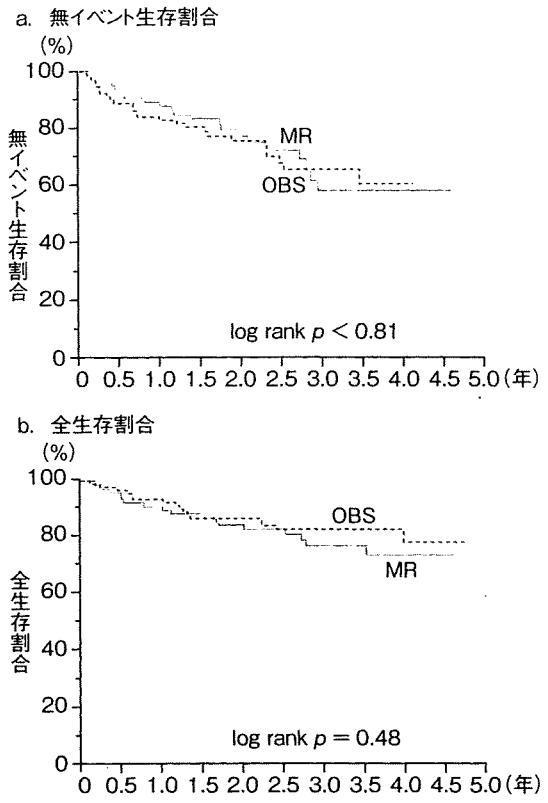


図2　R-CHOP 療法後の rituximab による維持療法の有無における生存曲線

OBS：observation，MR：maintenance rituximab
（文献4より引用）

それ以外の状況（CHOP 療法後に維持療法を併用した場合の OS，R-CHOP 療法後に維持療法を行った場合の FFS と OS）では生存の改善は認められなかった（図2）．これらの比較試験の結果から，CD20 陽性 DLBCL に対する標準的治療は R-CHOP 療法による初回治療であり，rituximab による維持療法は不要とされている．

3）CD20 陽性 DLBCL 以外の進行期中悪性度リンパ腫に対する標準的治療

DLBCL は，中悪性度リンパ腫の7～8割を占めている．その DLBCL の9割以上は CD20 を発現しており，進行期中悪性度リンパ腫において大規模な比較試験から得られたエビデンスのほとんどは CD20 陽性 DLBCL に対するものとオーバーラップしていることとなり，それ以外の病型に対してはレベルの高いエビデンスが存在しないのが現状である．

CD20 陰性の中悪性度リンパ腫の主な組織型には，peripheral T-cell lymphoma, not otherwise specified（PTCL-NOS，末梢性 T 細胞リンパ腫・非特異型），angioimmunoblastic T-cell lymphoma（AITL，免疫芽球性 T 細胞リンパ腫），anaplastic large cell lymphoma（ALCL，異型大細胞リンパ腫）といった T 細胞リンパ腫が含まれている．一部の例外を除いて，一般的に T 細胞リンパ腫は B 細胞リンパ腫よりも予後不良とされている．

国際的に行なわれた PTCL に関する後方視的な解析でも，T 細胞リンパ腫の約45％を占める PTCL-NOS と AITL で，5年 OS がそれぞれ32％であり，全体で見ても，たとえ IPI の low risk 群であっても DLBCL と比べると予後不良であった[5]．T 細胞リンパ腫の12％程度を占める ALCL は，anaplastic lymphoma kinase（ALK）蛋白の過剰発現の有無により ALK 陽性と ALK 陰性の2つに分けられる．この分類は病理組織学的な分類だけでなく，好発年齢や予後といった臨床的な分類の意味ももっている．

先述の T 細胞リンパ腫に関する国際的な解析では，ALK 陽性例と陰性例では，年齢中央値が34歳 vs 58歳，5年 OS が70％ vs 49％と，陰性例のほうが高齢者に多く予後不良であることも示されている[6]．CHOP 療法が進行期中悪性度リンパ腫の標準的治療であると結論づけた比較試験の結果より，これらの対象に対しても CHOP 療法が行なわれることが多い．しかし，この解析では PTCL-NOS と AITL に対する治療に key drug であるアントラサイクリン系の薬剤を用いても生存に寄与しないことも報告されている（図3）．その一方で，CHOP 療法よりも強力な多剤併用療法を行なってもこれらの病型の予後は改善しないという後方視的な報告もある[7]（図4）．結局，CD20 陽性 DLBCL 以外の進行期中悪性度リンパ腫に対する標準的治療は，CHOP 療法以上に有用であると証明された治療はないというのが現状である．なお，中悪性度リンパ腫の予後予測モデルには通常は IPI が用いられているが，PTCL に特化した予後予測モデル（Prognostic Index for PTCL：PIT）がイタリアのグループより提唱さ

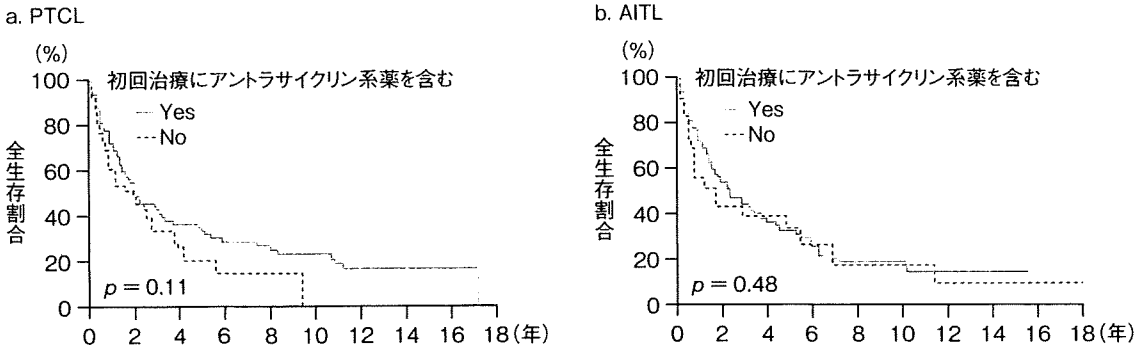


図3 アントラサイクリン系薬の有無による生存曲線

(文献6より引用)

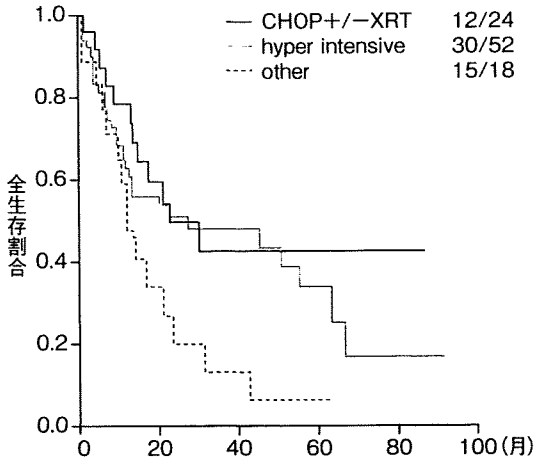


図4 MD Anderson Cancer Center で行われたT細胞リンパ腫に対するCHOP療法または強力な治療による生存曲線

(文献7より引用)

れている.

年齢, performance status, LDH高値, 骨髄浸潤の有無の4つの因子で4群に分けると, 5年と10年のOSがそれぞれgroup 1(予後不良因子:0)で62.3%と54.9%, group 2(予後不良因子予後不良因子:1)で52.9%と38.8%, group 3(予後不良因子:2)で32.9%と18.0%, group 4(予後不良因子:3~4)で18.3%と12.6%と層別化することができる[8](図5). 現在, この予後予測モデルを用いて治療が層別化されることはないが, 前向きな臨床試験による治療開発への応用が期待される.

b 試験的治療

1) 地固め療法としての自家造血幹細胞移植併用の大量化学療法

CHOP療法は標準的な化学療法レジメンであるが, IPIのhigh intermediate~high risk例や大多数のPTCLに対しては3割程度しか長期予後が期待できない. そのため, 予後不良と考えられる中悪性度リンパ腫に対しては, 可能であるならば治癒を目指した強力な治療を行なうべきという考え方がある. その代表的な治療法が, 自家造血幹細胞移植を併用した大量化学療法である. これまでに初回治療としての通常量の化学療法と大量化学療法の比較試験がいくつか報告されている.

GELAで行なわれた, ACVBP療法で奏効が得られた後に通常量の化学療法による地固め療法または自家造血幹細胞移植併用の大量化学療法を行なうという比較試験で, IPI全リスクを対象とした比較では両群に差はないもののhigh intermediate~high risk例では5年EFSで59% vs 39%, 5年OSで65% vs 52%と有意に大量化学療法群がまさっていた[9]という報告がされたが, その一方で, 同グループでその後に行なわれた, 最初からhigh intermediate~high risk群を対象としてACVBP療法と大量化学療法を比較した試験では, 5年OSで60% vs 46%と大量化学療法よりもACVBP療法群の方がまさっていたとも報告されている[10].

この2つの試験の結果の違いに関してであるが, 前者の試験が行なわれたのはIPIが公表され

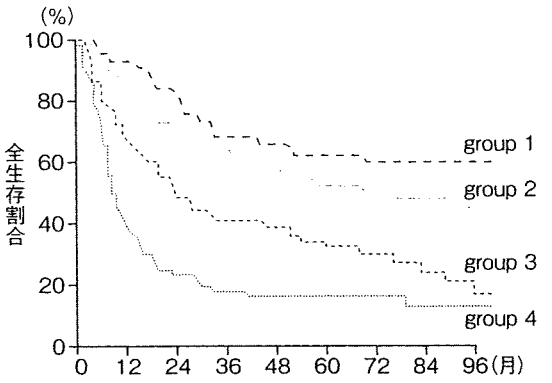
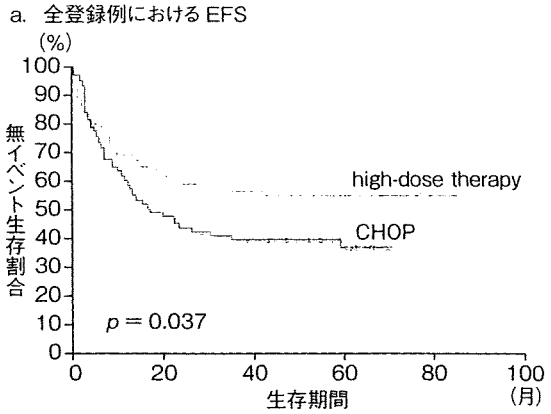


図5 PIT による因子数別の生存曲線

(文献8より引用)



