労災疾病臨床研究事業費補助金

# 胸膜中皮腫に対する新規治療法の臨床導入に関する研究

平成 27 年度　総括・分担研究報告書

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# Ⅰ．　総括研究報告

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# 【胸膜中皮腫に対する新規治療法の臨床導入に関する研究】

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**研究要旨**

近年新たながん免疫療法の有用性が報告されており、特に抗 PD-1 抗体はその有用性が期待されている。胸膜中皮腫におけるこれらの薬剤の有用性と安全性を評価すべく、医師主導治験を含めた臨床試験の実施について複数の製薬会社と交渉した結果、企業主体の治験が企画され実際に治験が開始された。また胸膜中皮腫患者における痛みや呼吸困難などの身体的苦痛に加え、精神的な苦痛や、社会的なストレスなどを客観的に評価するための尺度が必要と考え、胸膜中皮腫患者の QOL とその関連要因を明らかにするための調査票を用いた全国調査を企画し、調査を実施した。

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## A. 研究目的

悪性胸膜中皮腫患者の予後改善と生活の質の向上を図るため、新規免疫療法の有用性と安全性を評価する。また、中皮腫患者の身体的・精神的・社会的問題点を評価する緩和ケアのツールを作成し、導入する。

## B. 研究方法

手術不能あるいは術後の再発を来した悪性胸膜中皮腫患者で、ペメトレキセドによる化学療法に不応となった患者に対し、抗 PD-1 抗体の胸膜中皮腫に対する臨床での有用性を検討するための臨床試験をおこなう。がんの免疫療法のうち、免疫チェックポイントである PD-1 を標的とした抗 PD-1 抗体は、悪性黒色腫、非小細胞肺癌に対しすでに承認されているほか、他の固形腫瘍においても臨床試験において良好な治療成績が報告されつつあ

り、胸膜中皮腫においてもその有用性を期待する。並行して医師、看護師、薬剤師、ソーシャル・ワーカーなど多職種により中皮腫患者の身体的・精神的・社会的問題点を評価する緩和ケアのツール（中皮腫サポートマニュアル）を作成し、導入する。

研究初年度である今年度は、医師主導治験を念頭に、臨床試験を実施するための準備を開始した。また中皮腫サポートマニュアルの作成にあたり、実際の中皮腫患者が抱える問題点を抽出するための QOL 調査を企画した。

（倫理面への配慮）

本研究に関係するすべての研究者はヘルシンキ宣言および「臨床研究に関する倫理指針」（平成 20 年厚生労働省告示第 415 号）に従って本研究を実施する。臨床試験の実施に際しては、試験についての説明を行い、十分に考える時間を与え、患者が試験の内容をよく理解したことを確認した上で、試験への参加について依頼する。中皮腫サポートマニュアルの作成においては、緩和ケアをめぐる臨床倫理に則り、痛みやその他の身体的な症状の軽減と精神的、社会的、スピリチュアルな問題への支援を行い、安全かつエビデンスに基づく質の高いケアの提供を心がけるものとする。

本研究に関係するすべての研究者は、個人情報および診療情報などのプライバシーに関する情報は個人の人格尊重の理念の下、厳重に保護され慎重に取り扱われるべきものと認識し、万全な管理対策を講じ、プライバシー保護に努める。

## C. 研究結果

1）胸膜中皮腫における新規治療法について

近年新たながん免疫療法の有用性が報告されており、特に抗 PD-1 抗体はその有用性が期待されている。標準治療に不応となった現在闘病中の患者に、新たな治療の選択肢を提供し、かつ胸膜中皮腫におけるこれらの薬剤の有用性と安全性を評価すべく、医師主導治験を含めた臨床試験の実施について複数の製薬会社と交渉した。その結果、当該製薬会社が企業主体の治験を企画することとなり、そのうちの 1 つについて実際に治験が開始された。本研究組織の主任研究者、分担研究者も同治験に参画している。また従来のシスプラチンとペメトレキセドによる化学療法に、新たな分子標的薬剤であるベバシズマブを加えることで生存期間の延長効果が得られることが報告された。本邦においても同療法の有用性を評価すべく、臨床試験の実施について当該製薬会社と交渉中である。

2）胸膜中皮腫患者の QOL の向上について

胸膜中皮腫患者は疾病に起因する痛みや呼吸困難などの症状に加え、精神的な苦痛や、社会的なストレスなどさまざまな負担がかかる。胸膜中皮腫患者の QOL の向上を図るに際し、まず QOL を客観的に評価するための尺度が必要と考えた。そこで今年度は、胸膜中皮腫患者の QOL とその関連要因を明らかにするため、調査票を用いた全国調査を企画した。全国のがん拠点病院、緩和ケア病棟、訪問看護施設および、中皮腫患者会に研究への協力を呼び掛け、65 施設より研究協力への承諾をいただいた。すでに各施設を通じて、無記名にて患者さんに調査票を記入の上、郵送にて返送していただいており、横断的な解析にとりかかっている。

## D. 考察

悪性胸膜中皮腫に対する新規治療として、新規免疫療法の臨床導入と緩和ケアの向上を掲げた。免疫チェックポイントである PD-1 を標的とした抗 PD-1 抗体は、悪性黒色腫、非小細胞肺癌に対し有用性が報告されすでに承認されている。われわれは胸膜中皮腫においても有用性が期待できるものと考えたが、通常胸膜中皮腫は症例数が多くないため有用な薬剤が開発された場合でも企業主体の治験が企画されることが少ない。本補助金事業の発足にあたり、当該企業に治験実施について問い合わせをしたところ当初は胸膜中皮腫に対する治験の予定はないとの回答であった。われわれは胸膜中皮腫の治療の現状と新規治療法の開発の必要性、重要性を訴え、本補助金事業を基にした医師主導治験の実施を主眼に企業と交渉を重ねた。その結果企業主体の治験として胸膜中皮腫に対する治験が企画され、すでに一部は実施されるに至った。治験参加施設として着実に治験を遂行し、これらの薬剤の有用性と安全性を評価に寄与したいと考えている。

また中皮腫患者の身体的・精神的・社会的問題点を評価するための QOL 調査に着手した。胸膜中皮腫患者は病初期から痛みや呼吸困難などの症状を伴うことが多く、また石綿という産業物質で死に至る病になったゆえの精神的な苦痛や、補償申請に関連する社会的なストレスなどさまざまな負担がかかる。これらの評価においては、これまで他の癌腫において導入されている QOL の尺度をそのまま導入するだけでは不十分であり、胸膜中皮腫患者特有の QOL とその関連要因を明らかにするための指標が必要であると考える。幸い全国の多数の施設より研究協力への承諾をいただき、多くの患者さんから調査票を返送していただいている。次年度はこれらの患者さんの実際の声を基に、それらの因子を多職種にて評価するツールの作成に着手する予定である。

