本ワークショップには300名以上の参加者があった．日本からは，早慶から酒井，堀之内，小林が，また，東海大学医学部の川口章先生が参加した．昨年北京で開催された第11回国際血液代替物学会（11th-ISBS）では，中国でのHBOCsの研究が国家プロジェクトとして推進されていることが良く解ったが，今回も中国からの参加者も散見され，HBOCsの開発の結末がどうなるのか静観しているように思えた．北米の非細胞型の化学修飾HBOCsの開発で生じた問題点を凝視し，分子デザインから新たな展開を開始する時期に差し掛かったのかもしれない．

以下にプログラムに従って，各発表者の内容について，パワーポイント発表および小生のメモをもとに，重要なことを書き出した．しかし，小生の英語力の無さのため，聞き落としや誤解もあるし，時差ぼけの影響もあり，物足りないと感じられることもあろう．また，空欄の箇所もできてしまったことには，お詫び申し上げたい．詳細について知りたい読者には，FDAのホームページにTranscriptが掲載されているので，これを参照して頂きたい（http://www.fda.gov/cber/blood/hboc042908.htm）．

Tuesday, April 29, 2008

Opening remarks

Jesse L. Goodman (Center for Biologics Evaluation and Research, FDA)

Simone Glynn (National Heart, Lung and Blood Institute, NIH)

Jerry A. Homberg (Office of the Secretary and Office of Public Health and Science, HHS)

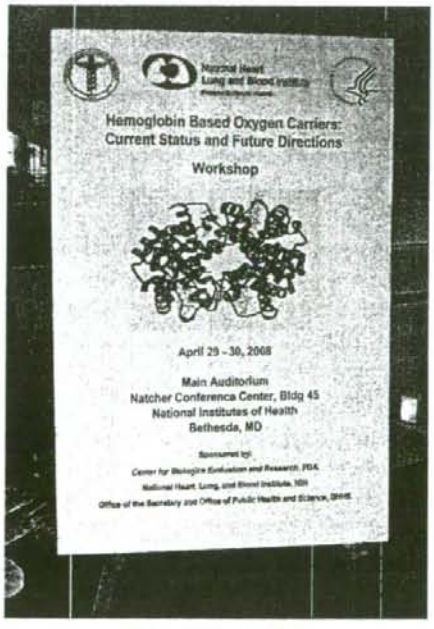

写真1．会場入口の案内

Session I, Workshop Overview and HBOCs Update

Overview of the Workshop

Moderator: Joseph C. Fratantoni (Maxcyte)

Overview of Oxygen Physiology

H.F. Bunn (Harvard Medical School)

血行動態が維持されなければ，酸素は運搬できない．NO捕捉を抑制すること，生理的に適ったP_{50}値，膠質浸透圧の調節が重要であることを主張．また，StamlerらのSNO-Hbの説について，Hbのβ93CysがSNO-Hbの発生部位なので，これをアラニンに置換したマウスを使って検討したところ，血管収縮などは全く無かったとの論文[9]が最近出ており，SNO-Hbの説を否定している．

HBOCs: Biochemical and Physiological Perspectives

A.I. Alayash (CBER, FDA)

Hbの毒性について詳細に説明した．先ず，Hemosol社のo-raffinose polymerized Hbについて．分子量分布が広く，monomeric Hbも含有する，ヘム構造が歪んだrhombic hemeの存在，T構造が固定されていて，酸素親和度が異様に低い（P_{50}が高い），様々な有用性が言われているβ93Cysが化学修飾のときの架橋点になっている．H_2O_2との反応により毒性の高いフェリルHbを産生する．心筋に対する毒性が組織病理から明らか．NO反応と自動酸化によるメト化を抑える方法が必要．Biopure社のOxyglobinについて，アスコルビン酸を体内で産生する動物種（例えばラット）であれば，血中でmetHbが還元される．しかし，ヒトではそうはいかない．HbとSOD/Catalaseの架橋，trolox, glutathioneなど抗酸化剤の利用を検討すべき．

Nitric Oxide and Nitrate Ions: Physiology, Pathology and Pharmacology

Alan N. Schechter (Molecular Medicine Branch, NIDDK, NIH)

HbとNOの反応の概説．NO吸入によるHbNOの増大と同時に，Nitrate（NO_3^-）が顕著に増大する．Nitrite（NO_2^-）はさほど増大しない．NitriteはdeoxyHbで還元されてNOになることが明らかになった．Nitriteの投与試験から血圧の降下を実証．NitriteをNOドナーとして利用できる．

Non-Clinical Testing: Strengths and Limitations

George P. Brio (Univ. of Ottawa and Univ. of Toronto)

従来のICH基準，およびGLP基準の非臨床動物実験（健常動物への単回投与，反復投与）では，安全性について十分な評価ができないのではないか．見落とす項目が出る可能性あり．疾病モデル動物の使用が必要．アカデミックな研究では，一般に効能についての研究が多く，また観察項目が限定されているため，一般性が持てない場合がある．血管内皮傷害は，様々な病態と

関係している．HBOCを投与するとNOが捕捉されるので，血管障害を助長する可能性がある．

Session II , Clinical Experience with HBOCs

Introduction to the Issues

Toby A. Silverman (CBER, FDA)

臨床試験の考え方について概説．Traumaに対する投与試験の難しさ．死亡率は明白なendpointであるが，短期生存と長期生存の結果が一致しない．QOLのある長期生存が患者およびその家族に最も重要である．輸血の出来ない場合（遠隔地など）を想定した臨床試験の倫理的・技術的問題．これまでの各社臨床試験の結果を解析し，その副作用をまとめた一覧表を提示（左心室心筋への影響，心筋虚血，血管収縮，消化器系の不快感/嘔吐/下痢/嚥下傷害/腹痛/腸管からのバクテリア侵入）．警告（Caveats）として，臨床試験の全てが公表されている訳ではない．今回ここに公表されているデータは，FDAに最終報告されたものとは違う．データ解析法や，解釈に問題がある場合もある．血中酵素濃度の上昇を，副作用とみなしていない場合がある．最後に課題として，もっとHBOCsの特徴について科学的に検証する必要あり．臨床上の利益を得る点ではendpointを明確にする必要あり．Survivalのみでは十分な評価ができない可能性があり，Surrogate markerを設定して，安全性と有効性を評価する必要がある．臨床的安全性の理解（最大投与量など），投与の損益の理解，論理的な臨床開発プログラムの決定，など．

Risk: Benefit considerations in Clinical Trials

Sara F. Goldkind (Office of the Commissioner, FDA)

FDAは，その薬剤を使用することの利益が，既知の毒性または副作用の可能性よりも重要であるかを検討する．また，病態が重篤であるか，他の治療法が無いかも考慮して，薬剤の危険性について回答するか判断する．

Presentations from Industry: Proposed Clinical Indications for HBOCs and Clinical Trial Experience to Date

Moderator: Barbara Alving (National Center for Research Resources, NIH)

Development of Hemospan

Peter Keipert (Sangart Inc.)

Dr. Robert M. Winslow 欠席のため代理としてDr. Keipertが発表した．様々なHBOCsがあるが，同じではない（Polymerized Hbsと一緒にして欲しく無いという意味）．Hemospanは若干の血圧亢進はあるが心拍出量を増大させ，末梢血管抵抗を低下させる．Autoregulatory Theoryの観点から重要なことは，分子サイズが大きいこと，酸素親和度が高いこと（P_{50}値が低い，5 mmHg），低Hb濃度（4.3 g/dL），膠質浸透圧が高いこと．NO結合速度と血管収縮は関係がない．Phase II（Sweden）では，整形外科手術（関節形成術）における投与試験を実施．血圧維持効果を実証．二例の死亡例あったが，Hemospanの投与とは無関係と判断．Phase III（欧州）を開始する予定．低血圧の予防（n = 370）と治療（n = 460）の試験について被験者登録名簿が今年完了する見込．Hemospanの用途は，ショックの蘇生，術中の血圧安定化，血液が利用できない場合の輸血迄のつなぎ，虚血領域への酸素ターゲティング．限界は，Hb濃度が低いので，血液希釈度が高いときに対応できない，膠質浸透圧が高いので，体液量過剰が起こりうる．

写真2．会場風景．左から，Dr. De Angelo, Dr. Estep, Dr. Greenburg, Dr. Abuchowski, Dr. Gould, Dr. Keipert, そしてDr. Goldkind

Clinical Development of Polyheme

Steven A. Gould (Northfield Laboratories, Inc.)

