Table 3: age older than 70

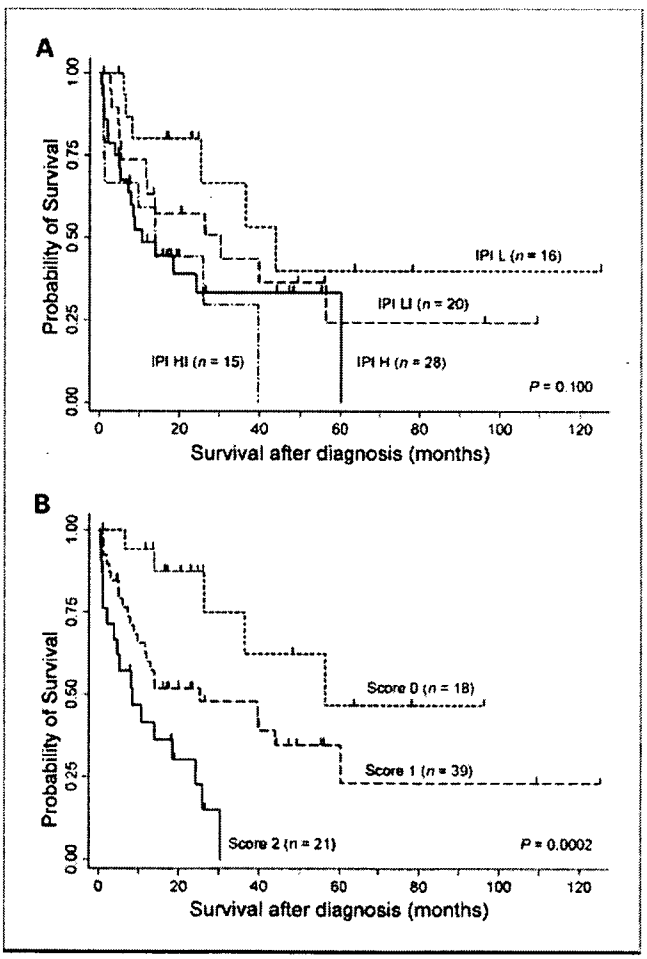

Fig. 3. Overall survival according to IPI (*A*) and prognostic model based on two simple clinical variables of age older than 70 y and the presence of B symptoms (*B*) in age-related EBV+ B-cell LPDs. This prognostic model is able to efficiently identify three groups of patients with different outcomes; patients with a score of 0 (*Score 0*, n = 18), no adverse factors; patients with a score of 1 (*Score 1*, n = 39), one factor; and patients with a score of 2 (*Score 2*, n = 21), two factors. Their median survival times were 56.3, 25.2, and 8.5 mo, respectively.