## E. 結論

胸膜中皮腫における現状の治療はその有用性がきわめて限られており、あらたな治療法の有用性、安全性の評価が不可欠である。また引き続き胸膜中皮腫に伴う身体的､精神的､社会的な苦痛を客観的に評価し対処するためのツールの開発に取り組む。

## F. 健康危険情報

臨床試験（治験）に際しては、新規抗悪性腫瘍薬の使用を想定している。その使用に際しては製薬メーカーから提供される取り扱い情報に基づき適正に取り扱う。また実際の投与に際しては、厚生労働省労働基準局より発出された「発がん性等を有する化学物質を含有する抗がん剤等に対するばく露防止対策について」（基案化発 0529 第 1 号）に則り各施設で定められた抗がん剤ばく露対策マニュアルを遵守し、医師、薬剤師、看護師が薬剤にばく露しないようにする。また患者やその家族に対しても、薬剤の取扱いに関する情報を周知する。

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## H. 知的財産権の出願・登録状況

### 1. 特許取得

該当するものなし。

### 2. 実用新案登録

該当するものなし。

### 3.その他

特記すべき事項なし。

(資料)

「胸膜中皮腫患者の QOL 調査票」

# 「胸膜中皮腫患者の QOL 調査票」

この調査は、胸膜中皮腫患者さんの生活の質を調べることで、よりよい治療や看護の提供を推進することを目的としています。お手数ですが、ご協力下さい。
調査結果は、学会や論文で公表されます。

・回答にかかる時間は 15 分ほどです。

・調査票は 9 ページです。その中には、似ている質問が繰り返されることがありますが、それぞれに分析方法が異なりますので、すべての質問にお答えください。

・ご自分で記入が難しい場合は、ご家族の方が読み書きをお手伝いくださって構いません。その場合は、ご家族のお気持ちでなく、患者さんご本人のお気持ちをお答えください。

# 胸膜中皮腫患者の QOL 調査

I.　患者さんご自身についてお答えください。

1. 年齢　　＿＿＿＿＿歳

2. 性別（一方に○をしてください）　　男　　女

3. 診断された年月　　＿＿＿＿＿年　＿＿＿＿月

4. これまでに受けた治療について、あてはまるものに○をしてください。

1）手術

・ 受けていない

・ 過去に受けた：＿＿＿＿年前に　（　胸膜肺全摘術　・　胸膜切除術　）

2）化学療法

・受けていない

・過去に受けた（　　年前）

・現在受けている

3）放射線療法

・受けていない

・過去に受けた（　　年前）

・現在受けている

4）痛みや息苦しさなどに対する治療（緩和ケアを含みます）

・受けていない

・過去に受けた

・現在受けている

5）現在服用中の薬の名前を書いてください

| |
|---|
| |

5. どれ位体を動かせるかについて、最も近いものの番号に〇をしてください。

1）全く問題なく活動できる。発病前と同じ日常生活が制限なく行える。

2）肉体的に激しい活動は制限されるが、歩行可能で、軽作業や座っての作業は 行うことができる。 例：軽い家事、事務作業

3）歩行可能で自分の身の回りのことはすべて可能だが作業はできない。
日中の 50%以上はベッド外で過ごす。

4）限られた自分の身の回りのことしかできない。
日中の 50%以上をベッドか椅子で過ごす。

5）全く動けない。自分の身の回りのことは全くできない。
完全にベッドか椅子で過ごす。

6. 中皮腫の症状がでて受診してから、診断されるまではスムーズでしたか？
最も近いものの番号に〇をしてください。

1）スムーズに診断された。
2）概ねスムーズに診断された。
3）あまりスムーズに診断されなかった。
4）なかなか診断されなかった。

7. 現在の治療に満足していますか？（あてはまるものに○をしてください）

| とても満足 | | 満足 | | どちらでもない | | 少し不満 | | 不満 |
|---|---|---|---|---|---|---|---|---|
| 5 | ——— | 4 | ——— | 3 | ——— | 2 | ——— | 1 |

8. 主治医との関係（あてはまるものに○をしてください）

| とても良い | | 良い | | どちらでもない | | あまり良くない | | 良くない |
|---|---|---|---|---|---|---|---|---|
| 5 | ——— | 4 | ——— | 3 | ——— | 2 | ——— | 1 |

9. ご家族はあなたを支えてくれますか？（あてはまるものに○をしてください）

| よく支えてくれる | | 支えてくれる | | どちらでもない | | あまり支えてくれない | | 支えてくれない |
|---|---|---|---|---|---|---|---|---|
| 5 | ——— | 4 | ——— | 3 | ——— | 2 | ——— | 1 |

10. アスベストで病気になったことについてどのようにお感じですか？

（あてはまるものに○をしてください）

| 激しい怒りを感じる | | 少し怒りを感じる | | どちらでもない | | あまり怒りを感じない | | 怒りは感じない |
|---|---|---|---|---|---|---|---|---|
| 5 | ——— | 4 | ——— | 3 | ——— | 2 | ——— | 1 |