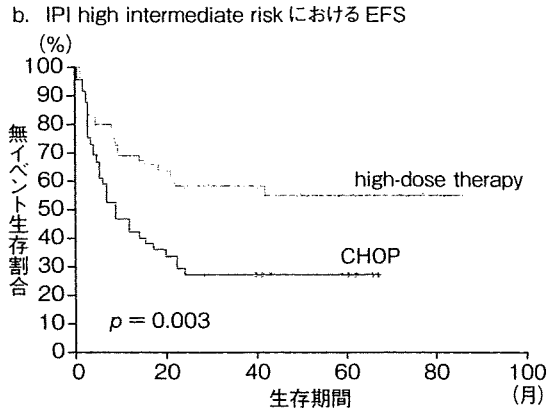


図6 CHOP 療法と大量化学療法の比較試験における生存曲線

(文献12より引用)

る前であったため，対象が同グループの基準による予後不良因子（ECOG の performance status が2～4，節外病変が2つ以上，骨髄または中枢神経浸潤を有する，バーキットリンパ腫またはリンパ芽球性リンパ腫）を1つ以上有する例であったのだが，それを試験終了後に公表された IPI を用いて解析したところ high intermediate～high risk に相当する例において大量化学療法の有用性が示されたという，サブグループ解析のような結果であったことが影響している可能性がある．

その他にイタリアのグループからは，IPI の high intermediate～high risk 例を対象に MACOP-B 療法を行なう群と治療期間を短縮した MACOP-B 療法の後に大量化学療法を行なう群との比較試験が報告されているが，そこでも両群に生存で差はなかったとされている[11]．しかし，これらの試験では大量化学療法前の初回治療の強度が，対象となる通常量の化学療法群と比較して十分ではなかったこと，通常量の化学療法群の治療レジメンが現在の標準的な治療と考えられているものとは異なっていることなどから，大量化学療法の意義を否定するものではなかったとも考えられている．

その後，標準的な化学療法レジメンである CHOP 療法を対象として大量化学療法の有用性を検討した比較試験がフランスの Groupe Ouest-Est des Leucemies et des Autres Maladies du Sang（GOELAMS）で行なわれ，そこでは統計学的な有意差こそ認められなかったものの5年 OS は71% vs 56%と大量化学療法群が良好であり，5年 EFS においては55% vs 37%と有意に大量化学療法群がまさっていたこと，なかでも high intermediate risk 例では5年 EFS が28% vs 56%，5年 OS が44% vs 74%と著名な差が認められたことが報告され（図6）[12]，CHOP 療法では治療効果が十分でない対象に対しては大量化学療法が有用である可能性が示された．しかし，この試験は IPI の high risk 群を「CHOP 療法の適応とすることは倫理的ではない」として対象から除外している一方で，IPI の low～low intermediate risk という，通常では初回治療後の奏効例のように地固め療法としての大量化学療法の適応としないような例も対象に含めており（試験に登録された例の約半数が IPI low～low inter-

mediate risk であった．high intermediate risk 例では大量化学療法群がまさっていたが，low～low intermediate risk 例では EFS，OS とも両群の間に差は認められなかった），結果の解釈には注意が必要であり，この結果だけで大量化学療法が CHOP 療法にまさると結論づけることはできないと考えられている．

B 細胞リンパ腫よりも予後不良である PTCL に対しても，初回治療から大量化学療法を導入することが検討されてきた．対象患者の少なさのため比較試験が成立困難であり，前向きな臨床試験は第Ⅱ相試験しかないのだが，その有用性を示唆する報告がある．ヨーロッパのグループから，PTCL と AITL 患者を対象とした地固め療法としての大量化学療法の第Ⅱ相試験が報告されている．そこでは，未治療の PTCL 患者に対して用量を高めた CHOP 療法（Mega CHOP）で初回治療を行ない，部分奏効以上の効果が得られた場合に自家造血幹細胞移植併用の大量化学療法が行なわれたが，大量化学療法後の完全奏効割合は 89％で，大量化学療法が行なわれた例（全体の 76％）の 2 年 OS が 84％，PFS が 56％，DFS が 63％という良好な結果であった[13]．未治療の AITL 患者に対して行なわれた試験では 3 年 OS が 60％，PFS が 55％という結果であり，同様に大量化学療法の有用性が示唆された[13]．しかし，別のグループから報告された試験では，全体の 4 年の PFS が 30％，OS が 39％と決して良好な結果ではないだけでなく，大量化学療法が行なえた例と行なえなかった例とで予後に違いはなく，PTCL に対する地固め療法としての大量化学療法にはまだ議論の余地があるとされている[14]．

前述の報告では，PIT の group 3～4 という予後不良例が 19％であったのに対して，この報告では group 3～4 が約半数も含まれていたという患者背景の違いが結果の違いに反映された可能性があるが，T 細胞リンパ腫に対する地固め療法としての大量化学療法の位置づけは B 細胞リンパ腫に対するものよりもさらに不明瞭である．

このように，地固め療法としての大量化学療法は有望な治療法であるが，現在も検討中の試験的治療なのである．なお，IPI low～low intermediate risk に対しては CD20 陽性であるならば R-CHOP 療法で約 80％以上の長期生存が期待できるため，適切に計画された臨床試験に登録する以外の状況で R-CHOP 療法以外の治療を選択する理由はない．

2）その他の試験的治療

a）新規薬剤の応用

近年の分子標的薬の進歩により，中悪性度非ホジキンリンパ腫の初回治療にも新規薬剤が応用されるようになってきている．PTCL の約半数に発現している CD52 に対するモノクローナル抗体である alemtuzumab と CHOP 療法の併用療法がイタリアのグループより報告されている．24 人の未治療の CD52 陽性の PTCL 患者に対して CHOP 療法 8 コースと alemtuzumab 4～8 回の併用療法を行なったところ，71％の完全奏効割合が認められたとされている[15]．しかし高い奏効割合の一方で，grade 4 の感染症として侵襲性アスペルギルス症，JC ウイルス脳炎，ブドウ球菌による敗血症，溶連菌による敗血症，ニューモシスチス肺炎を合併したことも報告されており，一般的に行なわれる治療になりうるかは疑問である．

DLBCL に対する新規治療としては，ヒト化 CD22 抗体である epratuzumab と R-CHOP 療法の併用の第Ⅰ相試験が報告されている．そこでは，未治療の DLBCL 患者に対する初回治療で 87％の奏効割合，2 年 PFS が 86％，OS が 86％と，良好な成績であったとされている[16]．

ほかには，vascular endothelial growth factor（VEGF，血管内皮細胞増殖因子）に対するヒト化モノクローナル抗体である bevacizumab と R-CHOP 療法の併用の試験で，未治療の DLBCL 患者に対して奏効割合が 85％，1 年 PFS が 77％という成績が報告されている[17]．これらの併用療法が，効果や費用の点から R-CHOP 療法に替わる治療となるかどうかはこれから検証されていくのだろうが，これからの治療の進歩の展望に期待が高まる．

b）Rituximab の急速投与

R-CHOP 療法は B 細胞リンパ腫の標準的治療である．悪性リンパ腫は年々発症頻度が増加しており，R-CHOP 療法の施行頻度もますます高まっていくものと考えられる．近年の急速な癌治療の環境整備により，抗癌化学療法は入院必須の治療

から外来でも可能な治療へと変化してきている.

標準的な投与法ではrituximabとCHOP療法はそれぞれ3〜4時間の点滴時間を必要とするため，外来で行なう場合，頻回な通院や設備の長時間の占有が余儀なくされる．外来化学療法の必要性が高まっている現在，これらの問題は軽視できない要素である．これに対し，カナダのBritish Columbiaのグループはrituximabの急速投与を行ない，その安全性を報告した．rituximabを総量250 mlになるように溶解し，最初の30分で20%，その後60分で残りの総量を投与するという方法で投与したところ，grade 3以上の輸注関連の有害事象が1件も認められなかったのみでなく，その他の軽度な有害事象も増加しなかった(corticosteroidを含んだ化学療法との併用時した患者が150人，維持療法としてrituximabを投与した患者が56人)[18].

その報告は，これまで1,200人以上の患者が同方法により治療され，治療資源の活用に有用であったと結ばれている．もちろん安易に模倣すべき方法ではないが，近年の外来化学療法の事情を考慮すると，今後このような治療研究も必要となるだろう.

c) PETの応用

近年，悪性リンパ腫の評価にPETが用いられるようになった．その感度の高さから，通常は治療前の病期決定や治療の効果判定に用いられるのだが，治療の途中でPETを行ない，治療の反応性を評価する研究も行なわれている.

Spaepenらは70人の中悪性度非ホジキンリンパ腫患者に対して治療を行ない，3コース後にPETを評価したところ，その時点でPETが陽性であったのが33人，陰性であったのが37人であり，陽性だった例では1人も長期奏効が見られなかったのに対して，陰性だった例は31人で奏効を維持しており，多変量解析では治療途中でのPET陽性/陰性はIPIよりも強い予後因子であったと報告した[19](図7)．この報告から，治療途中のPETは予後予測の方法の1つとして有用であるだけでなく，その結果により地固め療法としての大量化学療法の導入することや，治療を短縮し抗腫瘍薬の総投与量を減量するなど，治療の層別化に利用することの可能性も示唆された.

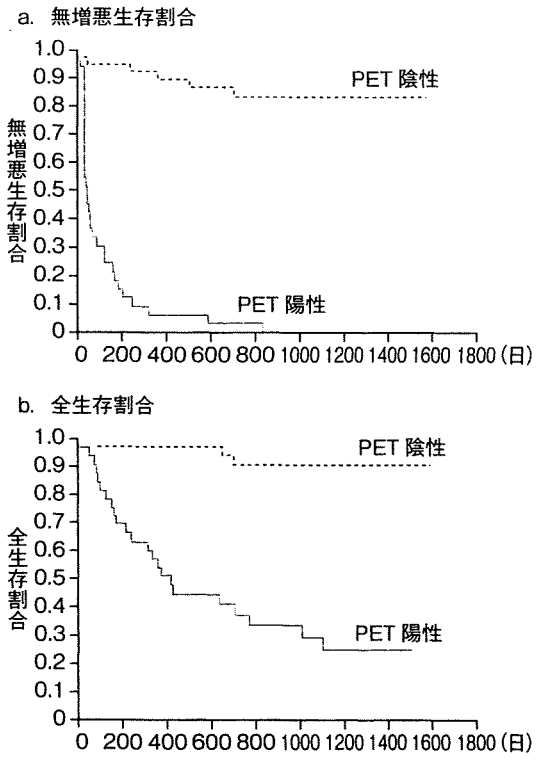


図7 治療途中のPET陽性/陰性別による生存曲線

(文献19より引用)

近年の学会では治療途中のPETに関する前向きの臨床試験が報告がされるようになってきているが，実地臨床において治療途中のPETの結果により戦略を変更するような治療を行なうには時期尚早である.