「Unmet medical needs」（いまだ満たされていない医学的ニーズ），つまり赤血球輸血が出来ない状況での対処法として，Polyhemeが有効である．I U =500 mL, [Hb] = 10 g/dL，1年間保存可能．Hospital Trauma Trial（n= 171）では，投与量に応じて血中Hb濃度が高くなること，死亡率の減少を確認[1]．Pivotal Phase III Trial（n = 720）重篤な外傷出血患者を対象とした試験では，過去に問題点が指摘された．実際には，124名の患者の登録が正しく行われなかったので，再度解析を行った．その結果，PolyHeme投与の方が，副作用の発症率が高い（肺炎，MOF，出血性ショック，呼吸障害，凝固亢進，敗血症，心筋梗塞）．それでも，赤血球輸血が出来ない状況での対処法として，Polyhemeは有効である．

HBOCs: Current Status and Future Directions

Abraham Abuchowski (Prolong Pharmaceuticals)

PEG-bovine Hbを開発したEnzon社の設立者．PEG修飾蛋白質3種類が既にFDAの認可を得ている（Adagen, Oncaspar, PEG-Intron）．PEG-HbについてはEnzon社の時代に，Phase Ib（n = 60）を実施．PEG-Hbの特徴: P_{50} = 14 - 20 mmHg, [Hb] = 4 - 6 %, met Hb < 10%, -20℃で6ヶ月以上安定，4℃で7日間安定．イヌ投与試験（30%血液交換）では特に問題なし．ブタ投与試験（80%血液交換）では，腎臓と脾臓に空包変性があるほかは特に副作用なし．Phase I試験（n = 34），1.67 - 8.33 ml/kgを投与．食道痙攣がみられた例があったが，鎮痙薬（levesinex）の投与で解消した．適応としては，放射線増感剤

があり，転移性疾患の患者に対するPhase Ib試験（n=33）では，2－8 ml/kgを投与した．副作用としては，緩慢な血圧上昇（24%），不全失語症（24%），吐き気（24），嘔吐（12%）．製品名AfterShock™は，PEG-Hbを高張食塩水溶液に溶解させた溶液．ショックの蘇生液として期待される．

Biopure HBOC-201

A. Gerson Greenburg (Biopure Corp.)

終始，過去のFDAの見解に対する反論．HBOCsの副作用について，全てのHBOCsが同じではない．安全性のシグナルが毒性と解釈されることがある．血管活性と主要臓器に対する毒性の因果関係は無い．HbOC-201は生命を維持するに十分な酸素運搬が出来る．FDAが要求した補足実験（ブタ10-30%交換輸血後の臓器血流量と酸素分圧の測定）の結果，主要臓器の血管収縮の証拠は無し．骨格筋では血管収縮あり．冠動脈の血管収縮なし．Pittmanらの微小循環計測の結果，血圧の亢進と骨格筋の血管収縮はあったが，腸管では血管収縮なし．臨床試験でも冠動脈の収縮は無い．血圧亢進はあっても，冠動脈性心臓病の患者に影響を与えない．動物実験では，血液が無い状態でも，左心室系の機能を維持できた．臨床試験において患者の管理方法に問題があったため，効能の評価に不整合が生じた．重篤な虚血状態に対しては，赤血球であろうとHBOCsであろうと副作用を低減させる事はできない．血液が無い時，HBOCsは利用できる利点がある．HBOCsの臨床試験は継続すべきである．今行うべきことは，安全性シグナルを正確に解釈する事，現在のマテリアルの最も適した用途を考える事，そしてHBOCsの開発を継続する事．

Lessons Learned from the Baxter Experience in the development of HBOCs

Timothy N. Estep (Chart Biotech Consulting)

Baxterの分子内架橋Hb（DCLHb）の副作用の一つに心筋損傷がある．実はpreclinical studyから解っていた．IND（investigational new drug）申請までの2年間にこの副作用の解析に費やした．その結果は，既に論文で公表されている[5]．サル，ブタで投与後24-28時間で発症するが，時間と共に治癒する．CK_{mb}，LDH_1はさほど上昇しなかった（但しoverallのCK，LDHは，ブタで上昇した）．心機能的には問題は無かった．心筋損傷は，SFHbでも認められた．またHbを重合，或はNO結合を遅くする事で低減された．NOS阻害剤であるL-NAMEの投与で同様の心筋損傷が認められた．しかしヒトでの確認は生検に困難を伴う．次に血管収縮について，NO反応性の制御，血管外漏出の抑制によって低減されるが，種差がある．何らかの薬剤の併用によって対処できる？．米国のPhase IIIでは，DCLHbの死亡率（46%）が生理食塩水投与（17%）に比較して高くなったため，試験を中止した．しかし，因果関係は解らない．事実，欧州での試験では，死亡率は42% vs. 38%で，さほど違いはなかった．重篤な外傷患者の登録方法，管理方法に問題があったか．また，蘇生方法自体の問題（overloadなど），Halothaneとの相互作用，LPSとの相互作用，外傷の種類（鈍的外傷が貫通外傷よりDCLHb投与後の死亡率が高い），外傷性患者を対象とする臨床試験の難しさもあったか．重篤な患者の方がTreatment groupになり易い？その他，副作用として，DCLHb投与によってMOFになり易い傾向，膵臓炎，NO捕捉による酸素消費量の増大?今後HBOCsの開発に必要なことは，全組織への血流分布の計測，臓器の病理検査，非侵襲のモニタリングなど．問題は多いが，unmet medical needs の対処法としてHBOCsは有望である．

Development of PHP as an NO Scavenger in the Treatment of Distributive Shock.

Joseph De Angelo (Apex Bioscience, Inc.)

PHP（pyridoxalated hemoglobin polyoxyethylene conjugated）は，血管外漏出し，細胞間質のNOと反応する．正常の場合には，NOは重要な働きを示すが，過剰量になると問題になる．この過剰産生されるNOの捕捉をPHPで行う．カテコールアミンが血液分布異常性ショックの治療に使用されるが，問題点もある．これに比較してPHPの方が，副作用が低減されるものと期待できる．臨床試験（SIRS患者，ショック患者に対する投与: 20 mgHb/kg/hr）の結果，MAPの急激な上昇がみられたが，28日生存率は，PHP 57.6%に対してPlacebo 58.6%で殆ど違いは無い．心筋虚血の発症例も，高めであった（淡々と発表をしていたが，PHPの利点が殆ど解らない発表であった．）．カテコールアミン耐性の患者を対象としたPhase IIIを計画中．

Panel Discussion

Wednesday, April 30, 2008

Session III, Clinical Findings and Mechanisms

Adverse Events (T/C)

	Baxter	Biopure	Hemosol	Northfield	Sangart
N	504/505	708/618	209/192	623/457	85/45
Death	78/61	25/14	1/4	73/39	2/0
HTN	76/38	166/59	113/75	NR	7/1
CHF	0/1	54/22	0/2	17/20	NR
Cardiac Arrest	NR	17/6	1/1	14/9	NR
MI	6/1	14/4	14/7	29/2	2/0
Arrhythmia	23/17	153/100	1/1	NR	15/5
CVA	NR	16/3	2/1	3/1	NR

写真3．各社HBOCsの臨床試験の結果総括．T/C, Treatment versus Control; MI, myocardial infarct 心筋梗塞; CHF, chronic heart failure, 慢性心不全; CVA, cerebrovascular accident, 脳血管障害; HTN, hypertension, 高血圧; cardiac arrest, 心停止; arrithmia, 不整脈

Functional Aspects of the HBOCs as a Class
Panel Discussion

Moderator: Harvey Klein (Dept. of Transfusion Medicine, Clinical Center at NIH)
冒頭，臨床試験の結果全てが公表されている訳では無い事，FDAも知らされていない結果がある事を公言した．

Demetrios Demetriades (Univ. of South California)
臨床試験の難しさ．外傷の種類によって死亡率が異なる(penetrating trauma, blunt trauma)．輸血において大量輸血を必要とした場合，パラメータとして，血漿または血小板：赤血球の比が死亡率を決める重要な項目であることを主張．この比が1：8のとき，死亡率は65%．1：1.4のとき19%にまで低下．

Daniel Freilich (Naval Medical Research Center)
軍服姿で発表．非臨床試験の結果から，外傷を対象とする臨床試験のプロトコルを決定しなければならない．効果を最大限に引き出すため，死亡率が高い重篤な出血性ショック患者を対象とする．輸血ができない状況を選択する．Hb濃度の高いHBOCsを用いる．損害を最小限にするため，高齢よりも若年層を対象とする．血圧の回復だけを指標としない．他の汎用輸液も併用する，担当者の危機管理意識，副作用が出たときには直ぐにHBOCsの投与を止める．副作用の報告は迅速に，血管活性の少ないHBOCsを選ぶ，止血ができたらニトログリセリンを投与して，HBOCsの血管活性の影響を低減させる．制御不能出血に使用する状況を考えている様子であった．

John Holcomb (Univ. of Texas Health Science Center at San Antonio)
大量出血患者がHb濃度が11 g/dLとすれば，Hbは十分にある．Bleeding problemとperfusion problemをどう考えるか．各社とも臨床第1相，第2相試験で，利点が得られなかったが，出血性ショック患者について現場で，試験対象患者基準と，除外基準をどうすべきであったかが，最も重要であった．

Charles Natanson (Critical Care Medicine, NIH)
現在のHBOCsの臨床試験の結果について，JAMAに発表の内容を説明（記述の通り）[11]．メタ解析の結果から，現行のHBOCsは死亡率が平均で30%上昇，心筋梗塞の発症率ともに高い．違うものを一色単にして解析して良いのかという議論もあるが，どれか一つの製品についてのデータを除去しても，同じ結果になった．P_{50}値との関係，公表データと未公表データの関係なども検討したが，結論からいうと，臨床試験プロトコル，製造者，物質の特性，結果の出典に拘わらず，死亡率，心筋梗塞の発症率はHBOCs群で高い．

Edward J. Norris (Johns Hopkins Univ. School of Medicine)
発表スライド無し．エホバの証人の患者100人に対し，総量200unitも投与してきた．HBOCsがunmet medical needsに対応できることを評価するべき．

Edward P. Sloan (Univ. of Illinois at Chicago)
Baxter社のDCLHbの臨床第三相試験：重傷出血性ショック患者に対する投与で，投与28日後の死亡率の評価では，Control群で32%に対し，DCLHb群で45%と高い値あった[12]．しかし，DCLHb投与に起因する血圧亢進は殆ど無かった．また，塩基欠乏，乳酸値についてもDCLHbとの因果関係は無かった．ショック指数について（SI = HR/BBPが ≧1は，非代償性ショック，SI ＜1は代償性ショック），DCLHbを投与することによるSIの変化は無かった．DCLHbの検討の結果，i) HBOC は重要である，ii) 論理的に想定されるDCLHbの影響が，*in vivo* でいつも観察される訳ではなかった，iii) 臨床的に有用なHBOC蘇生液を見つける必要あり．今後のHBOCs開発に対する忠告として，i) HBOCsの基礎研究と臨床試験は継続すべき．ii) 論理的に想定できる現象が臨床的に認められるか見極める，iii) 効能に関する質問に的確に答えるられるよう，臨床試験の方法の最適化を行う．