11. 労災の申請について　（当てはまる数字に○をしてください）

1）申請していない

2）申請中

3）認定された

4）申請したが認定されなかった

12. 石綿健康被害救済法の救済給付について（当てはまる数字に〇をしてください）

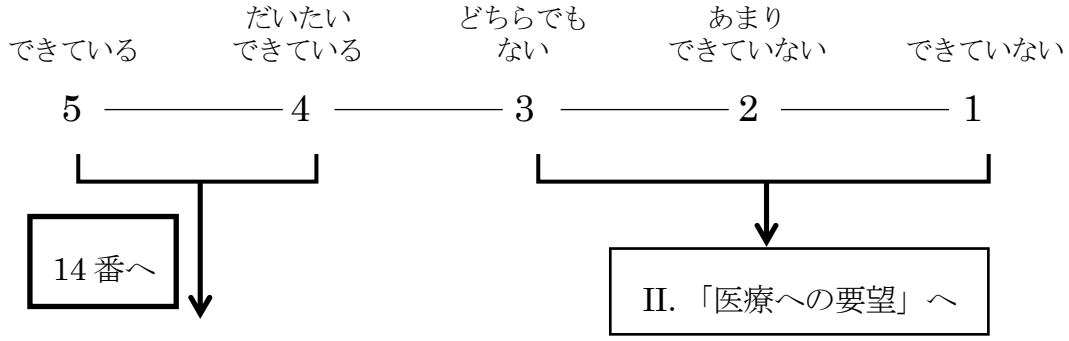
1）申請していない

2）申請中

3）認定された

4）申請したが認定されなかった

13. 病気や生活、人生について悩んだとき、誰かに気持ちを聞いてもらったり、相談できていますか？　（当てはまるものに〇をしてください）




14. それは、どなたですか？該当者全てに〇をしてください。

ご家族　　友人　　同僚　　医師　　看護師　　患者会

その他（　　）

## II.　医療へのご要望

1. 病気や治療について、不安なこと、困っていること、心配なことがおありでしたらお書きください。

2. 今後の治療選択にあたり、どのようなことに重きを置きますか？

当てはまるもの全てに○をしてください。

1）副作用があっても少しでも長く生きられる治療をしたい。

2）新しい薬の治療法の臨床試験に参加したい。

3）少し余命が短くなっても、体に負担のかからない治療をしたい。

4）その他

3. 診断や治療にあたって、あなたは医師にどんなことを望みますか？

またどのような態度や言葉で接して欲しいですか？

**III.** あなたの状態に、もっともよく当てはまる番号一つを〇で囲み、全設問にお答え下さい。「正しい」答えや「誤った」答え、といったものはありません。

| | まったくない | 少しある | 多い | とても多い |
|---|---|---|---|---|
| 1 重い買い物袋やスーツケースを運ぶなどの力仕事に支障がありますか。 | 1 | 2 | 3 | 4 |
| 2 長い距離を歩くことに支障がありますか | 1 | 2 | 3 | 4 |
| 3 屋外の短い距離を歩くことに支障がありますか。 | 1 | 2 | 3 | 4 |
| 4 一日中ベッドやイスで過ごさなければなりませんか。 | 1 | 2 | 3 | 4 |
| 5 食べること、衣類を着ること、顔や体を洗うこと、トイレを使うことに人の手を借りる必要がありますか。 | 1 | 2 | 3 | 4 |

| この一週間について | まったくない | 少しある | 多い | とても多い |
|---|---|---|---|---|
| 6 仕事をすることや日常生活活動に支障がありましたか。 | 1 | 2 | 3 | 4 |
| 7 趣味やレジャーをするのに支障がありましたか。 | 1 | 2 | 3 | 4 |
| 8 息切れがありましたか。 | 1 | 2 | 3 | 4 |
| 9 痛みがありましたか。 | 1 | 2 | 3 | 4 |
| 10 休息をとる必要がありましたか。 | 1 | 2 | 3 | 4 |
| 11 睡眠に支障がありましたか。 | 1 | 2 | 3 | 4 |
| 12 体力が弱くなったと感じましたか。 | 1 | 2 | 3 | 4 |
| 13 食欲がないと感じましたか。 | 1 | 2 | 3 | 4 |
| 14 吐き気がありましたか。 | 1 | 2 | 3 | 4 |
| 15 吐きましたか。 | 1 | 2 | 3 | 4 |
| 16 便秘がありましたか。 | 1 | 2 | 3 | 4 |

次のページにお進みください

| この一週間について | まったくない | 少しある | 多い | とても多い |
|---|---|---|---|---|
| 17 下痢がありましたか。 | 1 | 2 | 3 | 4 |
| 18 疲れていましたか。 | 1 | 2 | 3 | 4 |
| 19 痛みがあなたの日々の活動のさまたげになりましたか。 | 1 | 2 | 3 | 4 |
| 20 ものごとに集中しにくいことがありましたか。たとえば新聞を読むときや、テレビを見るとき。 | 1 | 2 | 3 | 4 |
| 21 緊張した気分でしたか。 | 1 | 2 | 3 | 4 |
| 22 心配がありましたか。 | 1 | 2 | 3 | 4 |
| 23 怒りっぽい気分でしたか。 | 1 | 2 | 3 | 4 |
| 24 落ち込んだ気分でしたか。 | 1 | 2 | 3 | 4 |
| 25 もの覚えが悪くなったと思いましたか。 | 1 | 2 | 3 | 4 |
| 26 身体の調子や治療の実施が、家族の一員としてのあなたの生活のさまたげになりましたか。 | 1 | 2 | 3 | 4 |
| 27 身体の調子や治療の実施が、あなたの社会的な活動のさまたげになりましたか。 | 1 | 2 | 3 | 4 |
| 28 身体の調子や治療の実施が、あなたの経済上の問題になりましたか。 | 1 | 2 | 3 | 4 |

**次の質問では、1から7の数字のうち、あなたにもっともよく当てはまる数字を○で囲んで下さい。**

29 この一週間のあなたの健康状態は全体としてどの程度だったでしょうか。

1 2 3 4 5 6 7

とても悪い とてもよい

30 この一週間、あなたの全体的な生活の質はどの程度だったでしょうか。

1 2 3 4 5 6 7

とても悪い とてもよい

## IV. 現在の療養生活をどのようにお感じになられていますか？

もっとも近い番号に○をおつけください。

| | 全くそう思わない | そう思わない | あまりそう思わない | どちらともいえない | ややそう思う | そう思う | 非常にそう思う |
|---|---|---|---|---|---|---|---|
| ○からだの苦痛が少ない | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○望んだ場所で過ごせている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○楽しみになることがある | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○医師を信頼している | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○人に迷惑をかけてつらいと感じる | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○ご家族やご友人と十分に時間を過ごせている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○身の回りのことはたいてい自分でできる | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○落ち着いた環境で過ごせている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○ひととして大切にされていると感じる | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○人生をまっとうできていると感じる | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○納得がいくまで治療を受けている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○自然に近いかたちで過ごせている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○大切な人に伝えたいことを伝えられている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○先ざきに起こることについて知りたいことを聞けている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○病気を意識せずに過ごせている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○他人に弱った姿をみせて辛いと感じている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○生きていることに価値を感じている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| ○信仰に支えられている | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