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ABSTRACT

The detection of minimal residual disease (MRD) in bone marrow is very important in the clinical management of malignant lymphoma. So far, the assessment of MRD in cases of diffuse large B cell lymphoma (DLBCL) has had some technical limitations, such as requiring patient-specific primers and complicated experimental steps. To resolve these problems, we applied a tumor-specific epigenetic alteration of the *p57KIP2* gene as a biomarker for detecting MRD in DLBCL. The methylation of the *p57KIP2* gene was analyzed in 63 cases of DLBCL by methylation-specific real-time quantitative PCR. Methylation of the *p57KIP2* gene was detected in 53 (84.1%) of these 63 cases of DLBCL. We could detect one *p57KIP2* gene-methylated cell among 10,000 unmethylated cells by the serial dilution experiment. This sensitivity is proved to be equivalent to that of detection of *bcl2/IgH* rearrangement by real-time quantitative PCR. This sensitivity could be converted to the detection of two methylated genomes per reaction. Using clinical material, the same results were confirmed. In this study, we established a convenient and universal method for detecting MRD in DLBCL. This technique is applicable for over 80% of patients with DLBCL. This could promote systemic MRD studies in the area of DLBCL.

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1. Introduction

The t(14;18)(q32;q21) translocation, which causes a *bcl2/IgH* gene rearrangement [1], is a characteristic chromosomal abnormality found mainly in B cell lymphomas and its detection and quantification is useful for monitoring minimal residual disease (MRD) in the bone marrow of follicular lymphoma cases [2–5]. However, in diffuse large B cell lymphoma (DLBCL), the frequency of this rearrangement is relatively low [6,7]. The most widely used method for detecting MRD in DLBCL is to identify the clonal B cells with an *IgH* variable region (VH) sequence similar to that of the original lymphoma lesion; this method was originally established to detect MRD in B-cell leukemia [8–10]. This technique is available for about 70% of DLBCL cases. The DNA sequence of the VH region for each patent is required and VH sequence-specific original primers must be designed. For real-time quantitative polymerase chain reaction (RQ-PCR), several probes are also required [10]. Thus, a universal and conventional method for detecting MRD in DLBCL is eagerly awaited.

Generally, malignancies have specific genetic or epigenetic alterations of oncogenes or tumor suppressor genes, absent in normal tissues. Several kinds of tumor suppressor genes were inactivated by aberrant DNA methylation in lymphoid malignancies [11,12]. Such DNA methylation has been used to detect malignant cells in many kinds of tumors. This concept was also applied to the RQ-PCR technique [13]. Quantitative analysis using methylation-specific RQ-PCR (MS-RQ-PCR) was able to detect minimal tumor cell contamination in blood plasma or urine in breast cancer, hepatocellular carcinoma, and prostate cancer [14–16], suggesting that the method is sufficiently sensitive and specific to detect several copies of a tumor genome. MS-RQ-PCR has already been used to detect MRD in acute myeloid leukemia [17].

The *p57KIP2* gene is a cyclin-dependent kinase inhibitor in the kinase-interacting protein (KIP) family. In particular, the *p57KIP2* gene is located at chromosome 11p15.5, a region implicated in sporadic cancers, including those of the breast [18], liver [19], and bladder [20]. We previously revealed that the promoter region of the *p57KIP2* gene is frequently methylated in B cell lymphomas [21]. The *p57KIP2* gene has been reported to be inactivated by DNA methylation in acute lymphocytic leukemia [22] and lung cancer [23]. The frequent inactivation and other aspects of this gene function suggest that *p57KIP2* gene is a strong candidate for a tumor

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Table 1
Primers and probes sequences used in this study.

Gene	Primer name	Sequence (5′→3′)
β-Actin	mACTB-U	TGGTGATGGAGGAGGTTTAGTAAGT
	mACTB-L	AACCAATAAAACCTACTCCTCCCTTAA
	Probe	FAM-ACCACCACCCAACACACAATAACAAACACA-TAMRA
p57KIP2	mP57-U	CGTATAAAGGGGGCGTAGGC
	mP57-L	CGCCTATCTCGTCCGAACG
	Probe	FAM-TTGGGCGTTTTATAGGTTAAGTGCGTTGT-TAMRA
BCL2-MBR	MBR	TTTAGAGAGTTGCTTTACGTGGC
	JH	ACTCACCTGAGGAGACGGTGAC
	Probe	FAM-TTTCAACACAGACCCACCCAGAGCC-TAMRA
BCL2-internal	BCL2-R1	GCAATTCCGCATTTAATTCATGG
	BCL2-R2	GAAACAGGCCACGTAAAGCAAC
	Probe	FAM-TCCAGATGGCAAATGACCAGCAGA-TAMRA

suppressor gene in DLBCL. Therefore, the aberrant DNA methylation of the *p57KIP2* gene could be a good biomarker for DLBCL.

We investigated the possibility of *p57KIP2* gene promoter methylation for MRD detection in B cell lymphoma, especially in DLBCL. A sensitive and specific method for detecting MRD in DLBCL using MS-RQ-PCR of the *p57KIP2* gene was established. This is a conventional procedure requiring just one set of common primers and one probe and is applicable for about 84% of patients with DLBCL.

2. Materials and methods

2.1. DNA preparation from clinical samples and cell lines

Genomic DNA was isolated by the standard method of proteinase K digestion and phenol–chloroform extraction. We examined the DNA from 63 biopsied lymph nodes of patients with DLBCL at the time of initial diagnosis, peripheral blood mononuclear cells (PBMNCs) of 54 healthy volunteers, and bone marrow mononuclear cells (BMMNCs) of 14 non-malignant hematologic diseases (aplastic anemia and idiopathic thrombocytopenic purpura). Informed consent was obtained from all patients and healthy subjects. For the determination of the detection limit, we used two cell lines that have fully methylated and unmethylated *p57KIP2* promoters: SU-DHL-6 and K562, respectively.

2.2. Bisulfite modification

For the methylation analysis, genomic DNA extracted from clinical samples and cell lines was processed for bisulfite modification using a Methylamp™ DNA Modification Kit (Epigentek, New York, NY) according to the manufacturer's protocol. The bisulfite-modified DNA was stored at −20 °C until use.

2.3. Primers and probes

The sequences of primers and probes used for real-time PCR are shown in Table 1. For the MS-RQ-PCR to analyze *p57KIP2* gene methylation, two sets of PCR primers and probes, designed specifically for the bisulfite-modified DNA sequences, were used as described [24]: a set representing fully methylated DNA for the promoter of the *p57KIP2* gene and an internal reference set for *β-actin* to normalize for input DNA. For the RQ-PCR to detect the major breakpoint region (MBR) of *bcl2/IgH* translocation, two additional sets of PCR primers and probes were used as described [5]: one set that amplifies the MBR region in *bcl-2* and a reference set that amplifies the *bcl-2* sequence as an internal control. The primers were obtained from SIGMA Genosys Japan (Hokkaido, Japan). The probes, labeled with FAM at the 5′ end and the quencher TAMRA at the 3′ end, were obtained from Applied Biosystems (Foster City, CA, USA).

2.4. Real-time methylation-specific PCR for methylation of the p57KIP2 promoter

Bisulfite-modified DNA was used as a template for fluorescence-based real-time PCR. The final volume for each PCR reaction was 25 μl, containing 400 nM of each primer, 200 nM of probe, 0.75 U of Platinum Taq polymerase (Invitrogen, Carlsbad, CA), 200 μM each of dNTPs, 2.5 mM ($p57^{KIP2}$ promoter) or 4.5 mM (*β-actin*) of $MgCl_2$, and 50 nM of ROX reference dye (Invitrogen). The reaction conditions were as follows: 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. The reactions were carried out in a 96-well reaction plate in a 7500 Real-Time PCR System (Applied Biosystems).

2.5. Real-time PCR for bcl-2/IgH MBR translocation

Genomic DNA was used as a template for fluorescence-based real-time PCR. The reaction mixture contained 400 nM (MBR) or 100 nM (internal bcl-2) of each primer, 250 nM (MBR) or 150 nM (internal bcl-2) of probe, and TaqMan Universal PCR Master Mix (Applied Biosystems). The reaction conditions were as follows: 50 °C for 2 min, 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s, and 60 °C for 1 min. The reactions were carried out in a 96-well reaction plate in a 7500 Real-Time PCR System (Applied Biosystems).

2.6. Sensitivity of real-time PCR assay

SU-DHL-6 DNA was serially diluted in 10-fold increments up to $1:10^5$ with K562 DNA. The SU-DHL-6 cell line has a fully methylated promoter region of *p57KIP2* and MBR translocation, while the K562 cell line is unmethylated and negative for translocation. For real-time methylation-specific PCR, the diluted DNA was modified with sodium bisulfite and then used as a template. For MBR real-time PCR, the diluted DNA was used without bisulfite modification. A conversion factor of 6.6 pg of DNA per diploid cell was used for expressing quantitative results in genome-equivalents [25]. One genome-equivalent was defined as the amount of a particular target sequence in a single reference cell.

2.7. Clinical characteristics of patients with DLBCL

The clinical parameters consisting international prognostic index (IPI) [26] of 63 DLBCL patients analyzed were collected. The difference of these parameters between p57KIP2 methylated and non-methylated patients were evaluated using the Mann–Whitney *U*-test for continuous variables and the χ^2-test for categorical variables.

3. Results

3.1. p57KIP2 gene promoter methylation in DLBCL

We examined 63 biopsied lymph nodes from patients with primary DLBCL at the time of diagnosis for *p57KIP2* DNA methylation by MS-RQ-PCR. Methylation of the *p57KIP2* gene was detected in 53 (84.1%) of these samples. The quantification was performed by comparison with the SU-DHL-6 cell line, which is fully methylated in the *p57KIP2* gene. The calculated amount of methylated *p57KIP2* gene was distributed from one to 1/64 fold of the SU-DHL-6 cell line among patients. Representative amplification plots of MS-RQ-PCR are shown in Fig. 1. We also analyzed PBMNCs from 54 healthy volunteers and BMMNCs from 14 non-malignant hematologic diseases, and the methylated *p57KIP2* gene was undetected (data not shown).

We compared the clinical variables consisting IPI and IPI itself between 53 methylated and 10 unmethylated patients, but statistical differences were not observed (Table 2).

3.2. Detection of the rearrangement of bcl2/IgH (MBR) by RQ-PCR in DLBCL

All 63 biopsied lymph nodes were subjected to the analysis of bcl2/IgH rearrangement by RQ-PCR. Seven (11.1%) of the 63 patients with DLBCL showed this rearrangement. This ratio was slightly

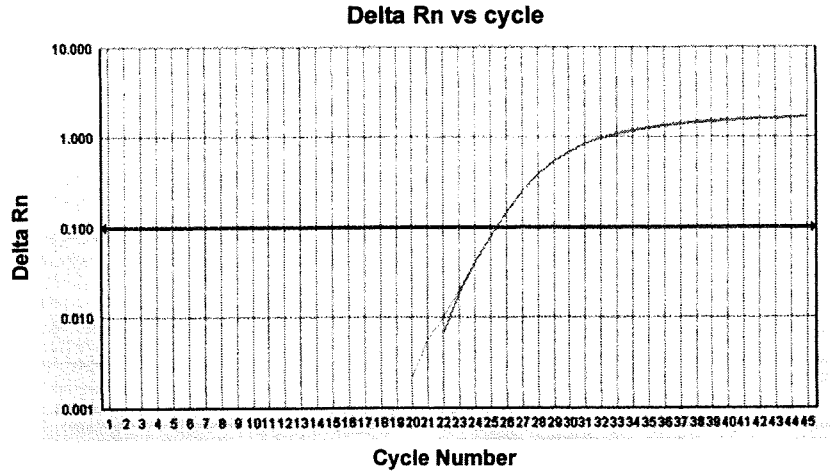

Fig. 1. A representative amplification plot for the p57KIP2 gene MS-RQ-PCR analysis of a DLBCL DNA sample. *X*-axis, the cycle number of quantitative PCR; *Y*-axis, ΔRn, the fluorescence intensity over the background.