Gus J. Vlahakas (Harvard Medical School)
Biopure社のHBOC-201の臨床第二相試験について．心臓手術後の血液希釈された状態に使用し，ヘマトクリットが回復するまでの一時的な酸素運搬体として使用し，同種血輸血を回避することが目的．特に心筋梗塞は認められなかった．しかし，HBOCsは血管収縮を起こす事が良く知られている．NOの結合，体動脈圧，肺動脈圧の上昇が報告されているが，NO結合と血管抵抗の上昇が，代謝性自動調節機能を覆すような作用を及ぼす事は無い．HBOCsの特性が有用である場面もありうる．血管収縮の他に，HBOCsはどのような不安定要素を持っているか．消化器系において，膵臓疾患（lipase, amylase 上昇），肝臓疾患（AST, ALT上昇），胸痛（食道の蠕動運動への影響）が報告されている．心筋梗塞について，DCLHbのサルを使った実験で最初に報告され，L-NAME投与のばあいと同様の症状であった．NOの結合が低減されたrHb2.0で低減された．

Stephen Cohn (Univ. of Texas Health Science Center at San Antonio)
米国では，Trauma Centerに1時間以内に到着出来るのは40%．従って田舎で外傷に遭うと，輸血が出来ない場合がありうる．

Organ Specific Aspects of Safety
Panel Discussion

Moderator : Richard Weiskopf (Univ. of California, San Francisco)

Renal (Perfusion) 腎・循環系
Andrew D. Baines (Univ. of Toronto)
急性腎傷害では，creatinine値は余り信頼性が無い．重要な初期マーカーとして，尿量，N-acetyl-beta-D-glucosaminidase (NAG), Urinary neutrophil gelatinase-associated lipocalin (NAGL), IL-18, Kidney injury molecule（KIM-1）などがある．

Gastrointestinal 消化器系
Mitchell P. Fink (Logical Therapeutics)
急性膵臓炎（Lipase, amylase上昇），肝細胞傷害（AST, ALT上昇），食道蠕動への影響

Cardiac 心臓系
David C. Warltier (Medical College of Wisconsin)
リコンビナントヘモグロビン: rHb1.1の投与により，HR低下，MAP上昇，LVEDP上昇．HBOCsの投与による心筋損傷は，Burhopらが初めて発表した．CPK上昇，壊死など．

Central Nervous System 中枢神経系
Raymond F. Regan (Thomas Jefferson Univ.)
神経系細胞培養系にHBOCsを添加した実験では，毒性は無いことを確認している．しかしMP-4（Sangart社製）の添加ではLDHの上昇が認められた．metHb生成が速いためか．臨床試験ではHBOCsの脳神経系への影響は良くわかっていない．

Shock Mechanism ショックの機序
Joseph E. Parrillo (Univ. of Medicine and Dentistry of New Jersey)
ショックの分類，病因，敗血症の際の心筋異常について概説．最近出版されたNEJM誌を参照[6]．

Pulmonary 肺系
Mark Gladwin (Natuional Heart, Lung and Blood Institute)
肺に関連するHBOCsの副作用として，肺高血圧症，心拍停止，肺炎，呼吸停止，ARDS（急性呼吸促迫［窮迫］症候群），MOF，血栓症などがある．

Session IV Finding a Way Forward: What is the Best Way Forward Scientifically and Ethically?
Moderator: George P. Brio (Univ. of Ottawa and Univ. of Toronto)

Biochemical Approaches and Mitigation Strategies for HBOCs

The Way Forward: Can Nitrite Modulate HBOC Toxicity?
Mark Gladwin (National Heart, Lung and Blood Institute)
亜硝酸イオン（NO_2^-）がdeoxyHbと反応して，NOを産生する反応の機序と，生理学的な役割，更には投与の効果について概説[7]．また，HBOCs投与後の血圧亢進を，NO吸入によって低減させる事に成功したZapolらの論文を紹介[8]．同様にNO_2^-を投与してやれば，HBOCsと反応してNOを産生し，血管収縮作用が低減出来るのではないか．

Strategies for Engineering Safer, More Efficient and More Stable Recombinant Hemoglobins for Use as O_2 Delivery Pharmaceuticals
John S. Olson (Rice University)
遺伝子組換えHbの分子設計において，P_{50}値の制御による酸素輸送量の調節，NO結合速度の制御による血管収縮の抑制が可能となっている（Somatogen-Baxter社の例）．また，自動酸化の低減，ヘム遊離の低減，などが検討されている．大量製造も可能ではあるが，実際には，費用の問題，E. coliからの産生であるので，LPSの除去を効率よく行うことが課題である．また，分子量を大きくするための工夫（化学修飾，カプセル化）も検討する必要がある．

Role of Microvascular Reactions in the Design of Hb Based Oxygen Carrying Plasma Expanders
Marcos Intaglietta (La Jolla Bioengineering Institute, University of California, San Diego)
各種HBOCsを投与した後の微小循環系の観察から明らかになったことは，血漿中のHb濃度は，血管収縮を誘発しうる重要因子である．血漿層の粘度調節は，血管収縮を回避するために重要．各種分子状HBOCsは，それぞれ異なった血管活性を示す．HBOCsによるNO運搬（Stamlerらの説）は不明．全てのHBOCsはNOを捕捉する．血漿層の粘度を増大することでNO産生を増加できる．日本のHb小胞体のほか，PEG-Hb, Polymerized Hbなど，様々なHBOCsを検討して明らかになったことは，分子設計にあたって，血漿増量のため膠質浸透圧（50 mmHg）は充分である．P_{50}値は12-16 mmHgにすることで，抵抗血管よりも下流の血管での酸素放出を可能とする．全血中のHb濃度は，Transfusion Triggerである7 g/dL以上に維持しつつ，血漿中のHb濃度は2 g/dL程度が良い．血液粘度は2-4 cP程度を目安とし，平滑筋に対するmechanotransduction効果を維持する．

Endogenous Hb Scavengers and HBOC Toxicity
Dominik J. Schaer (University of Zurich, Switzerland)
血中の遊離Hbの排泄には，ハプトグロビン（Hp）およびレセプターCD163が関与している．これらがどのようにHbの毒性を低減しているか．Hp, CD163とHBOCsの相互作用の強さは，その構造に依存し，修飾度が高いほど相互作用は低減する．マクロファージCD-163と相互作用をすると，HO-1が誘導され，酸化的傷害を低減する．HpはHbの血圧亢進の効果を低減させる．

Utility of Animal Models in HBOC Evaluation
Joy Cavagnaro (AccessBio)
前臨床試験について，動物実験では臨床の結果を100％予見することはできないし，たとえ臨床試験を行ってもその後，予想しないことも起こりうる．病態の動物モデルを用いた実験，種差の検討が必要である．毒性の許容範囲を明らかにしてそれを見るための動物実験モデルを作製することが重要．

Alternative Focused Clinical Designs
Jeffrey L. Carson
(University of Medicine and Dentistry of New Jersey)
臨床試験のエンドポイントは，臨床的な結果とすべきであり，「同種血輸血の低減」とするべきではない．

Panel Discussion
Jerry L. Carson (University of Medicine and Dentistry of New Jersey)
Joy Cavagnaro (AccessBio)
Ezekinel Emanuel (National Institute of Health)
臨床試験の倫理的問題について概説したJAMA誌の論文を配布[9]．
Thomas R. Flemng (University of Washington)
Marcos Intaglietta (La Jolla Bioengineering Institute, University of California, San Diego)
John S. Olson (Rice University)
Dominik J. Schaer (University of Zurich, Switzerland)
Gus J. Vlahakas (Harvard Medical School)
David C. Warltier (Medical College of Wisconsin)

Closing Remarks
Jay S. Epstein (Office of Blood Research Review)
二日間の討論の中で，全ての問題点に対して十分な答えを得る事は出来なかったかもしれないが，多くの情報を得ることが出来た．また，大まかな意見の一致が見られたのではないか．それは，重要なUnmet medical needsがあること，HBOCsの研究開発を先に進めるべきこと，そして，新しい戦略が引続き必要であること．組織委員会および，Dr. J. Goldsmithに謝意を表して，本会を閉会する．

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Cancer Chemother Pharmacol (2008) 62:331–333
DOI 10.1007/s00280-007-0636-z

ORIGINAL ARTICLE

Syndrome of inappropriate secretion of antidiuretic hormone after chemotherapy with vinorelbine

Hiroaki Kuroda · Masafumi Kawamura · Tai Hato · Kazunori Kamiya · Masahiro Kawakubo · Yotaro Izumi · Masazumi Watanabe · Hirohisa Horinouchi · Koichi Kobayashi

Received: 23 July 2007 / Accepted: 24 October 2007 / Published online: 8 November 2007

Abstract
Purpose To describe a case of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) after administration of vinorelbine (VNB) for recurrence of lung cancer.
Case A 76-year-old man underwent bronchial arterial infusion (BAI) of VNB for postoperative recurrence of lung cancer. Seven days later, hyponatremia and natriuresis developed. Based on his clinical and laboratory findings, we diagnosed him with SIADH. He improved within a couple of days with fluid restriction only.
Conclusions Administration of VNB may potentially cause SIADH. This is the second report of the SIADH caused by VNB. It is important to monitor the serum sodium level and clinical findings after chemotherapy with VNB.