V.「中皮腫・アスベスト疾患・患者と家族の会」について

当てはまる数字に〇をしてください。

1）知らない

2）聞いたことはあるが会員ではない

3）会員である

VI. その他なんでも自由にご意見をお書きください。

お答えの内容確認が必要になった場合に、ご連絡を差し上げてもいいですか？
どちらかに〇をしてください。

はい　　いいえ

↓

お電話番号をお書きください。

（　　）　　—

これで終わりです。返信用封筒に入れて投函してください。
ありがとうございました。

# Ⅱ．　分担研究報告

労災疾病臨床研究事業費補助金分担研究報告書

# 【胸膜中皮腫における患者の Quality of Life (QOL) 調査に関する研究】

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**研究要旨**

胸膜中皮腫患者の身体的苦痛、精神的な苦痛や、社会的なストレスなどを客観的に評価するための尺度が必要と考え、QOL とその関連要因を明らかにするための調査票を用いた全国調査を企画し、実施した。全国のがん拠点病院を中心とした 422 施設に調査協力の依頼を行い、協力の承諾を得られた 65 施設に対して質問票を配布し、胸膜中皮腫患者への調査票の配布を依頼した。これまでに 49 名の患者より質問票を回収し、中間解析を行った。

## A. 研究目的

胸膜中皮腫は、多くの場合 2 年以内に死亡し、様々な症状が重篤に出現するため、完治を目的とする治療だけでは患者と家族が直面する困難を解決することは難しい。英国の胸膜中皮腫ガイドラインは、診断と同時に緩和ケアを導入することで、QOL を保つことを奨励している。我が国においても、胸膜中皮腫患者の QOL が阻害されている可能性を示唆する研究が発表されており、患者の QOL の保持向上を可能とする、我が国の治療方法にあった患者支援体制の整備が急務である。しかしながら、胸膜中皮腫患者の QOL の実態を調査した研究は世界的に見てもみあたらない。そこで世界に先立ち、我が国における胸膜中皮腫患者の QOL について調査を行うこととした。

## B. 研究方法

QOL 調査には世界中でがん患者を対象に広く用いられている QOL 尺度である EORTC (The European Organization for Research and Treatment of Cancer) QLQ-C3 と、日本で作成された包括的 QOL 尺度である CoQoLo（患者による緩和ケアのアウトカム尺度）を用いた。EORTC QLQ-C30 は、がん患者の QOL を評価するための指標であり、81 の言語に翻訳され、その信頼性や妥当性が検証され、3,000 以上の試験で世界的に用いられている。5 つの機能スケール（身体、役割、認知、情緒、社会）と 3 つの症状スケール（倦怠感、悪心、痛み）および単一の症状項目から構成され、それぞれ 4 段階（1－全くない、2－少しある、3－多い、4－非常に多い）で評

価する。2 つの全般的な QOL スケールは 7 段階（1－とても悪いから 7－とてもよい）で評価する。これら 2 つの QOL 尺度に加えて年齢、性別、診断日、治療、ECOG (Eastern Cooperative Oncology Group) Performance Status (PS)、労災や石綿健康被害救済法の認定の有無などもあわせた自記式質問票を作成した。全国のがん拠点病院を中心とした 422 施設に調査協力の依頼を行い、協力の承諾を得られた 65 施設に対して質問票を配布し、胸膜中皮腫患者への調査票の配布を依頼した。また、中皮腫・アスベスト疾患患者と家族の会に依頼し、胸膜中皮腫患者の会員に対して質問票を配布した。胸膜中皮腫患者が質問票に記入後、事務局へ郵送することで回収した。

## C. 研究結果

2016 年 3 月 7 日までに 49 名の患者より質問票を回収でき、中間解析を行った。回答者は病院経由で質問票を渡された通院患者が主で、年齢は 42−86 歳（平均 69.8 歳）、男性 71.4%であった。PS が 0 か 1 で良好なものが 61.2%と多く、治療に関する不満も少なかった。労災または石綿健康被害救済法の認定者は 65.3%であったが、55.1%が診断から 12 ヶ月以下であるため、申請中のものも多かった。

EORTC QLQ-C30 の結果では nausea & vomiting（嘔気・嘔吐）, diarrhea（下痢）は平均 20 点未満と、特に訴えが少なく、他の症状スコアも 50 点を超える項目はなかった。一方、身体機能、役割機能、情緒的機能、認知機能、社会的機能はいずれも平均 50 点以上であった。

CoQoLo では、医師への信頼が平均 6.3 点と非常に高かった。逆に身体的苦痛、人に迷惑をかけること、病気を意識すること、弱った姿を見せることなどは平均 4 点未満と不良であった。

## D. 考察

中皮腫患者の身体的・精神的・社会的問題点を評価するための QOL 調査に着手した。胸膜中皮腫患者は病初期から痛みや呼吸困難などの症状を伴うことが多く、また石綿という産業物質で死に至る病になったゆえの精神的な苦痛や、補償申請に関連する社会的なストレスなどさまざまな負担がかかる。これらの評価においては、これまで他の癌腫において導入されている QOL の尺度をそのまま導入するだけでは不十分であり、胸膜中皮腫患者特有の QOL とその関連要因を明らかにするための指標が必要である。まだ中間解析ではあるが、全国の多数の施設より研究協力への承諾をいただき、多くの患者から調査票を返送していただいている。

このたびの解析では、嘔気・嘔吐や下痢などの症状をはじめ、全般的に訴えは少ない傾向であった。これまでの回答者は病院経由で質問票を渡された通院患者が中心で PS が 1 以下と比較的良好な方が 61.2%と多かったためと思われる。ただそれでも身体機能、日常役割機能、情緒的機能、認知機能、社会的機能はいずれも低下していることが示されており、中皮腫における QOL の阻害が示唆された。現時点でもさらに調査票は回収中であり、次年度はより詳細な解析を予定している。これまでのところ、比較的 PS が良好な患者からの回答が主であるが、今後は病状が進行している患者か

らの回答を得るための方策も考える必要がある。そしてこれらの調査により明らかにされた QOL 阻害要因を取り除くための方策を立案していく予定である。

## E. 結論

胸膜中皮腫患者においては身体機能、日常役割機能、情緒的機能、認知機能、社会的機能がいずれも低下している可能性がある。

## G. 研究発表

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**H. 知的財産権の出願・登録状況（予定を含む。）**

**1. 特許取得**

該当するものなし。

**2. 実用新案登録**

該当するものなし。

**3.その他**

特記すべき事項なし。

労災疾病臨床研究事業費補助金分担研究報告書

# 【岡山労災病院における胸膜中皮腫患者の QOL 調査】

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**研究要旨**

胸膜中皮腫患者に対し、治療の初期段階から適切な緩和ケアを導入するため、スクリーニングツールとして調査票を用いた聞き取り調査を行った。今後の早急の課題として、まず患者に疾患や治療、今後の経過、緩和ケア、経済的な支援などについて適切に情報提供できる体制が必要であると思われた。