Table 2
IPI score of DLBCL patients ($n = 63$).

	IPI				
	L	LI	HI	H	
p57KIP2 methylation					
+($n = 53$)	22	15	9	7	$p = 0.836$[a]
−($n = 10$)	4	2	3	1	

L: low risk; LI: low intermediate risk; HI: high intermediate risk; H: high risk.
[a] Mann–Whitney *U*-test.

lower than that of a previous report [27]. All of these seven patients also had *p57KIP2* methylation.

3.3. The sensitivity of MS-RQ-PCR for the p57KIP2 gene

We performed a dilution experiment using cell line DNA to determine the detection limit of the real-time PCR assay. We prepared serial dilutions of SU-DHL-6 with K562 DNA. SU-DHL-6 DNA has a fully methylated *p57KIP2* gene promoter and MBR translocation, while K562 has an unmethylated promoter and is negative for translocation. This serial dilution was prepared for the real-time PCR to detect both *p57KIP2* gene methylation and MBR translocation. All of the samples contained approximately equal amounts of DNA, as was evident from the overlapping *β-actin* and *BCL2*-internal curves (data not shown). For the MS-RQ-PCR of the *p57KIP2* gene and the RQ-PCR of the *bcl2/IgH* rearrangement, the first input DNA used as a template was approximately 112.5 ng and 100 ng, respectively. The target DNA could be detected at a dilution of 10^{-4} in both reactions (Fig. 2). This sensitivity corresponds to 1.7 genome-equivalents and 1.5 genome-equivalents, respectively. Considering the loss of DNA during the bisulfite modification, the real first input DNA was less than the calculated amount.

The sensitivity was also examined using a bone marrow sample of DLBCL that showed both *bcl2/IgH* rearrangement and *p57KIP2* gene methylation in the primary lymphoma lesion. The lymphoma cell invasion of bone marrow of this patent was negative by microscopic examination and flow cytometric analysis. We could detect *bcl2/IgH* rearrangement and *p57KIP2* gene methylation by RQ-PCR in the bone marrow of this patient. The calculated percentage of lymphoma cell contamination in the bone marrow was 0.1% based on *bcl2/IgH* rearrangement and 0.09% based on *p57KIP2* gene methylation. The bone marrow DNA of this patient was serially diluted and subjected to MS-RQ-PCR of *p57KIP2* and RQ-PCR of *bcl2/IgH* MBR rearrangement. We found similar sensitivity for the detection

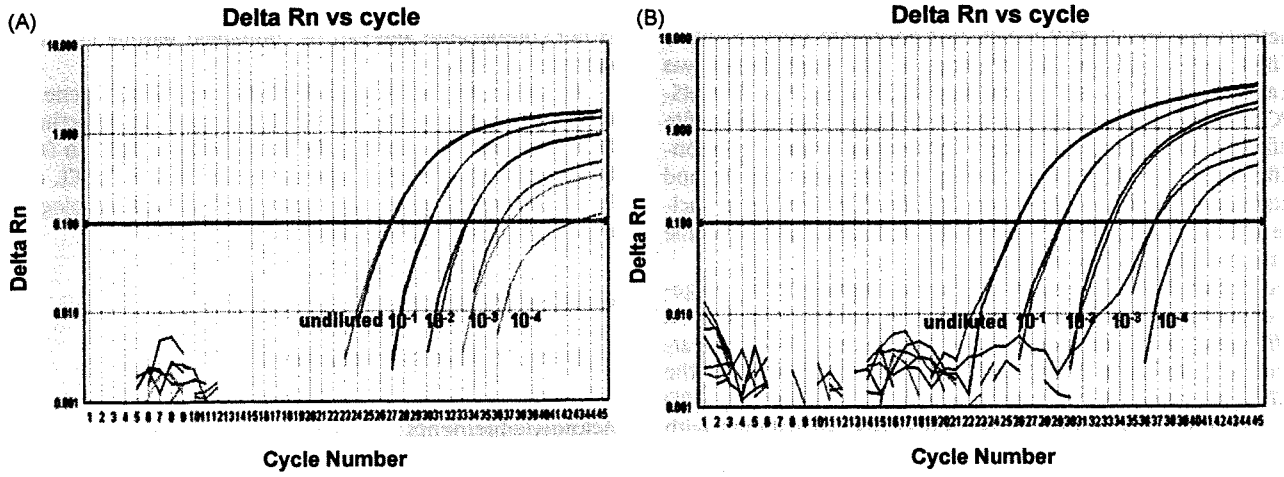

Fig. 2. A comparison of detection sensitivities between the MS-RQ-PCR analysis of p57KIP2 gene and RQ-PCR of major breakpoint region (MBR) of bcl2/IgH translocation. The genomic DNA of SU-DHL-6 (fully methylated p57KIP2 gene and carrying bcl2/IgH translocation) was serially diluted by the genomic DNA of K562 (absence of p57KIP2 gene methylation and bcl2/IgH translocation). (A) The amplification plot for p57KIP2 gene MS-RQ-PCR. (B) The amplification plot of bcl2/IgH translocation RQ-PCR. The target DNA was detected at a dilution of 10^{-4} in both reactions. *X*-axis, the cycle number of quantitative PCR; *Y*-axis, ΔRn, the fluorescence intensity over the background.

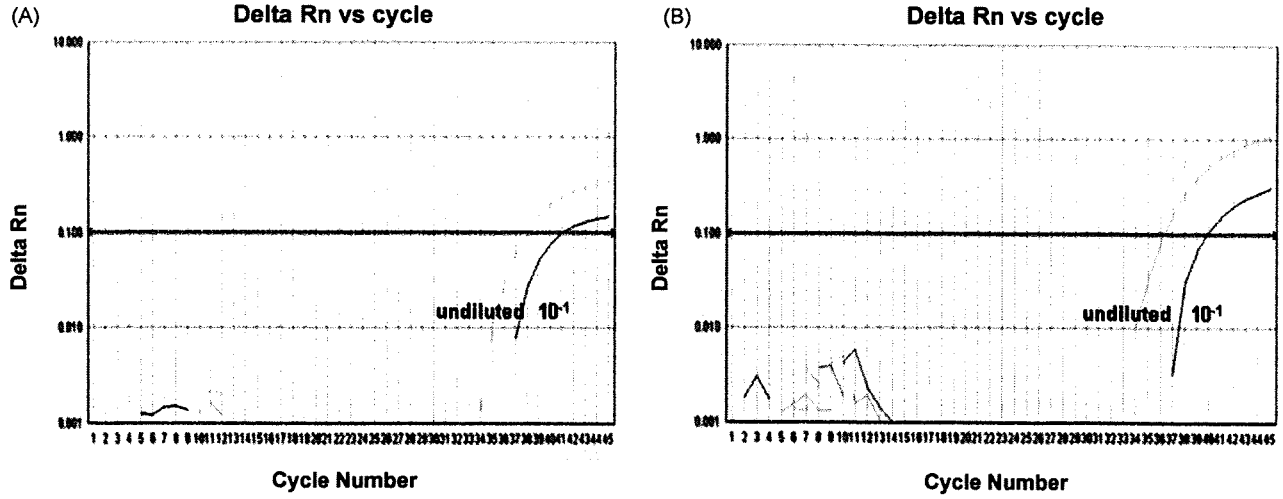

Fig. 3. The detection of MRD in bone marrow of a DLBCL patient with methylated p57KIP2 gene and bcl2/IgH translocation. (A) The amplification plot for p57KIP2 gene MS-RQ-PCR. (B) The amplification plot of bcl2/IgH translocation RQ-PCR. The percentages of the lymphoma cell bone marrow contamination were calculated as 0.09% and 0.1% by each method at undiluted condition, respectively. The target DNA was detected at a dilution of 10^{-1} in both reactions. *X*-axis, the cycle number of quantitative PCR; *Y*-axis, ΔRn, the fluorescence intensity over the background.

of lymphoma cell contamination in bone marrow by MS-RQ-PCR of *p57KIP2* (0.015%) compared to RQ-PCR of *bcl2/IgH* MBR rearrangement (0.01%) in clinical material (Fig. 3). Two genomes per reaction could be detected by both methods.

4. Discussion

Aberrant DNA methylation of a tumor suppressor gene is a tumor-specific biomarker and has been used to detect minimal tumor contamination.

In this study, the high frequency of methylation of the *p57KIP2* gene in DLBCL and the sufficient sensitivity for detecting MRD by MS-RQ-PCR of the *p57KIP2* gene was demonstrated. The *p57KIP2* methylation status did not affect the prognostic variables in DLBCL patients. This biomarker might be universal for DLBCL. We could not detect the methylated DNA fragments from 68 non-malignant subjects; therefore the low possibility of false positive was demonstrated. Previously, we reported that methylation of the *p57KIP2* gene was observed about in 55% of patients with DLBCL [21], but the present analysis showed a higher frequency. Methylation-specific RQ-PCR is a sensitive and specific method for detecting aberrant DNA methylation using a methylation-specific probe. Our previous method for detecting methylation was classical methylation-specific PCR, which used primers to anneal bisulfite modified DNA sequences. The presence of DNA methylation was determined just by the successful amplification by PCR. The MS-RQ-PCR method in this study uses primers that will anneal bisulfite modified DNA sequences and detect methylation by a methylation-specific probe. MS-RQ-PCR is thought to be more specific and sensitive than classical methylation-specific PCR. The higher incidence of detection of *p57KIP2* methylation in this study might come from the difference in the methods.

The methylation of the *p57KIP2* gene is a tumor-specific epigenetic alteration and is found in over 80% of patients with DLBCL. This frequency is almost equal to the incidence of *bcl2/IgH* rearrangement in follicular lymphoma. Thus, the methylation of the *p57KIP2* gene could be a very good biomarker for detecting MRD in the bone marrow of DLBCL. We found that all patients with DLBCL with *bcl2/IgH* rearrangement had *p57KIP2* DNA methylation; therefore, the combination of these two markers will not expand the applicability for MRD of DLBCL. The sensitivity for detecting *p57KIP2* methylation by MS-RQ-PCR and *bcl2/IgH* MBR rearrangement was compared. Both markers were equivalent in sensitivity for the detection of MRD. We could detect two copies of the *p57KIP2* methylated genome per reaction with 25 ng input DNA. This could be converted to a sensitivity for the detection of one lymphoma cell out of 10,000 unaffected cells. This ability for MRD analysis by *p57KIP2* methylation was also demonstrated in clinical samples. This sensitivity is thought to be sufficient for the clinical use in the detection of MRD in lymphoma.