Keywords Syndrome of inappropriate secretion of antidiuretic hormone (SIADH) · Vinorelbine (VNB) · Non-small cell lung cancer · Chemotherapy

Introduction

We present a case of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) in a 76-year-old man after administration of vinorelbine (VNB). He underwent bronchial arterial infusion (BAI) of VNB for postoperative recurrence of lung cancer. Seven days later, hyponatremia and natriuresis developed.

Based on his clinical and laboratory findings, we diagnosed him with SIADH. He improved within a couple of days with fluid restriction only. This is the second report of the SIADH caused by VNB therapy for lung carcinoma.

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Case report

A 76-year-old man underwent left upper lobectomy for lung adenocarcinoma (pathological staging T3N1M0, stage IIIA) and 14 months later, chest computed tomography (CT) showed bilateral pulmonary metastases. Five courses of chemotherapy with docetaxel (DOC) 100 mg and gemcitabine (GEM) 1,400 mg resulted in stable disease (SD) and he was begun on modified chemotherapy with vinorelbine (VNB 40 mg). After three courses of VNB, he was admitted to hospital, complaining of left chest pain and cough.

Chest X-ray and CT on admission showed the tumor occluding in the left main bronchus and complete atelectasis of the remaining left lower lobe (Fig. 1a, b).

Transbronchial interventions, such as tumor resection, injection of ethanol and YAG laser ablation, were performed repeatedly, after which the chest X-ray showed gradual restoration in his remaining left lobe. Irradiation (total 50 Gy) of the recurrent tumor was performed for 5 weeks and then chemotherapy with VNB 40 mg (BAI 20 mg + intravenous 20 mg) was repeated.

Seven days after administration of 40 mg VNB, he complained of anorexia, nausea and lethargy. His consciousness was clear. His physical and neurogenic examinations were almost intact. His plasma sodium concentration was 113.1 mEq/l, serum osmolality was 242 mOsm/kg lower

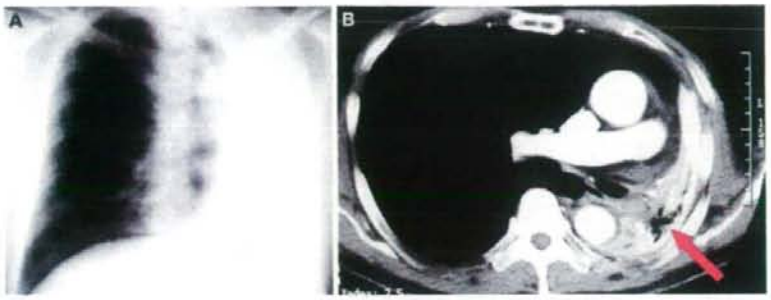

Fig. 1 a Chest X-ray on admission shows complete atelectasis of remaining left lobe. **b** Chest computed tomography scan shows the tumor occluding the left main bronchus (*arrow*)

than normal limit of 270 mOsm/kg and urine osmolality was 309 mOsm/kg higher than normal limit of 300 mOsm/kg. Urine sodium value was 20.2 mEq/l higher than normal limit of 20 mEq/l. His cortisol concentration value was normal. The plasma arginine vasopressin (AVP) concentration was 0.59 pg/ml, which was within normal limits, as were other parameters. Clinically, there were no symptoms related to adrenal or anterior pituitary dysfunction. The patient was euvolemic and renal function tests were within normal limits. VNB is considered to be strongly associated with SIADH, so he was diagnosed as having SIADH because his clinical features and laboratory data satisfied all standard criteria.

He was treated with water restriction, oral intake plus drip infusion into vein (DIV, total 750 ml/day). Within 2 days, his serum sodium concentration rose gradually and was restored to 130.3 mEq/l (Fig. 2). His mentation and appetite recovered in accordance with the increasing serum sodium concentration without central pontine myelinolysis. There was a possibility of developing SIADH again with a fifth cycle of VNB, so the chemotherapy agent was changed. He has been free of SIADH and has lived with lung cancer for 1 year.

Discussion

Schwartz et al. first reported SIADH in patients with lung cancer in 1957 [1]. Standard criteria include (1) hyponatremia,

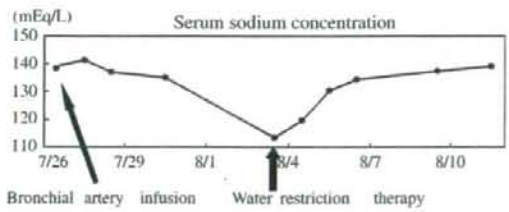

Fig. 2 Clinical course of serum sodium concentration 7 days after administration of vinorelbine. The patient complained of anorexia, nausea and lethargy and the sodium concentration was 113.1 mEq/l. He was treated with oral water restriction only and 2 days later, the serum sodium concentration was restored to 130.3 mEq/l

(2) plasma hypo-osmolality and urine hyperosmolality, (3) continuous secretion of sodium in urine, (4) normal renal function without hydration, (5) no adrenal gland dysfunction and (6) hyponatremia and hyposmotic pressure that recover with water restriction therapy without change in blood pressure [2]. The present patient fulfilled these criteria.

Various causes of SIADH have been reported, such as disorders of the cerebral nervous system, malignant tumors, diseases of the thoracic cavity, medicinal side-effects and idiopathic [3]. Approximately 75% of tumor-associated cases of SIADH are related to small-cell lung cancer (SCLC) [4]. The occurrence of SIADH with non-small-cell carcinoma (NSCLC) has been only rarely reported [5]. It has been reported that SIADH is caused by the tumor invading the vagus nerve or releasing ADH-like product. In the present study, imaging showed no evidence of invasion of the vagus nerve and we identified that the serum level of AVP was normal. We do not consider that its upregulation or secretion of ADH-like material occurred because the patient never experienced other electrolytic abnormalities and his serum sodium concentration was restored rapidly by water restriction therapy alone. There was no evidence of a relationship between progression of lung carcinoma and the onset of SIADH in this case.

Another possibility is that when the extension receptors of the left atrium detect hyperthoracic pressure and abnormal hemodynamics, they decrease the suppressor signal level and induce continuous release of ADH from the posterior lobe of the pituitary. But in the present case, bronchoscopic interventions were performed to target the local recurrence and restore his remaining left lobe. Syndrome of inappropriate secretion of antidiuretic hormone developed after 5 weeks of irradiation therapy following these interventional procedures. Even if there was a change in the respiratory circulation with release of the atelectasis, it is unlikely because of the time delay.

Enhancing release of AVP, potentiating the renal action of AVP and unknown mechanisms are reported as the main causes of SIADH by drugs [3]. Syndrome of inappropriate secretion of antidiuretic hormone associated with vinca

alkaloids, especially vincristine (VCR) and vinblastine (VBL), has been reported [6–8]. Garrett and Simpson first reported that SIADH occurred after a single treatment of VNB for breast cancer [9]. In addition, they reported that there was a slight structural difference between VNB and other vinca alkaloids, however, the precise mechanism is unclear and they may possess common neural or renal adverse effect profiles. In the present case, we firmly concluded that SIADH was induced by chemotherapy with VNB because concomitant medication was steroids only. In addition, SIADH occurred after four courses and not with earlier exposures of VNB. Although we think that repeated administration or retention of VNB may have been associated with SIADH, the precise mechanism of this SIADH was unclear.

In the report by Garrett and Simpson, SIADH from VNB did not respond to fluid restriction and patient had to be give 3% NS. But in our case, patient recovered with fluid restriction only. This difference with these two mechanisms was unclear. Furthermore, they also reported that prophylactic use of demeclocycline, which interacts with ADH at the renal collecting duct, might usefully prevent recurrence of SIADH associated with continuous treatment with VNB [9]. Stuart and Cuaso reported that SIADH was prevented by rigorous water restriction [10]. For our patients, we choose an alternative because of the high possibility of SIADH caused by VNB. If chemotherapy with VNB results in complete response or partial response, we would choose to readminister VNB with restriction of water, or use demeclocycline and monitor the sodium concentration.

We consider that VNB should be regarded as very likely to cause SIADH, but as this is only the second report of SIADH associated with use of VNB alone, it is a rare occurrence.

Conclusion

It is known that lung cancer can give rise to SIADH as a paraneoplastic syndrome and there are some anticancer agents that can potentially cause SIADH. It is important to monitor the serum sodium level and clinical findings after chemotherapy for lung cancer.

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European Journal of Cardio-thoracic Surgery 34 (2008) 196–199

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

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Surgical outcomes for pulmonary metastases from hepatocellular carcinoma

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Received 27 September 2007; received in revised form 24 March 2008; accepted 31 March 2008; Available online 1 May 2008

Abstract

Background: Although favourable prognosis following aggressive treatment of extrahepatic metastases from hepatocellular carcinoma (HCC) has been reported, surgical outcomes for pulmonary metastases are unclear. **Methods and materials:** Sixty-one patients (2.6%) of 2297 registered with the Metastatic Lung Tumor Study Group of Japan between 1990 and 2006, who underwent surgery for pulmonary metastases from HCC, were retrospectively reviewed from the registry. **Results:** The overall 5-year survival rate was 32.2%. The prognosis was significantly better for ≤ 2 lesions than for ≥ 3 lesions ($p = 0.046$), for ≤ 3 lesions than for ≥ 4 lesions ($p = 0.0070$), and for ≤ 4 lesions than for ≥ 5 lesions ($p = 0.029$). No other factors that influence outcomes were identified. A stepwise regression analysis showed three or less pulmonary metastases to be an independent factor for better prognosis ($p = 0.048$). **Conclusion:** With careful patient selection, comparatively good outcomes can be expected following surgical resection of pulmonary HCC metastases. Among them, patients with multiple metastases, if number of metastases is small such as four or less, can be expected to survive long after surgery.