## A. 研究目的

WHO（世界保健機関）は、がん緩和ケアを「生命を脅かす疾患による問題に直面している患者とその家族が抱えている身体的、精神的、社会的、スピリチュアルな苦痛を早期に診断し、適切に対応・治療することで QOL(生活の質)を向上させる医療」としている。がん緩和ケアは、1990 年頃までは「終末期医療」とされてきた。しかし WHO は 2002 年の改定で、その概念を「がん治療の早期から開始すべき積極的な医療」へと転換した。日本でも 2007 年、「がん対策推進基本計画」が策定され、「がん患者及びその家族が可能な限り、質の高い療養生活を送れるようにするため、治療の初期段階から緩和ケアの実施を推進していくこと」が掲げられた。

胸膜中皮腫は、多くの場合 2 年以内に死亡し、様々な症状を伴う。また胸膜中皮腫においては病初期より胸痛、息切れなどの症状を呈することが多く、そこへ不安、抑うつなどの心の苦痛、仕事や家族、経済的な問題などの社会的な苦痛、さらに、いわゆるスピリチュアルペインと呼ばれる苦痛も前面に出てくる。岡山労災病院ではがんサポートチーム（緩和ケアチーム）を中心に、胸膜中皮腫患者における早期からの緩和ケアの導入に取り組んでいる。このたびの研究では、病初期から中皮腫患者が抱える問題点を適切に抽出するため、QOL 調査

票を用いた聞き取り調査を導入した。

## B. 研究方法

岡山労災病院呼吸器科病棟に入院した悪性胸膜中皮腫の患者に対して「胸膜中皮腫患者の QOL 調査票」を用いて、2015 年 11 月より情報収集を開始した。入院中あるいは外来通院中の患者に質問用紙を渡し、記入された情報を元に看護師が直接内容を聞き取ることとした。

## C. 研究結果

これまでのところ、胸膜中皮腫患者 5 名に対し実際に聞き取り調査を行った。このうち 1 名に関しては毎月 1 回、計 6 ヶ月にわたり同じ調査票を用いて調査し、情報を収集した。

現在調査を行った人数：5 名　（述べ 10 名）

1. 年齢：　50 代　2 名、60 代　1 名、70 代　2 名
2. 性別：　男 4 名、女 1 名
4. 手術歴：　あり 1 名、なし 4 名
   - 化学療法：過去に受けた　1 名、現在受けている　4 名
   - 放射線療法：1 名　(脳転移に対してサイバーナイフ)
   - 症状緩和治療：受けている 4 名、以前受けた 1 名
5. 現在の活動：PS0：1 名　PS1：2 名　PS2：4 名　PS3：3 名
6. 受診してからスムーズに診断されたか：
   - スムーズだった　1 名、概ねスムーズだった　2 名、あまりスムーズに診断されなかった　1 名、なかなか診断されなかった　1 名
7. 現在の治療に満足していますか：
   - とても満足　2 名、どちらでもない　2 名
8. 主治医との関係は：
   - とても良い　2 名、どちらでもない　3 名（1 名良いと重複）
9. 家族は支えてくれますか：
   - 良く支えてくれる　4 名、どちらでもない　1 名
10. アスベストで病気になったことについてどのように感じていますか：
    - 激しい怒りを感じる　5 名
11. 労災申請：　申請中　2 名、認定済み　2 名
12. 石綿救済法の申請中：　1 名
13. 病気や人生について誰かに気持ちを聞いてもらっていますか：
    - できている　3 名
    - だいたいできている　1 名
    - あまりできていない　1 名（家族は支えてくれるが、話はあまりできない。）
    - できていない　1 名（家人が中皮腫について理解できていなかった為本人の訴えを信じられず、聴く姿勢がとれていなかった。）

14. 訴えを聞いてくれる相手は：

家族３名、　医師、看護師１名、　同僚１名、　他の患者　１名

II. 医療への要望

1. 病気や治療について、不安なこと、困っていること、心配なことは。(原文まま)

症状のコントロールが困難。(疼痛　食欲不振　不眠)

治るのか治らないのか。

今後どうなっていくのか心配。　２名

病気についての知識が無いのでこれからどのように進行するのか、

どうなるのか分からず不安だらけ。

2. 今後の治療は何に重きを置きますか：

副作用があっても長く生きられる治療　２名

治験に参加したい　４名

少し余命が短くなっても、身体に負担のかからない治療　３名

3. 診断や治療にあたって医師に望むこと(原文まま)：

・がんの余命宣告は、知らない方が希望が湧いて少しは生きられると思う。

・病人、患者の立場に立って考えて欲しい。親身に治療して欲しい。

・日常生活が問題なく送れるようにして欲しい。

・毎日声をかけて欲しい。患者とコミュニケーションをとって欲しい。

III. あなたの状態に、もっともよくあてはまる番号を一つお答え下さい。

| | まったくない | 少しある | 多い | とても多い |
|---|---|---|---|---|
| 1　重い買い物袋やスーツケースを運ぶなどの力仕事に支障がありますか。 | 3 | 1 | 1 | 4 |
| 2　長い距離を歩くことに支障がありますか。 | 0 | 3 | 3 | 4 |
| 3　屋外の短い距離を歩くことに支障がありますか。 | 1 | 5 | 3 | 1 |
| 4　一日中ベッドやイスで過ごさなければなりませんか | 4 | 2 | 3 | 1 |
| 5　食べること、衣類を着ること、顔や体を洗うこと、トイレを使うことに人の手を借りる必要がありますか。 | 7 | 3 | 0 | 0 |
| 6　仕事をすることや日常生活活動に支障がありましたか。 | 0 | 5 | 1 | 3 |
| 7　趣味やレジャーをするのに支障がありましたか。 | 1 | 2 | 1 | 4 |
| 8　息切れがありましたか。 | 1 | 1 | 7 | 1 |