So far, convenient and general methods for examining MRD in DLBCL have not been established. In follicular lymphoma, *bcl2/IgH* rearrangement has been utilized for MRD analysis because of the very high occurrence of this genomic alteration in this type of lymphoma. However, the rearrangement of *bcl2/IgH* is not especially frequent in DLBCL, with a range of 12–30% [27]. The identification of the clonal B cell with a *IgH* variable region (VH) sequence similar to that of the original lymphoma lesion was applied for the detection of MRD in DLBCL. This technique is appropriate about for 70% of patients with DLBCL. The VH region of each patent is required to be sequenced and its VH sequence-specific original primers must be designed. Furthermore, several specific probes are necessary for RQ-PCR. MS-RQ-PCR of the *p57KIP2* gene does not require patient-specific manipulations and one set of primers and probes are applicable for all cases of DLBCL that show aberrant DNA methylation of the *p57KIP2* gene. The bisulfite DNA modification is not complicated and can be completed within 1 h when using commercial kits.

As shown in this study, MS-RQ-PCR of *p57KIP2* gene methylation is a convenient and universal procedure for detecting MRD in DLBCL. Using this method, the monitoring of MRD in DLBCL will be generalized; therefore, the survey of MRD in DLBCL, especially in the setting of high-dose chemotherapy with autologous stem transplantation, will be promoted.

Conflicts of interest

There are no conflicts of interest.

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REVIEW ARTICLE

Fluorine-18-Fluorodeoxyglucose Positron Emission Tomography for Interim Response Assessment of Advanced-Stage Hodgkin's Lymphoma and Diffuse Large B-Cell Lymphoma: A Systematic Review

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A B S T R A C T

Purpose
To systematically review the prognostic accuracy of fluorine-18-fluorodeoxyglucose positron emission tomography (FDG-PET) for interim response assessment of patients with untreated advanced-stage Hodgkin's lymphoma (HL) or diffuse large B-cell lymphoma (DLBCL).

Methods
MEDLINE, EMBASE, SCOPUS, and Biologic Abstracts were searched for relevant studies. Two assessors independently reviewed studies for inclusion and extracted data. Relevant unpublished data were requested from the investigators if unavailable from publications. A meta-analysis of the prognostic accuracy was performed.

Results
Thirteen studies involving 360 advanced-stage HL patients and 311 DLBCL patients met our inclusion criteria. Advanced-stage HL studies included few unfavorable-risk patients. DLBCL studies were heterogeneous. FDG-PET had an overall sensitivity of 0.81 (95% CI, 0.72 to 0.89) and a specificity of 0.97 (95% CI, 0.94 to 0.99) for advanced-stage HL, and a sensitivity of 0.78 (95% CI, 0.64 to 0.87) and a specificity of 0.87 (95% CI, 0.75 to 0.93) for DLBCL. Meta-regression and subgroup analyses did not identify factors that affect prognostic accuracy.

Conclusion
For low- to intermediate-risk advanced-stage HL, FDG-PET performed after a few cycles of standard chemotherapy seems to be a reliable prognostic test to identify poor responders, warranting prospective studies to assess PET-based treatment strategies. For DLBCL, no reliable conclusions can be drawn due to heterogeneity. Interim PET remains an unproven test for routine clinical practice. Its use should be reserved for research settings where treatment regimens and imaging conditions are standardized.

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INTRODUCTION

Malignant lymphoma is the fifth most commonly diagnosed cancer in the United States.[1] With advances in treatments, Hodgkin's lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL) are potentially curable lymphomas.[2,3] However, challenges remain especially in the treatment for high-risk patients,[4,5] since more than half of these patients do not achieve long-term survival with currently available standard first-line chemotherapy. A possible treatment involves intensive and toxic polychemotherapy for advanced-stage HL[6] or first-line high-dose chemotherapy with stem-cell support for DLBCL,[7] depending on individual risk of treatment failure. Therefore, better identification of poor responders to first-line therapy is important to advance risk-adapted treatment strategies.

Fluorine-18-fluorodeoxyglucose positron emission tomography (FDG-PET) is a functional imaging test that has become widely used in the management of both HL and non-Hodgkin's lymphoma (NHL).[8] Studies that assessed FDG-PET as a prognostic tool performed during chemotherapy have reported the ability to predict poor outcomes.[8] However, the studies used different design, conduct, and reporting, making interpretation of the results difficult. In particular, inclusion of heterogeneous populations with different categories of disease (eg, limited-stage *v* advanced-stage HL or DLBCL

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Table 1. Studies of PET for Interim Response Assessment of Malignant Lymphoma Included in the Systematic Review

Study	Year	Country	Study Design	No. of Involved Institutions	Start of Follow-Up Period	Follow-Up (months)		Pretherapy Scan to Confirm FDG Avidity (%)
						Median	Range	
Advanced-stage HL + DLBCL								
Kostakoglu et al[32]	2006	USA	Retrospective	1	Start of therapy	21†	3-47	100
Advanced-stage HL								
Friedberg et al[33]	2004	USA	Prospective	3	Pre-therapy PET	24†	10-32	100
Hutchings et al[37]	2005	UK	Retrospective	1	Diagnosis of lymphoma	40†	6-125	100
Gallamini et al[30]	2006	Italy	Prospective	11	Diagnosis of lymphoma	20‖	2-46	100
Hutchings et al[13]	2006	Denmark	Prospective	3	Diagnosis of lymphoma	22	6-40	100
Zinzani et al[14]	2006	Italy	Prospective	1	NR	18	12-27	100
Gallamini et al[29]	2007	Italy + Denmark	Prospective	14	Diagnosis of lymphoma	26†	4-62	100
DLBCL								
Spaepen et al[34]	2002	Belgium	Prospective	1	End of therapy	36††	19-51	97†
Haioun et al[31]	2005	France	Prospective	4	Study enrollment	24†	NR	100
Mikhaeel et al[12]	2005	UK	Retrospective	1	Diagnosis of lymphoma	24†	NR	100
Fruchart et al[35]	2006	France	Prospective	1	Start of therapy	19	2-35	100
Querellou et al[38]	2006	France	Retrospective	1	Start of therapy	15†¶	9-28	100
Ng et al[36]	2007	Australia	Retrospective	1	Start of therapy	28	2-81	Partial

(continued on following page)

v other aggressive NHLs) clearly affects the clinical applicability of the study results because each category has different clinical profiles (eg, treatment strategies, response, and prognosis). In this systematic review, we assessed the prognostic accuracy of FDG-PET performed during first-line therapy to predict disease progression or relapse in patients with advanced-stage HL and DLBCL, paying particular attention to the clinical applicability of the reported results.

METHODS

Data Sources and Searches

We searched Ovid MEDLINE and EMBASE from 1966 through July 2006,[9] and PubMed from August 2006 through July 2007 without language restriction. The search strategy can be found in online-only Appendix Table A1. This search was augmented by searches of SCOPUS and Biologic Abstracts. We also examined the reference lists of eligible studies, review articles, and textbooks.

Study Selection

Two reviewers (T.T., H.N.) screened abstracts and determined eligibility. Full-text articles were reviewed when abstracts did not provide sufficient information for determination. We included studies that evaluated FDG-PET performed between the first and the fourth cycle of first-line chemotherapy for patients with advanced-stage HL or DLBCL. We included both prospective and retrospective studies, and we considered clinical follow-up with or without pathologic confirmation to be a reference standard. We included studies that evaluated at least 10 patients and included at least five patients who progressed during chemotherapy or relapsed through clinical follow-up. We accepted studies in which patients received high-dose chemotherapy followed by autologous stem cell transplantation as long as it was administered as a part of primary therapy or consolidation therapy after standard induction chemotherapy. We excluded abstracts, editorials, comments, letters, and review articles. We excluded studies that enrolled patients with HIV-associated or post-transplant lymphoproliferative disorders.

Many studies did not meet all the inclusion criteria, but did partially include a relevant patient population. For these studies, we contacted the authors for relevant individual patient or subgroup data. When there was no response after 4 weeks, another correspondence was sent. When there was no response after the third communication attempt, we considered the request rejected.

Data Extraction and Quality Assessment

Two independent, board-certified hematologists (T.T., H.N.) abstracted relevant data. We extracted patients' demographic and clinical characteristics including the International Prognostic Scores (IPS) for advanced-stage HL[4] or the International Prognostic Indexes (IPI) for DLBCL,[5] therapeutic interventions, interim PET results, and final clinical outcomes. We subdivided the treatment failures into three categories based on the relative timing to the completion of first-line therapy: during therapy, after 1 year from diagnosis or the start of therapy, and in between. When the timing of completion of first-line therapy was unclear, we arbitrarily considered the treatment period to be 6 months. We also extracted the number of cases in remission but censored from follow-up within 1 year from the start of therapy (early censoring). One nuclear medicine specialist (T.N.) evaluated the technical specification and quality of PET procedures using recommended guidelines.[10] Reviewers were not blinded to the name of the journal. Inconsistencies between reviewers were either clarified by the authors or resolved by consensus.

To evaluate the quality, applicability, and reporting of the studies, we used QUADAS, a recently proposed tool to assess the quality of studies of diagnostic accuracy included in a systematic review.[11] Details on how we scored each item can be found in online-only Appendix Table A2. We assessed only published data and did not use unpublished data because the latter was not available from all the studies.