Keywords: Hepatocellular carcinoma; Pulmonary metastasis; Extrahepatic metastasis; Surgery; Metastasectomy

1. Introduction

Advances in localised treatments for hepatocellular carcinoma (HCC) have seen better local control achieved in recent years. The prognosis remains poor for extrahepatic recurrence however, although some studies have reported improved outcomes following aggressive treatment of extrahepatic metastases [1]. Pulmonary metastases account for over 50% of extrahepatic HCC metastases [2,3]. The primary lesion in more than 80% of HCC with extrahepatic HCC metastases is advanced, stage III or IVa, so there are few opportunities for aggressive surgical treatment of extrahepatic metastases [3]. Surgical resection is contraindicated in most cases of pulmonary HCC metastases due to the number of lesions. There have accordingly been few studies of the results of surgical treatment of pulmonary metastases. In this study we analysed the results of surgical procedures on pulmonary HCC metastases in 61 patients to determine prognostic factors.

2. Methods and materials

From 2297 patients registered with the Metastatic Lung Tumor Study Group of Japan from January in 1990 to May in 2006 who underwent resection of metastatic lung tumours, the subjects of this study were the 61 patients (2.6%) who underwent surgery for pulmonary HCC metastases. Information regarding subject gender, date of birth, date and time of hepatic surgery, date of detection of pulmonary metastases, number of lesions on right and left, maximum tumour diameter, date and time of resection of pulmonary metastases, and the type of procedure performed, were recorded in the registration form. Detection of pulmonary metastases was performed by CTscan. Multi-detector CTscan

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doi:10.1016/j.ejcts.2008.03.056

has become available since late 1990s. Video-assisted thoracic surgery (VATS) has been undergone since 1996. Choice of surgical procedure was left to each surgeon. Although the patients were required to have sufficient liver function for pulmonary resection, information of their liver condition was not mandatory for registration because of chest surgeons' registration. Patients were followed-up basically with chest CT scan twice a year for the recurrence of pulmonary metastases. Outcome surveys were subsequently conducted at 1-year intervals. In general, we followed the indications for surgical resection of pulmonary HCC metastases proposed by Thomfold et al. [4], that the primary lesion is under control or is planned to be under control, there are no metastases to other organs, and the patient's general condition is good enough to withstand surgery [4]; we also included patients with bilateral disease. We counted two stage procedures for bilateral pulmonary metastases as a single procedure, and we defined re-do surgery for recurrent pulmonary metastases as surgery for pulmonary metastases newly detected after the initial procedure. The disease-free interval (DFI) was defined as the time between the day of hepatic surgery and the day of detection of pulmonary metastases, giving a DFI of 0 if pulmonary metastases were discovered prior to or at the time of the initial hepatic surgery. Cumulative survival rates were calculated using the Kaplan–Meier method, and comparisons among the survival curves were made using the log-rank test. Multivariate analysis for prognostic factors was assessed using a stepwise regression. A p value <0.05 were considered as a statistically significant difference. Statistical analysis was performed using the SPSS Base 11.0J software package (SPSS Inc., IL, USA).

3. Results

There were 46 male and 15 female subjects, ages ranging from 27 to 80 (mean 60 years). Patient background characteristics are shown in Table 1. There were no operation related deaths. Preoperative evaluation of hilar and mediastinal lymph node metastasis was recorded in 44 cases. Among them actual lymphatic metastases were proven histologically in four cases. Pulmonary metastases were already present at the time of diagnosis of HCC in six patients, of whom pulmonary surgery was performed first in one patient. The cumulative 1-year survival rate after the initial pulmonary surgery was 69.8%, the 3-year survival rate 46.9%, and the 5-year survival 32.2%. The cumulative 1-year survival rate after hepatic surgery was 93.2%, the 3-year survival rate 74.0%, the 5-year survival 50.3%, and the 8-year survival 33.3% (Fig. 1). Of the eight subjects who survived at least 5 years following pulmonary surgery, three survived as cancer bearers.

We examined outcomes according to each parameter: gender, age (<60 vs ≥60), DFI (≤12 m vs ≥13 m, ≤24 m vs ≥25 m), number of pulmonary metastases at time detection (solitary vs multiple, $n = 1–2$ vs ≥3, $n = 1–3$ vs ≥4, $n = 1–4$ vs ≥5), maximum tumour diameter (<2 cm vs ≥2 cm), procedure (wedge resection vs segment resection/lobectomy), and number of pulmonary procedures (single vs multiple). As shown in Table 2, no significant difference was seen between

Table 1
Clinical characteristics of subjects with pulmonary metastases from hepatocellular carcinoma

Gender	
Male	46
Female	15
Age (range 27–80, average 60)	
Age <60	28
Age ≥60	31 + (unknown 2)
Disease-free interval (DFI) (range 0–180, average 28.7 m)	
0	6
0 < DFI ≤ 12	15
12 < DFI ≤ 24	16
24 < DFI	24
Number of pulmonary metastatic lesions (range 1–20, average 2.2)	
1	32
2	15
3	7
4	2
5~	5
Laterality of pulmonary metastases	
Right	30
Left	18
Bilateral	13
Mode of resection	
Wedge resection	47
Segmentectomy or lobectomy	14
Maximum diameter of pulmonary metastasis (MD) (range 0.4–4.8 cm, average 2.3 cm)	
MD < 1.0 cm	5
1.0 cm ≤ MD < 2.0 cm	23
2.0 cm ≤ MD < 3.0 cm	10
3.0 cm ≤ MD	18 + (unknown 5)
Number of pulmonary operations	
One	49
Two	9
Three	2
Four	1

solitary metastasis and multiple lesions ($p = 0.203$), whereas the prognosis was significantly worse for ≥3 lesions than for ≤2 lesions ($p = 0.046$), for ≥4 lesions than for ≤3 lesions ($p = 0.007$) (Fig. 2) and for ≥5 lesions than for ≤4 lesions ($p = 0.029$). No other factors that influence outcomes were identified. A stepwise regression analysis showed three or

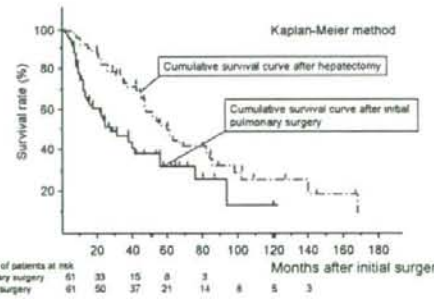

Fig. 1. Cumulative survival curves after hepatectomy and after initial pulmonary resection. Five-year survival rate after initial pulmonary metastasectomy is 32.2% and 5-year survival rate after hepatectomy is 50.3%.

Table 2
Cumulative overall survival results using log-rank analysis (Kaplan–Meier method)

Parameter	No. of patients	5-year survival rate	Significance (p)
Male vs female	46 vs 15	24.0% vs 51.4%	0.15
Age			
Age <60 vs Age ≥60	28 vs 31	26.1% vs 35.6%	0.46
DFI			
DFI ≥ 13 m vs DFI ≤ 12 m	40 vs 21	35.5% vs 21.0%	0.17
DFI ≥ 25 m vs DFI ≤ 24 m	24 vs 37	34.8% vs 28.1%	0.96
Number of pulmonary metastases			
Solitary vs multiple	32 vs 29	42.7% vs 20.0%	0.20
n = 1–2 vs $n \geq 3$	47 vs 14	37.2% vs 12.8%	0.046
n = 1–3 vs $n \geq 4$	54 vs 7	37.5% vs not reached	0.007
n = 1–4 vs $n \geq 5$	56 vs 5	36.0% vs not reached	0.029
Maximum diameter of pulmonary metastasis (MD)			
MD < 2 cm vs 2 cm ≤ MD	28 vs 28	37.2% vs 26.3%	0.41
Lung procedure			
Wedge vs segmentectomy or lobectomy	47 vs 14	34.5% vs 20.8%	0.98
Number of pulmonary lesions resected			
Single vs multiple	49 vs 12	35.7% vs 16.2%	0.98

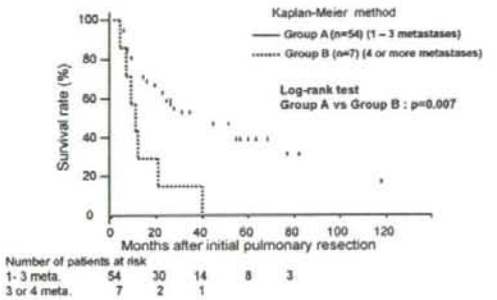

Fig. 2. Cumulative survival curves of patients with 1–3 metastases and with four or more metastases.

less pulmonary metastases to be an independent factor for better prognosis (p = 0.048) (Table 3).

4. Discussion

Treatment outcomes for HCC hepatic primary lesions have improved remarkably with recent advances in a variety of therapeutic modalities, including resection, hepatic artery injection sclerotherapy, ethanol injections and ablation therapy. This has led to consideration of more aggressive treatments for extrahepatic metastases. There have been some reports of prolonged life expectancy following aggressive therapy, including localised treatments, for extrahepatic metastases [1]. Pulmonary lesions are the most common extrahepatic HCC metastases, accounting for 50–60% of the total [2,3]. In most cases, surgery is contraindicated due to multiple lesions, so surgical treatment for pulmonary HCC metastases has yet to be fully evaluated.