| | | | | |
|---|---|---|---|---|
| 9　痛みはありましたか。 | 0 | 3 | 7 | 0 |
| 10　休息をとる必要がありましたか。 | 0 | 4 | 4 | 1 |
| 11　睡眠に支障がありましたか。 | 1 | 3 | 1 | 5 |
| 12　体力が弱くなったと感じましたか。 | 0 | 2 | 2 | 6 |
| 13　食欲がないと感じましたか。 | 0 | 3 | 2 | 5 |
| 14　吐き気がありましたか。 | 4 | 6 | 0 | 0 |
| 15　吐きましたか。 | 9 | 1 | 0 | 0 |
| 16　便秘がありましたか。 | 3 | 4 | 1 | 2 |
| 17　下痢がありましたか。 | 5 | 5 | 0 | 0 |
| 18　疲れていましたか。 | 1 | 3 | 1 | 4 |
| 19　痛みがあなたの日々の活動のさまたげになりましたか。 | 1 | 3 | 5 | 1 |
| 20　ものごとに集中しにくいことがありましたか。例えば新聞を読むときや、テレビを見るとき。 | 1 | 2 | 1 | 6 |
| 21　緊張した気分でしたか。 | 2 | 2 | 3 | 3 |
| 22　心配がありましたか。 | 1 | 2 | 1 | 6 |
| 23　怒りっぽい気分でしたか。 | 4 | 4 | 2 | 0 |
| 24　落ち込んだ気分でしたか。 | 2 | 0 | 2 | 6 |
| 25　もの覚えが悪くなったと思いましたか。 | 2 | 0 | 1 | 7 |
| 26　身体の調子や治療の実施が、家族の一員としてのあなたの生活のさまたげになりましたか。 | 1 | 2 | 2 | 5 |
| 27　身体の調子や治療の実施が、あなたの社会的な活動のさまたげになりましたか。 | 2 | 0 | 1 | 7 |
| 28　身体の調子や治療の実施が、あなたの経済上の問題になりましたか。 | 3 | 1 | 3 | 3 |

26 に関して

・妻の精神状態が不安定になり、心療内科を受診するようになった。妻の付添いの為に他の家族に負担をかけている。

・遠方での治療の為家族との時間がなくなる。2 名

・家事ができなくなった。その為夫が家事をおこなわなければいけなくなった。

27 に関して

・町内の役員ができなくなった。2 名　その分家族が役割を代行し、負担をかけている。

・仕事を休まなければいけない。

28 に関して

・労災認定されると問題ないが医療費がかかる。

・交通費

・今まで自分で行っていた庭木の剪定などが出来なくなり業者に委託するため、お金がかかる。
・救済法について今まで聞いたこともなく、医療費を払っていた。今回治験の為転院し、初めて医療費の支援がある事を聞いた。

29　この一週間のあなたの健康状態は全体としてどの程度だったでしょうか。

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| とても悪い | 3名 | 2名 | 3名 | 1名 | | とてもよい |

30　この一週間、あなたの全体的な生活の質はどの程度だったでしょうか。

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| とても悪い | 2名 | 5名 | 2名 | 1名 | | とてもよい |

IV. 現在の療養生活をどのようにお感じになられていますか？

| | 全くそう思わない | そう思わない | あまりそう思わない | どちらともいえない | ややそう思う | そう思う | 非常にそう思う |
|---|---|---|---|---|---|---|---|
| ○からだの苦痛が少ない | 2 | 1 | 2 | 2 | 1 | 0 | 0 |
| ○望んだ場所で過ごせている | 1 | 0 | 1 | 4 | 0 | 2 | 0 |
| ○楽しみになることがある | 2 | 2 | 1 | 2 | 0 | 2 | 0 |
| ○医師を信頼している | 0 | 0 | 0 | 0 | 0 | 2 | 6 |
| ○人に迷惑をかけてつらいと感じる | 0 | 1 | 0 | 2 | 0 | 1 | 3 |
| ○ご家族や友人と十分に時間を過ごせている | 2 | 1 | 0 | 3 | 0 | 2 | 0 |
| ○身の回りのことはたいてい自分でできる | 0 | 0 | 1 | 1 | 2 | 3 | 1 |
| ○落ち着いた環境で過ごせている | 0 | 0 | 0 | 3 | 3 | 2 | 0 |
| ○ひととして大切にされていると感じる | 0 | 0 | 0 | 1 | 2 | 4 | 1 |
| ○人生をまっとうできていると感じる | 1 | 2 | 1 | 2 | 2 | 0 | 0 |
| ○納得がいくまで治療を受けている | 0 | 0 | 0 | 1 | 1 | 4 | 2 |
| ○自然に近いかたちで過ごせている | 1 | 0 | 1 | 2 | 3 | 1 | 0 |
| ○大切な人に伝えたいことを伝えられている | 0 | 0 | 1 | 3 | 0 | 4 | 0 |
| ○先ざきに起こることについて知りたいことを聞けている | 0 | 1 | 2 | 2 | 2 | 1 | 0 |
| ○病気を意識せずに過ごせている | 6 | 1 | 0 | 1 | 0 | 0 | 0 |
| ○他人に弱った姿をみせて辛いと感じている | 0 | 3 | 1 | 0 | 1 | 1 | 2 |
| ○生きていることに価値を感じている | 1 | 0 | 0 | 1 | 0 | 0 | 6 |
| ○信仰に支えられている | 4 | 0 | 1 | 1 | 2 | 0 | 0 |

## D. 考察

中皮腫患者が抱える問題点を病初期から適切に抽出するため、QOL 調査票を用いた聞き取り調査を導入した。まだ導入したばかりであるが、1）さまざまな症状の出現が日常生活の妨げになっていること、2）疾患に対する知識不足により本人は不安が強く、また家族も疾患を正しく認識できていないため本人の訴えに寄り添えない状況があること、3）中皮腫と診断され労災認定された場合療養給付が受けられるが、それでも病気療養により日常生活において他人に依頼しなければならないことも増え、金銭的負担となるケースがあること、4）石綿救済法に対する認識はいまだ十分ではなく、知らされていない患者がいること、などが明らかとなった。今後の早急の課題として、まず患者に疾患や治療、今後の経過、緩和ケア、経済的な支援などについて適切に情報提供をできる体制が必要であると思われた。また実際の聞き取り調査に当たり、調査に参加した看護師からは、調査票をもとに情報収集を行うことで、普段であれば聴きづらい内容に関しても聞くことができる、これらの聞き取り調査から得られる情報を具体的に治療や看護に活かすことができるのではないか、などの声が聞かれた。次年度以降、できるだけ多くの患者に早期から聞き取り調査を行うこと、また診断時から治療導入、病状の寛解あるいは進行などさまざまな状況においても継続的に調査を継続することで中皮腫患者が抱える様々な問題点をより具体的に抽出することが可能となるものと思われた。

## G. 研究発表

### 1. 論文発表

該当するものなし。

### 2. 学会発表

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## H. 知的財産権の出願・登録状況（予定を含む。）

### 1. 特許取得

該当するものなし。

### 2. 実用新案登録

該当するものなし。

### 3.その他

特記すべき事項なし。

## Ⅲ．　研究成果の刊行に関する一覧表

＜研究成果の刊行に関する一覧表＞

【書 籍】

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| 加藤勝也. | 胸膜肥厚・腫瘤の鑑別はどこでするのか？－石綿関連胸膜病変の鑑別を中心に. | 酒井文和 | 呼吸器画像診断のコツ. －見る・診る・語る | 克誠堂出版 | 東京 | 2015 | 123-133 |