Data Synthesis and Statistical Analysis

For each study, we constructed a 2×2 contingency table consisting of true positive (TP), false positive (FP), false negative (FN), and true negative (TN), where all patients were categorized according to whether they were PET positive or negative, and whether they experienced treatment failure. In the main analysis, we employed the entire clinical follow-up as the reference standard. In sensitivity analysis, we categorized patients using shorter clinical follow-up as the alternative reference standard to focus on very early treatment failures (only during therapy or < 6 months), or early treatment failures (< 12 months). We counted patients in remission during the specified follow-up period as no treatment failure even if they eventually experienced treatment failure thereafter. We counted early censorings as no treatment failure in the main analysis. In sensitivity analysis to explore a worst-case scenario, early censorings were excluded from the analysis, and then counted as FP if they had negative PET results and were lost to follow-up early without treatment

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Table 1. Studies of PET for Interim Response Assessment of Malignant Lymphoma Included in the Systematic Review (continued)

Study	No. of Chemotherapy Cycles Before PET Scan	Duration Between Chemotherapy and PET Scan (days)	No. of Total Participants*	Women		Age (years)	
				No.	%	Median	Range
Advanced-stage HL + DLBCL							
Kostakoglu et al[32]	1	8-15 for HL, 15-22‡ for DLBCL	34§	23†	49	48.2†‖	18-76
Advanced-stage HL							
Friedberg et al[33]	3¶	NR	22	NR†	36	NR†	18-60
Hutchings et al[37]	2 or 3	8-15	28	42†	49	36.7†	15-73
Gallamini et al[30]	2	11.6‖	108	57	53	32.6‖	14-79
Hutchings et al[13]	2	8-15	46	28†	36	36	18-74
Zinzani et al[14]	2	NR	40	21	53	32	14-48
Gallamini et al[29]	2	NR	106#	127†	49	32†	14-79
DLBCL							
Spaepen et al[34]	3 or 4#	14†† or 21‡	47	18†	26	40†	3-78
Haioun et al[31]	2	13-14†† or 20-21‡	83	34†	38	53†	17-78
Mikhaeel et al[12]	2 or 3	NR	57	56†	46	55†	20-84
Fruchart et al[35]	2 or 3	12†† or 18‡	35	13†	33	56†	24-77
Querellou et al[38]	2, 3, or 4¶‡‡	15-21‡	21	NR†	33	NR†	17-75
Ng et al[36]	2, 3, or 4§§	12-14†† or 19-21‡	44	21	48	60	27-83

Abbreviations: FDG, fluorodeoxyglucose; ACVBP, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin's lymphoma; NR, not reported; PET, positron emission tomography; R, rituximab.
*Only advanced-stage HL or DLBCL patients were included in this systematic review.
†Data abstracted from total participants of original report, not exclusively for relevant patient population.
‡For tri-weekly cycle chemotherapy [eg, (R-)CHOP].
§Including 10 advanced-stage HL patients and 24 DLBCL patients.
‖Mean.
¶Patients underwent PET at the midpoint of the whole chemotherapy cycles (the end of the second cycle for 4-cycle chemotherapy regimens, the third cycle for 6-cycle regimens, and the fourth cycle for 8-cycle regimens).
#Only patients not included in the previous reports[13,30] were left.
**Only patients in long-term remission.
††For bi-weekly cycle chemotherapy [eg, (R-)ACVBP].
‡‡Eleven patients underwent PET at the end of the fourth cycle.
§§Eleven patients underwent PET at the end of the fourth cycle.

failure. Three studies reported intermediate PET results as minimal residual uptake (MRU).[12–14] We considered this category negative scan in the main analysis because this was how investigators analyzed the results. In sensitivity analyses, MRU results were excluded from analysis, considered positive, considered positive in the case of treatment failure and negative in the case of continuing remission (best-case scenario), and considered negative in the case of treatment failure and positive in the case of continuing remission (worst-case scenario).

We calculated sensitivity, specificity, and likelihood ratios (LRs) for each study. For the estimation of 95% CI, we used the binominal Wilson method for sensitivity and specificity, and normal approximation for LRs. Then we combined summary statistics, 95% confidence regions of summary sensitivity and specificity, and summary receiver operating characteristic (ROC) curves by the hierarchical SROC method,[15] which takes into account both within-study and between-studies variation. We fitted the model by using maximum likelihood estimation implemented in the GLLAMM algorithm[16] in STATA (version 9.2; Stata Corp, College Station, TX), and depicted the summary ROC curves and confidence regions for summary sensitivity and specificity.[17] We estimated the Q^* statistic,[15] the point on the curve where sensitivity equals specificity, as global measures for the summary ROC.

To explore heterogeneity, we performed subgroup analyses by visual assessment of ROC plots and univariate meta-regression analyses. In the meta-regression, we incorporated study design or clinical characteristics as covariates into the bivariate model using Meta-Analyst (Tufts Medical Center, Boston, MA). Our preplanned analyses included characteristics of study design (prospective *v* retrospective), whether studies included more than 10 patients with treatment failure, rates of treatment failure, adoption of combined FDG-PET and computed tomography (FDG-PET/CT), the mean number of chemotherapy cycles before PET, timing of PET scan after the administration of chemotherapy, percentage of high or high-intermediate risk for DLBCL, and percentage of rituximab (R) use for DLBCL. We also performed posthoc analyses on the use of high-dose chemotherapy. Two-sided *P* values lower than .05 were considered to be statistically significant.

Results

Search Results

Online-only Appendix Figure A1 summarizes the search results. We retrieved 23 full reports for further review and contacted nine authors for additional data. We excluded three studies that presented the same participants as previous reports,[18–20] three studies that did not provide information to calculate prognostic accuracy,[21–23] two studies that adopted nondedicated PET scanner,[24,25] one study with fewer than 10 relevant participants,[26] one study with fewer than five patients who progressed or relapsed,[27] and one study that evaluated patients during salvage therapy.[28] One study[29] presented updated results combining previous reports from two independent groups[13,30] together with 106 newly evaluated patients from both groups. In this report, we included only the added subpopulation as an independent study. Three studies reported FDG-PET results at completion of second cycle and fourth cycle of chemotherapy.[13,14,31] We abstracted data only on the second cycle in these studies. One study evaluated

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Table 2. Patient Characteristics of Studies of Positron Emission Tomography for Interim Response Assessment of Malignant Lymphoma

Study	Year	No. of Participants Included	Clinical Staging*	Staging Before Therapy (No.)	Standard Prognostic Scores (No.)	Therapy	Use of Rituximab (%)
Advanced-stage HL			Inclusion criteria of advanced-stage		International Prognostic Scores		
Friedberg et al[33]	2004	22	IIB-IVB, any stage with bulky disease		NR	ABVD × 6 or MOPP/ABVD × 6 ± radiotherapy	—
Hutchings et al[37]	2005	28	IIB-IVB, any stage with bulky disease		NR	ABVD × 6 to 8 ± radiotherapy	—
Gallamini et al[30]	2006	108	IIB-IVB, IIA with adverse prognostic factors†		0 pts: 28, 1 pt: 34, 2 pts: 29, 3 pts: 10, 4 pts: 3, ≥ 5 pts: 4	ABVD × 6 or COPP/EBV/CAD × 6 ± radiotherapy	—
Hutchings et al[13]	2006	46	IIB-IVB		Median 3 pts	ABVD × 6 to 8 or comparable anthracycline-containing regimen ± radiotherapy	—
Kostakoglu et al[32]	2006	10	III-IV, any stage with bulky disease‡		0 pts: 3, 1 pt: 2, 2 pts: 4, 4 pts: 1	ABVD × 6	—
Zinzani et al[14]	2006	40	IIB-IVB		NR	ABVD × 6	—
Gallamini et al[29]	2007	106	IIB-IVB, IIA with adverse prognostic factors†		0 pts: 38, 1 pt: 70, 2 pts: 87, 3 pts: 42, 4 pts: 13, ≥ 5 pts: 10§	ABVD × 6, ABVD-like regimen × 6, or COPP/EBV/CAD × 6 ± radiotherapy	—
DLBCL					International Prognostic Indexes		
Spaepen et al[34]	2002	47		IA: 1, IIA: 15, IIB: 6, IIIA: 14, IIIB: 2, IVA: 14, IVB: 20§	L: 26, L-I: 22, H-I: 17, H: 17§	CHOP × 8, biweekly CHOP × 6, CHVmPBV × 8, or COP/COPADM/CYM × 6‖	0
Haioun et al[31]	2005	83		I-II: 8, III-IV: 82§	L: 14, L-I: 23, H-I: 30, H: 23§	(R-)CHOP × 8, R-ACVBP × 4¶, or ACVBP × 4 or ACE × 4#	45
Mikhaeel et al[12]	2005	57		I: 21, II: 14, III: 9, IV: 13	NR	(R-)CHOP × 6 or PMitCEBO × 6**	16
Fruchart et al[35]	2006	35		I-II: 13, III-IV: 27§	L: 13, L-I: 2, H-I or H: 15§	(R-)CHOP × 8 or (R-)ACVBP × 4††	74
Kostakoglu et al[32]	2006	24		I: 2, II: 11, III: 10, IV: 1	L: 16, L-I: 8	R-CHOP × 6 to 8	100
Querellou et al[38]	2006	21		I: 3, II: 2, III: 4, IV: 15§	L: 8, L-I: 5, H-I: 6, H: 5§	(R-)CHOP × 8, R-COP × 6, or (R-)CEEP × 4‡‡	90
Ng et al[36]	2007	44		I: 16, II: 9, III: 5, IV: 14	L: 17, L-I: 9, H-I: 12, H: 1, NA: 5	(R-)CHOP or CHOP-like regimen × 6 to 8, (R-)Hyper-CVAD × 8, or biweekly (R-) CHOP × 6 ± radiotherapy§§	40

Abbreviations: ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; ACE, doxorubicin, cyclophosphamide, etoposide; ACVBP, doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone; pts, patients; CAD, lomustine, doxorubicin, vindesine; CEEP, cyclophosphamide, epirubicin, vindesine, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CHVmPBV, cyclophosphamide, doxorubicin, teniposide, prednisone, bleomycin, vincristine; COP, cyclophosphamide, vincristine, prednisone; COPADM, cyclophosphamide, vincristine, prednisolone, doxorubicin, high-dose methotrexate; COPP, cyclophosphamide, vincristine, procarbazine, prednisone; CVAD, cyclophosphamide, vincristine, doxorubicin, dexamethasone; CYM, cytrabine, high-dose methotrexate; DLBCL, diffuse large B-cell lymphoma; EBV, epirubicin, bleomycin, vinblastine; H, high risk; H-I, high-intermediate risk; HL, Hodgkin's lymphoma; L, low risk; L-I, low-intermediate risk; MOPP, nitrogen mustard, vincristine, procarbazine, prednizone; NR, not reported; PMitCEBO, cyclophosphamide, mitoxantrone, etoposide, prednisolone, vincristine, bleomycin; R, rituximab.

*According to the Ann Arbor staging system.

†> 3 nodal cites, subdiaphragmatic involvement, bulky disease, erythrocyte sedimentation rate > 40 mm/hour.

‡Selected post hoc because of no information on B symptoms.

§Abstracted from total participants of original report, not exclusively for relevant patient population.

‖Some underwent high-dose chemotherapy followed by autologous stem-cell transplantation as consolidation therapy.

¶All received an eight-cycle biweekly consolidation therapy consisting high-dose methotrexate, etoposide, ifosfamide, and cytarabine after the ACVBP regimen.

#All underwent high-dose chemotherapy followed by autologous stem-cell transplantation with or without rituximab maintenance therapy.

**A portion of patients (n = 16) with limited-stage disease underwent 2 to 4 cycles of (R-)CHOP followed by involved field radiation therapy instead of full course (R-)CHOP.

††Patents with one age-adjusted international prognostic risk factor received an eight-cycle consolidation therapy, and patients with two or three factors underwent high-dose chemotherapy followed by autologous stem-cell transplantation.

‡‡All underwent high-dose chemotherapy followed by autologous stem-cell transplantation.

§§A portion of patients (n = 13) with limited-stage disease underwent 2 to 4 cycles of (R-)CHOP or similar regimens followed by involved field radiation therapy instead of full-cycle chemotherapy.