Tomimaru et al. reported a series of 615 patients who underwent radical resection for HCC, 34 of whom developed pulmonary metastases during the postoperative follow-up period. Pulmonary metastases were resected in eight of the 14 patients with one or two pulmonary lesions, and outcomes were markedly better in the resection group than in the nonresection group [5]. This result indicates that surgical resection is effective in this highly selected group of patients with 1–2 pulmonary metastases, but at the same time leaves us with the suspicion that surgical resection may be indicated only in patients with no more than two pulmonary HCC metastases. Lam et al. reported a favourable 5-year survival rate of 67% in patients (n = 9) who underwent resection of solitary lesions [6]. Our analysis did not show any significant difference in the 5-year survival rate between subjects with solitary lesions (n = 32) and with multiple lesions (n = 29) (p = 0.203). On the other hand, there were significant differences in prognosis between ≥3 lesions and ≤2 lesions (p = 0.046), between ≥4 lesions and ≤3 lesions (p = 0.0070), and between ≥5 lesions and ≤4 lesions (p = 0.029). These data suggest that there is a relationship between metastatic number and prognosis and some patients with multiple metastases, if number of metastases is small such as four or less, can be expected to survive long after surgery. Actually about 90% of subjects had no more than three pulmonary metastases in this study. Multivariate

Table 3
Relationships of individual variables to patient prognosis (stepwise regression model)

Variable	Score	p
No. of metastases		
$n \leq 3$	3.908	0.048
Female	2.041	0.15
DFI ≤ 12 m	2.213	0.14
<60 (years)	0.806	0.67
Re-do operation	0.001	0.98
Segmentectomy or lobectomy	0.047	0.83
2 cm ≤ MD	0.535	0.77

DFI, disease-free interval; MD, Maximum diameter of pulmonary metastasis.

analysis also showed that three or less pulmonary metastases was an independent better prognostic factor for surgical treatment ($p = 0.027$).

In their studies of outcomes following surgical treatment of pulmonary HCC metastases, Koide et al. reported a 5-year survival rate (overall survival) of 26.8% for 14 subjects [7], and Nakajima et al. reported 23.8% for 20 subjects [8]. These are both single center therapeutic results. Our data, although collected from multiple institutions, yield a similar 5-year survival rate of 32%. All of the published studies to date have had insufficient patient numbers to properly assess the therapeutic effect of surgical resection for pulmonary HCC metastases. Koide and Nakajima both used roughly the same indications for surgery as we did, however, indicating that a 5-year survival rate of around 30% can be achieved with patients selected in this way.

Of the 12 subjects who underwent repeat surgery for recurrent pulmonary metastatic disease, only one survived for 5 years. This study does not demonstrate any efficacy for re-do surgery. There were no surgery-related deaths, however, and a number of studies have shown better outcomes for aggressive treatment of extrahepatic metastases [9–12], so there are no convincing reasons why repeat surgical resection should be contraindicated for recurrent pulmonary metastases.

5. Conclusions

With careful patient selection, comparatively good outcomes can be expected following surgical resection of pulmonary HCC metastases. Among them, patients with multiple metastases, if number of metastases is small such as four or less, can be expected to survive long after surgery.

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MODERN PATHOLOGY

Original Article

Modern Pathology (2008) **21**, 992–1001; doi:10.1038/modpathol.2008.79; published online 30 May 2008

Histopathological features and prognostic significance of the micropapillary pattern in lung adenocarcinoma

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Received 3 January 2008; Revised 16 April 2008; Accepted 17 April 2008; Published online 30 May 2008.

Abstract

The micropapillary pattern is characterized by small papillary tufts with no fibrovascular core lying in spaces and has been reported as an aggressive variant of carcinoma in several organs. We investigated the histopathobiological properties of the micropapillary pattern with immunohistochemistry, serial sections, and electron microscopy in lung adenocarcinoma. We further analyzed its clinicopathological character and prognosis. The subjects included 383 adenocarcinoma cases, of which 184 (48%) were micropapillary pattern-positive and 199 (52%) were micropapillary pattern-negative. On histology, micropapillary tufts seemed to float in the alveolar space or spaces encased by connective tissues, whereas serial sections revealed that most tufts had continuity with other tufts and even with the main tumor. Positive staining for the adhesion molecules E-cadherin and β-catenin suggested the preservation of tight adhesion, and electron microscopy showed the existence of intercellular junctions. Negative staining for laminin and loss of basement membrane as determined by electron microscopy suggest a loss of cell–matrix contact. Positive staining for Ki-67 indicates that cells constituting micropapillary tufts retained their proliferation potency. There were no CD34-positive cells in micropapillary tufts, and the loss of the vascular core was confirmed. In micropapillary pattern-positive cases, lymphatic invasion was identified significantly more frequently than in micropapillary pattern-negative cases ($P<0.001$), even at stageIA (without lymph node metastasis, $N=197$) ($P<0.001$). The 5-year and 10-year overall survival rates of the micropapillary pattern-positive stageIA group were 77.6 and 67.6%, respectively, which were significantly less than those of the micropapillary pattern-negative stageIA group (98.1 and 98.1%) ($P=0.001$). In conclusion, cells constituting the micropapillary pattern are likely to have acquired anchorage-independent growth and a potential for high malignancy.

Keywords: lung adenocarcinoma, micropapillary pattern, histopathology, prognosis, immunohistochemistry, electron microscopy

Disease-Free Interval Length Correlates to Prognosis of Patients Who Underwent Metastasectomy for Esophageal Lung Metastases

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Background: Pulmonary metastasectomy is a standard method for treatment of selected pulmonary metastases cases. Nevertheless, because prognosis for patients with lung metastases from esophageal cancer who have undergone pulmonary metastasectomy is poor, candidates for this method of treatment are rare. Therefore, the efficacy of surgical treatment for pulmonary metastatic lesions from esophageal cancer has not been thoroughly examined.

Methods: Between March 1984 and May 2006, 57 patients underwent resection of pulmonary metastases from primary esophageal cancer. These cases were registered in the database developed by the Metastatic Lung Tumor Study Group of Japan and were retrospectively reviewed from the registry. After excluding eight cases because of missing information, we reviewed the remaining 49 cases and examined the prognostic factors for pulmonary metastasectomy for metastases from esophageal cancer.

Results: There were no perioperative deaths. After pulmonary metastasectomy, disease recurred in 16 (33%) of the 49 patients. The overall 5-year survival was 29.6%. Median survival time was 18 months. The survival of patients with a disease-free interval (DFI) less than 12 months was significantly lower than patients with a DFI greater than 12 months. Through multivariate analysis, we identified DFI as a clinical factor significantly related to overall survival ($p = 0.04$).

Conclusions: We identified that patients with a DFI less than 12 months who underwent pulmonary metastasectomy for metastases from esophageal cancer had a worse prognosis. Pulmonary metastasectomy for esophageal cancer should be considered for selected patients with a DFI ≥12 months.

Key Words: Esophageal cancer, Pulmonary metastasis, Metastasectomy.

(*J Thorac Oncol.* 2008;3: 1046–1049)

Pulmonary metastasectomy is a standard method of treatment for selected pulmonary metastases cases.[1] When patients are appropriately selected for this treatment, the overall 5-year survival after pulmonary metastasectomy is about 30 to 40%.[1,2] In general, because prognosis for patients who have undergone this method of treatment is poor with disease frequently recurring, pulmonary metastasectomy is not a frequently chosen method of treatment for lung metastases from esophageal cancer. Consequently, survival after surgery for pulmonary metastases from esophageal cancer has not been thoroughly examined. In Japan, the annual report by the Japanese Association for Thoracic Surgery does not document patients who underwent metastasectomy for metastasized esophageal cancer.[3] Because the outcome of pulmonary metastasectomy for metastases from esophageal cancer has not been thoroughly investigated, it is controversial whether surgery is an effective treatment for metastatic esophageal cancer. To identify prognostic factors of pulmonary metastasectomy for metastases from esophageal cancer, in the present study, we reviewed cases registered in the Metastatic Lung Tumor Study Group of Japan database of patients who underwent metastasectomy for metastasized esophageal cancer.

PATIENTS AND METHODS

The Metastatic Lung Tumor Study Group of Japan developed a database for registration of lung metastases cases. These patients all underwent surgical resection. The database documents the following parameters: gender; age; histology; status of the primary tumor; treatment for the primary tumor; date of primary surgery; kind of surgery; curability; date of metastasis; disease-free interval (DFI); side, size and numbers of resected metastases; date of metas-

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Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864/08/0309-1046

tasectomy; and follow-up. Between March 1984 and May 2006, 57 patients underwent resection of pulmonary metastases from primary esophageal cancer. These cases were registered in the Metastatic Lung Tumor Study Group of Japan database and were retrospectively reviewed from the registry. Preoperative examination, surgical indication, and operative procedure were at the discretion of each institution.

After excluding eight cases because of missing information such as number of resected metastases, age, or DFI, we examined the remaining 49 cases (46 males and 3 females) in our study. Surgery alone for the primary tumor was performed in 26 cases (53%), surgery and chemoradiotherapy were performed in 7 cases (14%), surgery and radiotherapy were performed in 6 cases (12%), surgery and chemotherapy were performed in 3 cases (6%), radiotherapy alone was performed in 2 cases (4%), and treatment data were not available for 5 cases (10%). We examined the following variables (Table 1): age (≥70 or <70), number of resected metastases (solitary or multiple), resected side (unilateral or bilateral), tumor size (≥3 or <3cm), DFI (≥12 or <12 months), surgical procedure (partial resection, segmentectomy, or lobectomy), and curability (complete or incomplete).

The present study was analyzed using anonymized data that were collected in each institution. Therefore, informed consent was not specifically obtained and institutional review board approval was not necessary.