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# Ⅳ.　研究成果の別刷

# 15 胸膜肥厚・腫瘤の鑑別はどこでするのか？
## —石綿関連胸膜病変の鑑別を中心に—

加藤 勝也

## はじめに

胸部単純X線写真やCTなどを主とした画像診断に際し，胸膜肥厚所見を認めた場合，良性か悪性か，腫瘍性か非腫瘍性かを鑑別する必要が生じる。その鑑別のポイントを示すことが本稿の目的であるが，炎症性から腫瘍性までさまざまな胸膜肥厚病変を呈するモデルとして，石綿関連胸膜病変がある。まずは石綿関連胸膜病変における，良性・悪性病変の所見について，その鑑別点を述べ，それに基づいて一般的な良悪胸膜病変の鑑別を示していく。

石綿関連胸膜病変としては，次のような疾患が挙げられる。まず良性病変として，胸膜プラーク，良性石綿胸水，びまん性胸膜肥厚があり，悪性病変としては中皮腫がある。まずは，これら石綿関連胸膜疾患について，画像的にどのように鑑別していくべきなのか以下に示していくこととする。

## 胸膜プラーク

胸膜プラークは胸膜肥厚斑または限局性胸膜肥厚とも呼ばれる。胸膜プラークは石綿以外の鉱物でも生じ得るが，現在の日本には石綿以外は存在しない。よって事実上わが国においては，胸膜プラークは石綿曝露に特異的所見と考えてよい。画像による胸膜良悪病変の鑑別の際に，胸膜プラークは特異的所見を呈し，また日常診療で遭遇する機会も意外に多く，その所見に精通しておく必要がある。

胸膜プラークは石綿低濃度曝露によっても生じるため，環境曝露や傍職業性曝露でも生じることがあり，明確な石綿の職業性曝露歴を認めないこともままある。一般的には石綿曝露から少なくとも10年以上経過して生じ，以後時間の経過とともに徐々に増大する[1]。胸膜プラークは限局性，板状の胸膜肥厚であり，その大部分は壁側胸膜に生じる。厚みは1 mm以下のものから10 mm以上のものまであり多彩であるが，1〜5 mm程度の厚さのものが多い。好発部位としては胸壁背外側第7〜10肋骨レベル，前外側6〜9肋骨レベル，横隔膜ドーム部，傍椎体領域などが知られており，肺尖部や肋骨横隔膜角部には通常みられない[2]。石灰化の頻度は10〜15%程度とされおり，石綿曝露から20年程度経って出現し，時間の経過とともに石灰化を伴う頻度が増加するといわれている[3]。

胸部CTではプラークは限局的な板状胸膜肥厚として描出される。2〜3 mm程度以上の厚みを持った病変であれば明瞭に描出可能である（図1）。筋肉よりもやや高吸収を呈し，厚みが1〜2 mm以下のような薄いプラーク症例（図2）では診断に迷うこともあるが，3 mm以上の厚みを持つような病変で前記のような好発部位に

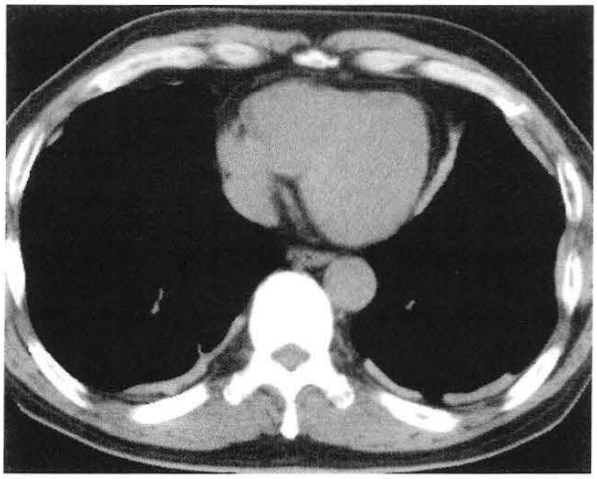


**図 1　典型的な胸膜プラーク**

70 歳代男性（単純 CT）：比較的厚い限局的な板状の胸膜肥厚を認める。典型的な胸膜プラークの所見である。内部均一で，筋肉よりやや高吸収である。

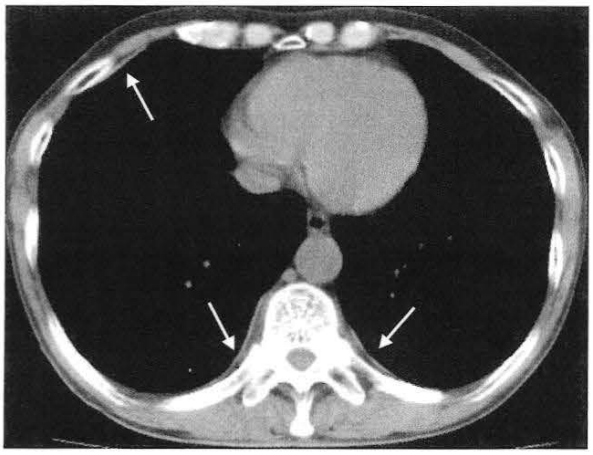


**図 2　薄い胸膜プラーク**

60 歳代男性（単純 CT）：傍椎体部や前外側部など好発部位に多発する薄い胸膜プラークを認める。やはり限局状，板状の胸膜肥厚で筋肉に比し同程度からやや高吸収である。

多発している場合には，ほかの胸膜病変と迷うことなく画像のみで診断可能である[4]。胸膜プラークは長期の経過で石灰化を来すが，石灰化を伴う胸膜肥厚を呈し鑑別が必要となる病変として，陳旧性結核性胸膜炎がある。鑑別点であるが，通常結核性胸膜肥厚は片側性で比較的広範に及ぶ肥厚であり石灰化が臓側胸膜側にも生じる（図 3）。これに対し胸膜プラークの石灰化は全層にわたって生じる症例もあるが，特に厚みのある症例では壁側胸膜側に部分的な石灰化を生じることが陳旧性結核性胸膜炎との重要な鑑別点となる[5]（図 4）。