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PET at varied timing ranging from the first to fifth cycle.[36] We contacted the investigators for individual patient data, and excluded one patient who underwent PET at the fifth cycle. We found one study[14] through hand searching of the reference lists. As a result, we included 13 studies: eight studies[13,14,29,30,32-35] that met all eligibility criteria and five studies[12,31,36-38] with unpublished data available through contacting the authors (Table 1).[12-14,29-36,38]

Study Characteristics

Thirteen included studies had 360 advanced-stage HL patients and 311 DLBCL patients (Table 1). Eight reports were prospective single- or multi-institutional studies enrolling adults or adolescents. Only one study evaluated both adults and children.[34] Most of the patients in the HL studies underwent PET after receiving two cycles of first-line chemotherapy, while the number of cycles before the PET scan varied in DLBCL studies. In three DLBCL studies, 25% to 52% of included patients underwent PET after the fourth cycle.[34,36,38] One study evaluated PET after one cycle.[32] In general, participants underwent PET during the second week of intended chemotherapy cycle for biweekly chemotherapies (eg, doxorubicin, bleomycin, vinblastine, dacarbazine [ABVD] or (R-) doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone [ACVBP]) and during the third week for triweekly regimens (eg, (R-) cyclophosphamide, doxorubicin, vincristine, prednisone [CHOP]). Four studies performed CT for a portion of patients at the same timing as interim PET but they did not perform direct comparison between the two tests.[13,30,36,38]

For advanced-stage HL studies, fewer than 10% of included patients had unfavorable risk by standard prognostic tool (IPS > 3 points; Table 2). Progression or relapse rates were between 20% and 30% except for one study of 50%.[32] All studies adopted currently available standard first-line chemotherapy: six to eight cycles of ABVD or comparable regimens with or without radiotherapy. For DLBCL studies, the percentage of patients with unfavorable prognosis (high-intermediate to high risk by IPI) ranged from 0% to 59%, with progression or relapse rates of 27% to 47%. Full course (R-) CHOP and (R-) ACVBP were the two most widely adopted regimens. Two studies employed abbreviated course of (R-) CHOP or comparable regimens followed by involved-field radiation for patients with limited-stage disease.[12,36] No patients received rituximab in one study.[34] In four studies, some patients received consolidation auto-transplant after induction chemotherapy.[31,34,35,38]

Concerning imaging techniques and technologies, included studies generally followed guidelines by the Society of Nuclear Medicine (Table 3). One study exclusively adopted combined PET/CT scanner.[38] In five studies, some patients underwent combined PET/CT while the others were evaluated with stand-alone dedicated PET scanner.[13,29,30,32,36] All but one study[34] adopted attenuation correction for image reconstruction.

In general, multiple experienced nuclear medicine physicians interpreted PET results with pretherapy baseline scan as reference. All studies adopted qualitative positive and negative diagnostic criteria with various definitions (online-only Appendix Table A3). Only two studies clearly reported the referential backgrounds to define positive lesion. Five studies defined MRU criterion,[12-14,29,37] which was eventually reported as negative in three studies.[13,14,29] No study reported between-observer variability.

Quality Assessment of Published Studies

Only two studies[13,35] reported all items of the QUADAS tool (online-only Appendix Table A4). Reporting was especially limited in

Table 3. Technical Specification of PET for Interim Response Assessment of Malignant Lymphoma

Study	Year	Preparation: Measurement of Blood Glucose	Procedure: Type of PET Scanner	Procedure: Time of Scan After Injection (minutes)	Procedure: Attenuation Correction	Procedure: Image Reconstruction Method	Procedure: Administered Activity (MBq)
Advanced-stage HL + DLBCL							
Kostakoglu et al[32]	2006	Yes	PET-CT or dedicated	60	Yes	OSEM	370-444
Advanced-stage HL							
Friedberg et al[33]	2004	Yes	Dedicated	50	Yes	OSEM	370
Hutchings et al[37]	2005	Yes	Dedicated	60	Yes	NR	350
Gallamini et al[30]	2006	Yes	PET-CT or dedicated	60	Yes	OSEM or RAMLA	370/70, 259/70, 2*
Hutchings et al[13]	2006	NR	PET-CT or dedicated	45-90	Yes	OSEM	400
Zinzani et al[14]	2006	NR	Dedicated	70-90	Yes	NR	6†
Gallamini et al[29]	2007	Yes	PET-CT or dedicated	60	Yes	OSEM or RAMLA	370/70, 259/70, 2*
DLBCL							
Spaepen et al[34]	2002	Yes	Dedicated	60	No	OSEM	370-555
Haioun et al[31]	2005	Yes	Dedicated	60	Yes	OSEM	2†
Mikhaeel et al[12]	2005	NR	Dedicated	60	Yes	NR	350
Fruchart et al[35]	2006	NR	Dedicated	60	Yes	OSEM	2.5†
Querellou et al[38]	2006	Yes	PET-CT	73 ± 15‡	Yes	OSEM	5.0-7.6†
Ng et al[36]	2007	Yes	PET-CT or dedicated	60-70	Yes	OSEM	5†

Abbreviations: CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; HL, Hodgkin's lymphoma; NR, not reported; OSEM, ordered subsets expectation maximization; PET, positron emission tomography; RAMLA, row-action maximum likelihood algorithm; SUV, standard uptake value.

*Three hundred seventy MBq/70 kg at the centers that used a GE scanner, 259 MBq/70 kg at the centers that used a Philips scanner, and 2 MBq/body weight kg at the centers that used a C-PET scanner.

†Administred activity was reported as the amount per body weight MBq/kg; eg, 360 MBq was administered to a 60 kg patient for 6 MBq/kg).

‡Mean ± standard deviation.

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three retrospective studies.[14,36,37] Physicians' knowledge of interim PET results may affect their assessment of patients' response as well as their treatment decisions, introducing biases.[39] Only three prospective studies[13,29,35] explicitly adopted blinding of clinicians to interim PET results to deal with these biases. In three prospective studies,[14,30,31] although they did not explicitly report the use of blinding, interim PET was not utilized to alter the preplanned treatment strategies. In two retrospective studies,[32,38] interim PET results had no effect on the treatment decisions. Because the assessment of treatment failure is not always objective, the absence of blinding can still potentially influence the way treating physicians judge the final clinical outcome in favor of interim PET, especially when the outcome is equivocal.[11,39] Although all the studies adopted the standard guidelines on response assessment[40,41] as the reference standard, they did not specify minimum follow-up period or situations where pathological confirmation was required. Four studies[29,32,33,38] employed post-therapy or follow-up PET to complement post-therapy response assessment. Because post-therapy response assessment with PET is still imperfect,[9] the applied reference standard could overestimate prognostic accuracy.[39]

Sensitivity, Specificity, LRs, and Summary ROC Curves

For advanced-stage HL, studies reported sensitivity from 0.67 to 1.00 and consistently high specificity from 0.94 to 1.00 for interim FDG-PET (Table 4; Fig 1). Summary estimates were 0.81 for sensitivity (95% CI, 0.72 to 0.89), 0.97 for specificity (95% CI, 0.94 to 0.99), 28.4 for positive LR (95% CI, 14.2 to 56.7), and 0.19 for negative LR (95% CI, 0.12 to 0.30). We did not estimate summary ROC curves because data points were closely clustered together with limited variations, a situation in which the hierarchical model could not produce reliable estimates (Fig 2).

DLBCL studies reported wide-ranging sensitivity (0.50 to 1.0) and specificity (0.73 to 1.00) values for interim FDG-PET (Table 4; Fig 1). Combined estimates had a sensitivity of 0.78 (95% CI, 0.64 to 0.87), a specificity of 0.87 (95% CI, 0.75 to 0.93), a positive LR of 5.9 (95% CI, 2.8 to 12.3), and a negative LR of 0.26 (95% CI, 0.15 to 0.46). The Q* statistic for the summary ROC curve was 0.82 (Fig 2).

In sensitivity analyses, the summary prognostic accuracy was stable for both advanced-stage HL and DLBCL regardless of how MRU results or early-censored cases without treatment failure were counted (results not shown). Regarding alternative reference standards based on the duration of clinical follow-up, subgroup data were available for five advanced-stage HL studies (n = 232)[13,14,30,32,37] and five DLBCL studies (n = 181)[12,32,35,36,38] (online-only Appendix Table A5). All DLBCL studies had improvement in sensitivity with loss of specificity when only progression during first-line therapy was counted by the alternative reference standard. A similar tendency was

Table 4. Study Results of Positron Emission Tomography for Interim Response Assessment of Malignant Lymphoma

Study	Year	Total No.	Progression or Relapse (%)	TP	FN	FP	TN	Sensitivity	95% CI	Specificity	95% CI	Positive Likelihood Ratio	95% CI	Negative Likelihood Ratio	95% CI
Advanced-stage HL															
Friedberg et al[33]	2004	22	23	4	1	1	16	0.80	0.28 to 1.00	0.94	0.71 to 1.00	13.6	1.9 to 95.7	0.21	0.04 to 1.23
Hutchings et al[37]	2005	28	32	6	3	0	19	0.67	0.30 to 0.93	1.00	0.82 to 1.00	26.0	1.6 to 416.8	0.36*	0.15 to 0.84
Gallamini et al[30]	2006	108	19	18	3	2	85	0.86	0.64 to 0.97	0.98	0.92 to 1.00	37.3	9.4 to 148.4	0.15	0.05 to 0.42
Hutchings et al[13]	2006	46	28	10	3	1	32	0.77	0.46 to 0.95	0.97	0.84 to 1.00	25.4	3.6 to 178.9	0.24	0.09 to 0.64
Kostakoglu et al[32]	2006	10	50	5	0	0	5	1.00	0.48 to 1.00	1.00	0.48 to 1.00	11.0	0.8 to 158.0	0.09	0.01 to 1.31
Zinzani et al[14]	2006	40	23	8	1	0	31	0.89	0.52 to 1.00	1.00	0.89 to 1.00	54.4	3.4 to 861.6	0.15†	0.04 to 0.67
Gallamini et al[29]	2007	106	20	15	4	4	83	0.79	0.54 to 0.94	0.95	0.89 to 0.99	17.2	6.4 to 46.0	0.22	0.09 to 0.53
DLBCL															
Spaepen et al[34]	2002	47	47	20	2	0	25	0.91	0.71 to 0.99	1.00	0.86 to 1.00	46.3	3.0 to 724.1	0.11	0.03 to 0.36
Haioun et al[31]	2005	83	39	20	12	14	37	0.63	0.44 to 0.79	0.73	0.58 to 0.84	2.3	1.4 to 3.8	0.52	0.32 to 0.83
Mikhaeel et al[12]	2005	57	38	15	7	8	27	0.68	0.45 to 0.86	0.77	0.60 to 0.90	3.0	1.5 to 5.8	0.41‡	0.22 to 0.78
Fruchart et al[35]	2006	35	29	9	1	6	19	0.90	0.56 to 1.00	0.76	0.55 to 0.91	3.8	1.8 to 7.8	0.13	0.02 to 0.86
Kostakoglu et al[32]	2006	24	38	9	0	1	14	1.00	0.66 to 1.00	0.93	0.68 to 1.00	10.1	2.2 to 46.8	0.06	0.00 to 0.83
Querellou et al[38]	2006	21	29	3	3	1	14	0.50	0.12 to 0.88	0.93	0.68 to 1.00	7.5	1.0 to 58.6	0.54	0.24 to 1.21
Ng et al[36]	2007	45	27	8	4	4	28	0.67	0.35 to 0.90	0.88	0.71 to 0.97	5.3	2.0 to 14.5	0.38	0.17 to 0.86

Abbreviations: DLBCL, diffuse large B-cell lymphoma; FN, false negative; FP, false positive; MRU, minimal residual uptake; TN, true negative; TP, true positive.
*The likelihood ratios for a MRU and a negative scan were 0.35 (95% CI, 0.05 to 2.5) and 0.33 (95% CI, 0.09 to 1.1), respectively, if these two categories were estimated separately.
†The likelihood ratios for a MRU and a negative scan were 1.1 (95% CI, 0.14 to 9.7) and 0.06 (95% CI, 0.00 to 0.84), respectively, if these two categories were estimated separately.
‡The likelihood ratios for a MRU and a negative scan were 0.96 (95% CI, 0.25 to 3.6) and 0.29 (95% CI, 0.12 to 0.73), respectively, if these two categories were estimated separately.