Statistical Analysis

Overall survival was analyzed by the Kaplan-Meier method, and differences in variables were calculated by the log-rank test. The date of pulmonary resection was defined as the starting point. Cox's proportional hazards model was used for multivariate analysis. The data were calculated using version 5.0 of the StatView software package (SAS Institute Inc, Cary, NC). A p value of less than 0.05 was defined as indicative of statistical significance.

TABLE 1. Survival of 49 Patients According to Clinical Factors of Pulmonary Metastases

Variables	n (%)	5-yr Survival (%)	p
Age (yr)			
≥70	13 (27)	32.9	0.928
<70	36 (73)	27.8	
Number			
Solitary	39 (80)	27.4	0.797
Multiple	10 (20)	42.9	
Resected side			
Unilateral	44 (90)	29.3	0.621
Bilateral	5 (10)	30.0	
Tumor size[a]			
≥3 cm	10 (21)	40.0	0.640
<3 cm	38 (79)	26.7	
DFI			
≥12 mo	28 (57)	39.2	0.048
<12 mo	21 (43)	15.7	
Surgical procedure			
Partial and segment	31 (63)	36.4	0.338
Lobectomy	18 (37)	22.9	
Curability			
Complete	45 (92)	31.4	0.990
Incomplete	4 (8)	25.0	

[a] No cases were available.
DFI, disease-free interval.

RESULTS

The median interval between treatment of esophageal cancer and diagnosis of pulmonary metastasis (disease-free interval) was 14 months (range: 0–124 months). There were no perioperative deaths. The median age of patients at the time of pulmonary metastasectomy was 65 years (range: 35–82). The median number of resected metastatic lesions per patient was one (range: 1–5). The metastases ranged in size from 0.4 to 5.5 cm, and the median size was 2.0 cm. The metastases were squamous cell carcinoma in 48 cases and adenocarcinoma in one case. The surgical procedure was wedge resection in 23 cases (47%), lobectomy in 16 cases (33%), segmentectomy in 8 cases (16%), and bilobectomy in 2 cases (4%). The median follow-up period after the first pulmonary resection was 18 months (range: 0–206 months). Recurrence developed in 16 (33%) of the 49 patients. Recurrences were as follows: lung, nine; lymph node, three; neck, one; distant metastasis, one; stomach, one; and unknown, two. The overall 5-year survival after pulmonary metastasectomy was 29.6% (Figure 1). Median survival time was 27 months. We investigated the relationships between prognostic factors and survival (Table 1). Patients with a DFI less than 12 months had a significantly worse prognosis, as assessed by survival rates, than patients with a DFI greater than 12 months (Figure 2). Multivariate analysis of these variables was performed using Cox's proportional hazards model for disease-specific survival. A DFI less than 12 months was shown to be an independent prognostic factor ($p = 0.04$) (Table 2). At the time of submission, 28 patients examined in our study have died. Although 23 patients died of esophageal cancer, 7 patients were not available for recurrent sites. Five patients have died of other diseases (two cases

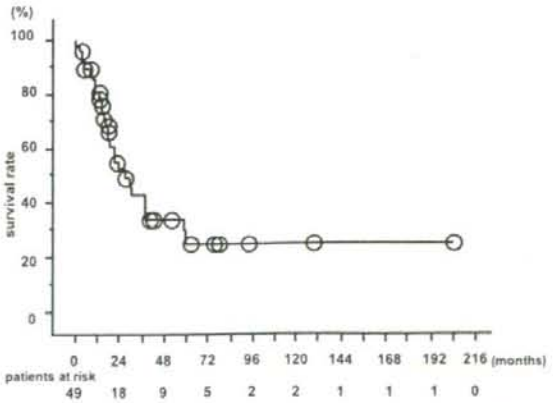

FIGURE 1. Overall survival of the 49 patients after pulmonary metastasectomy. The 5-year survival was 29.6%.

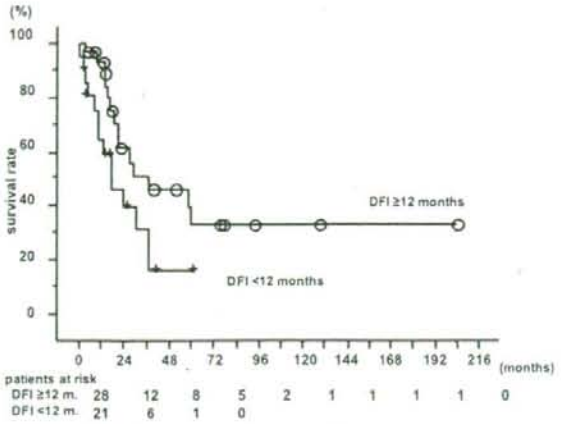

FIGURE 2. Overall survival after pulmonary metastasectomy according to DFI. Survival curves of patients with DFI <12 months and ≥12 months. DFI, disease-free interval.

TABLE 2. Relationships of Individual Variables to Survival (Cox's Proportional Hazards Model)

Variable	Risk Ratio	95% CI	*p*
≥70 yr	1.01	0.41–2.50	0.983
Multiple metastasis	1.67	0.30–9.19	0.557
Bilateral metastasis	1.19	0.19–7.53	0.853
Tumor size ≥3 cm	0.76	0.25–2.35	0.635
DFI <12 mo	2.30	1.04–5.09	0.040
Partial and segment	0.60	0.22–1.65	0.180
Incomplete resection	1.00	0.22–4.56	0.881

CI, confidence interval; DFI, disease-free interval.

were pneumonia, two cases were cerebral infarction, and one case was myocardial infarction).

DISCUSSION

Patients who are candidates for pulmonary metastasectomy for metastases from esophageal cancer are a minority. Analysis of the outcomes of surgery for pulmonary metastases from esophageal cancer has not been published. Quint et al.[4] showed that 29 of 147 (20%) patients with newly diagnosed metastasized esophageal cancer had lung metastasis. Although autopsy studies showed that the frequency of esophageal lung metastasis was 50%,[5] there was not a high percentage of esophageal cancer relapse after esophagectomy. Kyriazanos et al.[6] revealed that 12 of 151 (8%) patients who underwent a curative esophageal resection had lung metastases. Within our study the number of adenocarcinoma of the esophagus was very small. Because the frequency of adenocarcinoma of the esophagus is low in Japan, we do not speculate about the scarce incidence of lung metastasis from adenocarcinoma of the esophagus.

Matsubara et al. showed that 38 of 230 patients (17%) who underwent surgery for esophageal cancer with extended lymph node dissection had distant metastases and 14 (6%) patients had lung metastases. In their article, the outcomes after recurrence were dismal, and no patients were alive 5 years after detection of recurrence. Nevertheless, they showed that the 1-year survival of the patients who had recurrent lesions and were treated with resection and adjuvant therapy was 83%. They concluded that when recurrent lesions were localized macroscopically, surgical removal of the recurrent lesions was an effective treatment.[7] Through our analysis, we found a 5-year survival of 29.6% after pulmonary metastasectomy, which indicates that pulmonary metastasectomy is a promising treatment for metastases from esophageal cancer. Nevertheless, as it is not easy to differentiate esophageal metastases from primary lung squamous cell carcinomas, it is possible that our data might include primary lung squamous cell carcinoma. Survival after metastasectomy might be lower than what our data indicate. Virgo et al mentioned that genetic markers are needed to confidently distinguish between metastases and primary solitary nodules.[8] Further investigation is needed to clarify this matter.

An article from the international registry of lung metastases states that the 5-year survival was 37% after pulmonary metastasectomy. In addition, the article showed that among cases of complete resection, the 5-year survival was 33% for patients with a DFI of 0 to 11 months and 45% for those with a DFI of more than 36 months. Furthermore, the 5-year survival was 43% for single lesions and 27% for 4 or more lesions.[1] DFI and number of pulmonary metastases are significant prognostic factors. Because our present data show that the median DFI is 14 months, we categorized DFI as ≥12 or <12 months. Regarding the DFI, our study suggests that patients with a DFI less than 12 months have a poor prognosis. Osugi et al. showed that 83% of recurrences presented within 24 months after esophagectomy and that the chance of survival of patients whose disease recurred within 24 months after esophagectomy was better than that of patients who suffered recurrence within 24 months. Regarding follow-up studies after esophagectomy, meticulous care should be taken to detect hematogenous recurrence.[9]

In general, incomplete resection is a dismal prognostic factor in lung metastasectomy. We could not demonstrate whether surgical curability is a prognostic factor. McDonald et al. reported that incomplete resection appeared to have no influence on overall survival in metastatic breast cancer. They suggested that this could be due to the systemic nature of the disease at the time of thoracotomy with unsuspected occult metastasis in other areas.[10] Nevertheless, in our study, only four patients underwent incomplete resection. Because the report from The International Registry of Lung Metastases stated that cases with incomplete resection clearly had worse prognoses,[1] we speculate that patients with lung metastases from esophageal cancer have the same tendency.

Although our present study was multi-institutional, we could not analyze in detail all of the records for each patient. From this point of view, because our findings were based on a limited number of cases, pulmonary metastasectomy for lung metastases from esophageal cancer is still highly controversial. Nevertheless, we identified that patients with a DFI less than 12 months had a worse prognosis, as assessed by

survival rates, than patients with a DFI greater than 12 months.

Consequently, although metastases from esophageal cancer are a minority, we think that pulmonary metastasectomy for esophageal cancer should be considered for selected patients with a DFI $\geq$12 months. As this study is small, further clinical studies will be needed.

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An Alternative Method for Screening EGFR Mutation Using RFLP in Non-small Cell Lung Cancer Patients

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Introduction: Epidermal growth factor receptor (EGFR) mutations are strong determinants of tumor response to EGFR tyrosine kinase inhibitors in non-small cell lung cancers (NSCLCs). Currently available methods of EGFR mutation detection rely on direct sequencing. Here, we describe the use of an alternative way to screen EGFR mutations.

Methods: A total of 109 frozen tumor specimens from NSCLC patients were obtained. For mutational analysis of EGFR exons 18, 19, and 21, reverse transcription-polymerase chain reaction was performed on the cDNA using original primers designed for restriction fragment length polymorphism (RFLP).

Results: EGFR mutations were detected in 37 patients (34%) by both RFLP and direct sequencing except one case in which it was detected only by RFLP. EGFR mutations were more frequently observed to be significant by multivariate analysis in patients with adenocarcinoma (OR = 5.56), no-smoking history (OR = 4.34), and 65-year-old or younger (OR = 2.64), but not in women (OR = 1.14). Among 37 patients, 18 were treated with gefitinib and 9 responded to the treatment. One patient without any mutation responded.

Conclusion: RFLP is a useful method for screening EGFR mutations and can also be applied to predicting the sensitivity of NSCLC patients to EGFR-tyrosine kinase inhibitors.

Key Words: EGFR mutation, Non-small cell lung cancer, RFLP.

(*J Thorac Oncol.* 2008;3: 1096–1103)

Lung cancer is the most common cause of death in both men and women worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 80% of these cases.[1] Recently, two drugs, gefitinib (Iressa) and elrotinib (Tarceva), which target the epidermal growth factor receptor (EGFR) tyrosine kinase (TK), were approved in different countries to treat NSCLC.[2,3]

In 2004, three separate studies reported that mutations in the EGFR gene in lung carcinomas made the disease more responsive to treatment with TK inhibitors.[4–6] Since then, a multitude of data has emerged from different groups around the world.

Most EGFR somatic mutations were exclusively detected in adenocarcinomas, including bronchiolo-alveolar carcinomas. The mutations were detected in exons 18, 19, and 21, which encode the intracellular kinase domain. The mutations detected in exon 18 had substitution of the amino acid G719 in the P-loop, whereas those detected in exon 21 had substitution of an amino acid in the activation domain (L858 and L861). The mutations in exon 19 were in-frame deletions that may alter the structure of αC helices. All of the EGFR mutations affect amino acids near the ATP-binding pocket that is targeted by gefitinib. Functional assays revealed that the hotspot mutants of EGFR had a higher EGF-independent activation than did the wild-type EGFR.[4–7]

EGFR mutations are predominantly found in Asians, women, adenocarcinomas, and never-smokers, which explains the association between the clinical predictors and gefitinib sensitivity.[4–6,8–10]

Direct gene sequencing is a standard method for detecting gene mutations. However, it is not suitable for clinical pretherapeutic screening of patients because it is time-consuming, costly, and sometimes unreliable. Thus, an easy and reliable method for detecting EGFR mutations that can be used clinically is needed.

The aim of this study was to establish an easy and reliable method with which to screen EGFR mutations. We studied a large series of consecutive NSCLC patients for EGFR mutations in exons 18, 19, and 21 using a comparative approach between 2 techniques: direct sequencing of polymerase chain reaction (PCR) products and restriction fragment length polymorphism (RFLP) analysis.

PATIENTS AND METHODS

Cell Lines and Plasmids Containing Wild-Type and Mutant EGFR Genes

Three NSCLC cell lines, SK-MES-1, H1650, and H1975, were purchased from American Type Culture Col-

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Disclosure: The authors declare no conflicts of interest.

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ISSN: 1556-0864/08/0310-1096

lection (Manassas, VA). SK-MES-1 has wild-type EGFR. H1650 contains an E746-A750 deletion mutation in exon 19. H1975 contains an L858R point mutation in exon 21 and a secondary T790M point mutation which is related to the resistance to gefitinib and erlotinib in exon 20.[11,12] pL420 plasmids containing wild-type, G719C and L858R mutant EGFR genes (generous gifts from Dr. Matthew Myerson, Dana-Farber Cancer Institute, Boston) were used to validate the RFLP assay for corresponding point mutations in exon 18 and exon 21, respectively.

Extraction of Nucleic Acids and Restriction Fragment Length Polymorphism for EGFR Mutants

Genomic DNA was isolated from tumors and lung cancer cell lines using the DNeasy Mini Kit (Qiagen, Munich, Germany), according to the manufacturer's protocol. Total RNA was also isolated from the same samples using the RNeasy Mini Kit (Qiagen) and cDNA was synthesized using an Omniscript Reverse Transcription kit (Qiagen).

We have designed original primers against cDNA for RFLP to detect mutations (Figure 1). The following primers containing appended M13 forward or reverse primer tails for direct sequencing were used for PCR amplification: exon 18 (forward, 5′-TGTAAAACGACGGCCAGTCCCTGGGGATC-GGCCTCTTCATGCGA-3′; reverse, 5′-CAGGAAACAGCT-ATGACCTATACACCGTGCCGAACGCACCGGGG-3′), exon 19 (forward, 5′-TGTAAAACGACGGCCAGTGATCA-AAGTGCTGGGCTCC-3′; reverse, 5′-CAGGAAACAGCT-ATGACCACGGTGGAGGTGAGGCAGAT-3′), exon 21 (forward, 5′-TGTAAAACGACGGCCAGTAACACCGCA-GCATGTCAAGAT-3′; reverse, 5′-CAGGAAACAGCTA-TGACCATTCCAATGCCATCCACTTGAT-3′), exon 20 (forward, 5′-TGTAAAACGACGGCCAGTCCTCGATGA-AGCCTACGTGATG-3′; reverse, 5′-CAGGAAACAGCTA-TGACCGGCAGCCGAAGGGTATGAGCTG-3′). The PCR reaction was performed on 1 μL of template cDNA, as prepared above, to which were added 10× buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$), 0.2 mM of both dNTP and 0.25 U AmpliTaq Gold, and 0.2 μM forward and reverse primers in a 50 μL reaction volume. The "hot start" PCR cycling parameters were: one cycle of 95°C for 15 minutes, 40 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 1 minute, followed by one cycle of 72°C for 3 minutes.

On the other hand, for the additional experiment to compare the sensitivity of the assay between cDNA and genomic DNA, we also performed RFLP against genomic DNA for exon 19 and exon 21. We chose external and nested primers designed by Paez JG[5] for PCR on genomic DNA. We use the following primers in external PCR: Exon 19, (forward, 5′-AAATAATCAGTGTGATTCGTGGAG-3′; reverse, 5′-GAGGCCAGTGCTGTCTCTAAGG-3′), Exon 21, (forward, 5′-GCAGCGGGTTACATCTTCTTTC-3′; reverse, 5′-CAGCTCTGGCTCACACTACCAG-3′). And we used in nested PCR: Exon 19, (forward, 5′-GTGCATCGCT-GGTAACATCC-3′; reverse, 5′-TGTGGAGATGAGCAG-GGTCT-3′), Exon 21, (forward, 5′-GCTCAGAGCCTGG-CATGAA-3′; reverse, 5′-CATCCTCCCCTGCATGTGT-3′). External-round PCR reaction was performed on 0.1 μg of genomic DNA with the same protocol as described above. For nested-round PCR reaction, 3 μL of the external-round PCR product was amplified in a second 50 μL reaction mixture using nested primers assembled as external-round PCR reaction, described above.

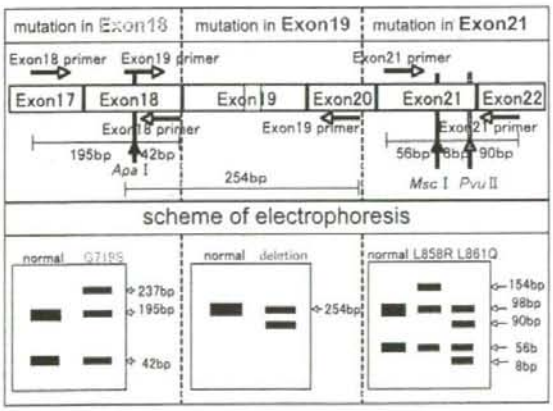

FIGURE 1. Scheme of digestion of PCR products and gel electrophoresis. PCR products were digested with corresponding enzymes (without digestion for exon 19), then were run on 2% agarose gel and the existence of mutations was assessed. Both *Apa*I and *Msc*I digest wild type EGFR allele, while *Pvu*II digests mutant EGFR allele.

Mutation Assay for G719X in Exon 18

The restriction enzyme *Apa*I digests the GGGCCC sequence in the amplicon of the wild-type allele. In contrast, the mutant allele was not digested because of the base substitution of G to X at the second base of GGGCCC. The PCR products after digestion were run on 2% agarose gel and the existence of the mutation was assessed (Figure 1).

Mutation Assay for Deletion in Exon 19

Because the range of exon 19 deletions containing commonly deleted codons 746 to 751 was reported to be from 9 to 18 bp, differences in the sizes of the PCR products enabled us to distinguish mutant from wild-type. The PCR products were run on 2% agarose gel and the existence of exon 19 mutations was assessed (Figure 1).

Mutation Assay for L858R and L861Q in Exon 21

The restriction enzyme *Msc*I was used to digest the TGGCCA sequence in the amplicon of the wild-type allele. In contrast, mutant type (L858R) was not digested because of the base substitution of T to G at the first base of TGGCCA. On the other hand, 2582T>G mutation creates a new *Pvu*II restriction site, CAGCTG, that can be used for a PCR-RFLP assay to distinguish L861Q mutant allele from wild-type. The PCR products were digested simultaneously with the restriction enzymes *Msc*I and *Pvu*II and run on 2% agarose gel, and the existence of these mutations was assessed (Figure 1).