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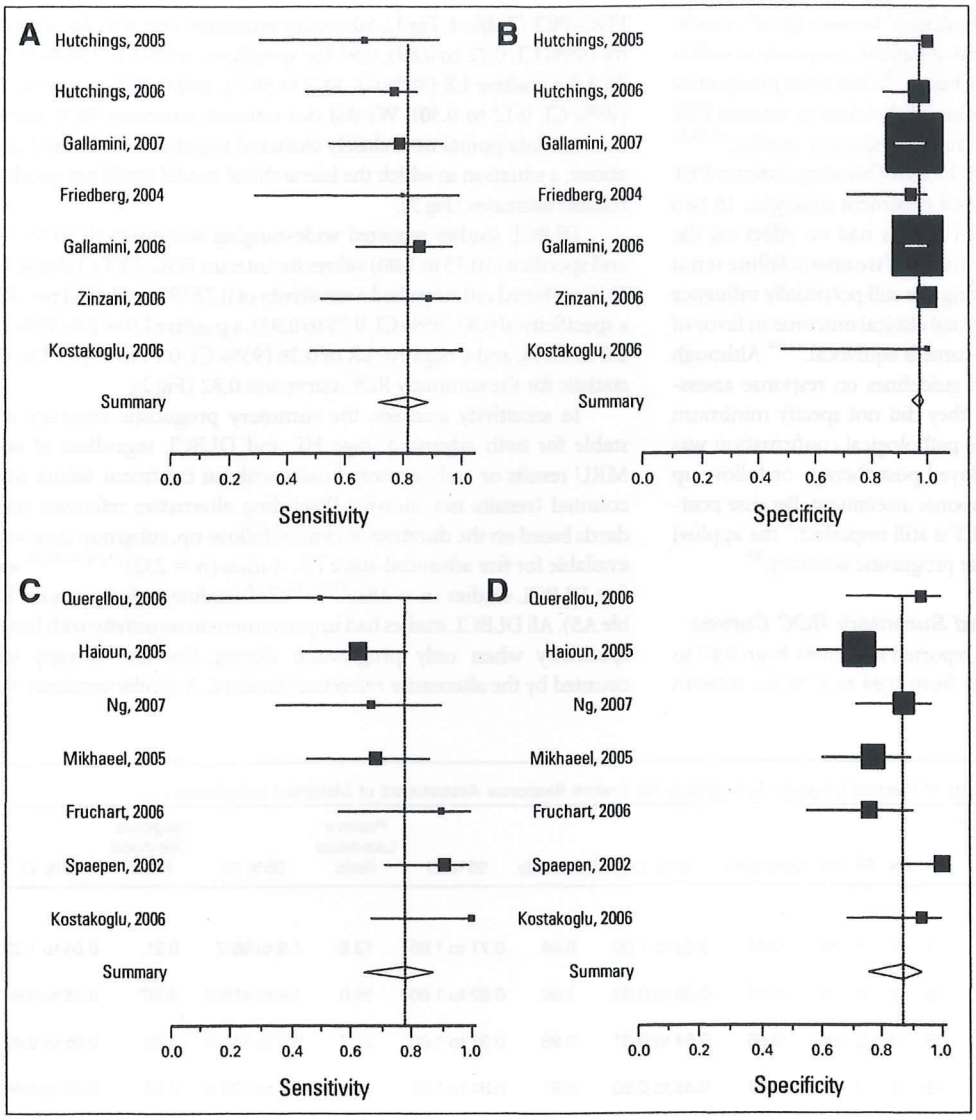

Fig 1. Sensitivity and specificity for (A, B) advanced-stage Hodgkin's lymphoma and (C, D) diffuse large B-cell lymphoma. The size of the square plotting is proportional to the number of patients with treatment failure for sensitivity and in remission for specificity. The horizontal lines are the 95% CIs. The vertical lines represent the summary estimates.

observed in all but one[30] advanced-stage HL studies (online-only Appendix Fig A2).

Subgroup Analyses and Meta-Regression Analyses

We did not perform subgroup analyses for advanced-stage HL because there were too few data points and there was little variation of the results across studies (Fig 1). Visual assessment of the ROC plots of DLBCL studies did not identify meaningful subgroups (data not shown). Meta-regression analyses on both advanced-stage HL and DLBCL did not find any clinical or test characteristics to explain the observed variability (data not shown).

DISCUSSION

This systematic review of interim response assessment of FDG-PET for patients with untreated advanced-stage HL showed that studies consistently reported high specificity and positive LRs. Although study quality was limited in some studies, as demographic and clinical characteristics of included patients were reasonably comparable over the studies, our results should generally be applicable to adult and adolescent patients with low- to intermediate-risk (IPS 0 to 3) receiving standard full course ABVD or comparable regimens. Because the summary positive LR is very high, positive PET results after a few cycles of chemotherapy would probably have an excellent ability to predict poor responders. Patients with negative PET, which predicts good response during the therapy, still have a moderate risk of post-treatment relapse since the summary negative LR is 0.19.[42]

The reported sensitivity and specificity of DLBCL studies of interim FDG-PET varied. This review also identified considerable clinical heterogeneity in these studies. For example, studies included patients with varied risk of treatment failure and adopted various therapeutic interventions. Also, studies were heterogeneous in how PET was used, such as the number of chemotherapy cycles before PET

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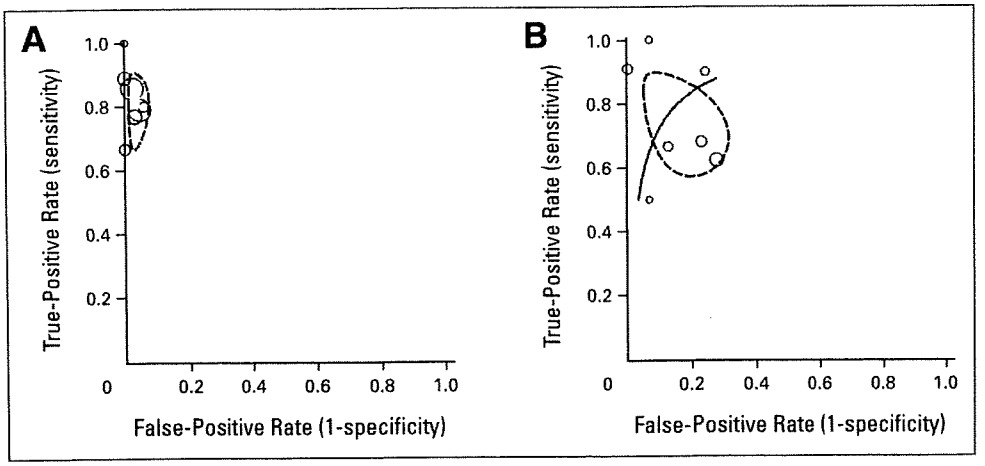

Fig 2. Receiver operating characteristic (ROC) plotting for (A) advanced-stage Hodgkin's lymphoma and (B) diffuse large B-cell lymphoma. Individual study estimates of sensitivity and 1 – specificity are shown (open circles). Summary ROC curve is presented only for DLBCL. Closed square represents the summary estimates. Dashed boundary represents the 95% confidence region for the summary sensitivity and specificity.

and the timing of scanning during the chemotherapy cycle.[43] Thus, our summary estimates should be interpreted carefully. Although we performed subgroup analyses and meta-regression analyses, we could not identify characteristics to explain the variability.

This study has several important limitations. Because only 13 studies with pertinent data were included in the meta-analysis, it may lack the power to detect clinically meaningful factors. In sensitivity analyses, fewer studies were available; therefore, the results may be less reliable. Although we did not independently estimate the summary LR for a MRU result, this distinct category may carry a worse prognosis than a clearly negative scan as reported.[12,14] Also, our results are likely subject to overestimation due to methodologic limitations in original studies, such as the absence of blinding of interim PET results to clinicians to assess final clinical outcomes.[11] Further, because of lack of data, we did not address the comparison between FDG-PET and CT or FDG-PET/CT and PET alone[38]; this review cannot answer whether PET is better than CT or whether the combined modality is superior to stand alone PET. In addition, this review did not specifically focus on limited-stage lymphoma; thus our results cannot answer the clinical question of whether early-interim PET can reliably identify good responders with localized disease. Finally, although three advanced-stage HL studies[13,29,30] and one DLBCL study[31] reported interim FDG-PET scan as a statistically significant independent prognostic factor in addition to IPS and IPI, respectively, we did not directly address this issue. For advanced-stage HL, because the included studies had few poor-risk (IPS 4 to 7) patients, our results may be less applicable to high-risk populations.

Interim PET should remain at this time as a test to be evaluated as part of clinical research where treatment regimens and imaging conditions are standardized; thus it should not be employed in the routine setting. This review supports conducting prospective trials for advanced-stage HL patients especially with low- to intermediate-risk (IPS 0 to 3) that incorporate early altering treatment to more intensive approach on the basis of positive FDG-PET results. For DLBCL, there is insufficient data to support similar trials. Additional prospective prognostic accuracy studies in the setting of conventional strategy would be needed to elucidate subgroups and timings of interim PET to better identify poor responders. Also, outside of study protocols where treatment strategies are explicitly defined on the basis of scan results, biopsy should be considered for positive PET findings if they are used to prompt a change in patient management. This is especially relevant if there is discrepancy between the scan results and other clinical data. Although biopsy cannot provide quantitative information as to how much residual tumor exists, it still is the most reliable way to confirm the presence of disease.

Authors' Disclosures of Potential Conflicts of Interest

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