またプラークの厚さが 1 cm を超えるような症例，かなり左右差がある症例や，さらには完全に片側性の症例，葉間胸膜にもプラークを認める症例なども頻度は低いが存在することを念頭におく必要がある。いずれも胸膜中皮腫や胸膜播種が鑑別を要する悪性疾患となる。まず，1 cm を超えるような厚いプラークについてであるが，中皮腫をはじめとする腫瘍性病変との鑑別点は，造影効果の有無である。プラークは硝子化病変で造影効果をほとんど伴わない。これに対し腫瘍である中皮腫やその他腫瘍性病変では造影効果を認める[6]。また，中皮腫では形態的に板状というよりも結節状を呈している。しかし，結節状の形態を呈するプラークも時にあり（図 5），この鑑別は意外に難しい。また FDG-PET で集積を認めれば，限局的な胸膜肥厚の場合腫瘍性病変の可能性が高くなる[7]（図 6）。胸膜プラークは通常壁側胸膜に生じるとされるが，まれに臓側胸膜にも生じ，その場合葉間胸膜にも生じ得る（図 7）。通常は葉間胸膜にプラークを生じるような症例では，その他部位にはっきりとしたプラーク所見を認めることが多く，また中皮腫や癌性胸膜炎のように胸水を伴わない症例が大部分である。さらに数年の経過でほとんど変化しない。基本的なことであるが，経過観察における増大の有無というのは重要な所見であり，良悪の鑑別に悩む際には経過観察するというのは 1 つの有効な選択肢である。ただ，胸膜中皮腫の場合まれに 1 週間単位で急速増大を認めるような症例があり，経過観察する場合には，まずはあまり期間をあけずに急速進行性の病変ではないことを確認しておくべきである。

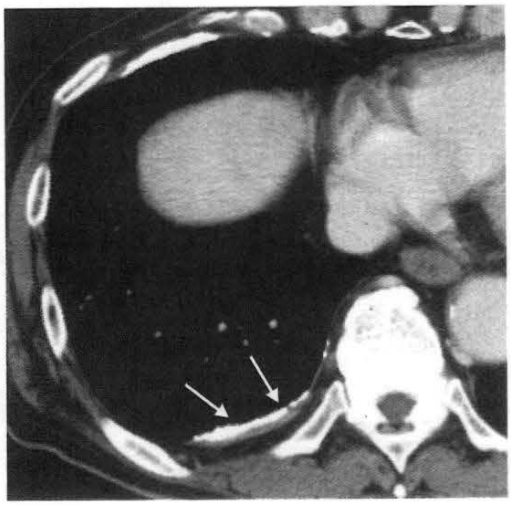


図3　陳旧性結核性胸膜炎

70歳代女性(単純CT)：右側胸膜肥厚を認め，石灰化を伴っているが，後で示す胸膜プラークの症例と異なり，臓側胸膜側に沿った石灰化所見を認める（→）。

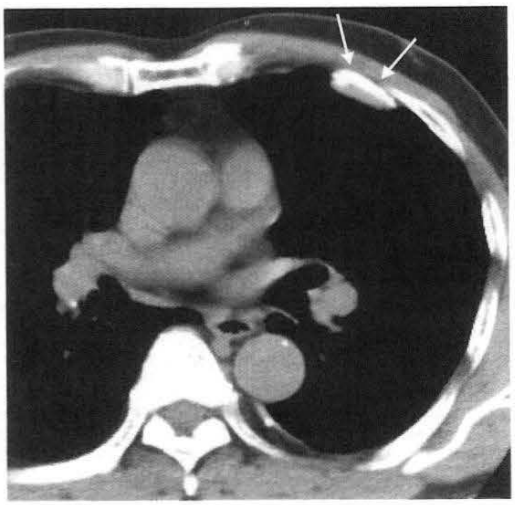


図4　石灰化胸膜プラーク

80歳代男性（単純CT）：左前側に厚い胸膜プラークを認める。石灰化を伴っているが先ほどの陳旧性結核症例と異なり壁側胸膜側に石灰化所見を認める（→）。

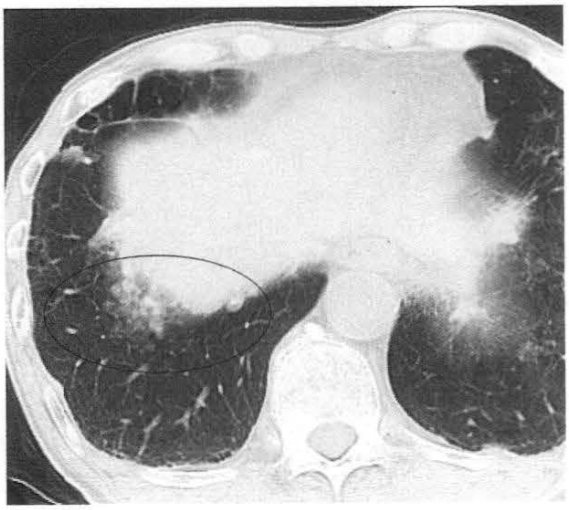


図5　粒状から結節状の胸膜プラーク

70歳代男性（単純CT肺野条件）：右横隔膜上に小粒状影から結節影が集簇したような形態の胸膜プラークを認める。その他にも両側横隔膜上，左背側など多発胸膜プラークを認める。

## 胸膜中皮腫

中皮腫は胸膜，心膜，腹膜，精巣鞘膜などに生じ，胸膜が最も頻度が高い。80～90%程度以上が石綿曝露によるものとされており，低濃度曝露でも生じ，曝露後40年程度経て発症することが多いが，10年程度で発症する例もある。大部分の症例で胸水を伴う。発見時に胸水を認めない症例も時に存在するが，そのほとんどで経過中に一度は胸水を合併するとされる[8]。予後は非常に悪く上皮型中皮腫は12カ月，肉腫型は6カ月程度で2年生存率が30%程度である[9]。

胸膜中皮腫の典型的CT像は片側性胸水，広範なびまん性の不整結節状胸膜肥厚像である。典型例では患側胸郭の容量は低下し，胸膜はびまん性に厚く不整に肥厚し，肺を環状全周性に取り巻き，葉間胸膜にも進展する（図8）。びまん性不整胸膜肥厚を呈する頻度が高いが，時には胸膜肥厚が目立たず，多発腫瘤を形成するような症例もある（図9）。われわれが行った胸膜中皮腫211例の診断確定時CT所見の検討では，先に示したような典型的な悪性所見を呈する例が78%程度と大部分を占めた[10]。しかし注意すべき点は，残りの22%では画像上胸膜不整をまったく伴わないか，良性胸膜病変も十分考え

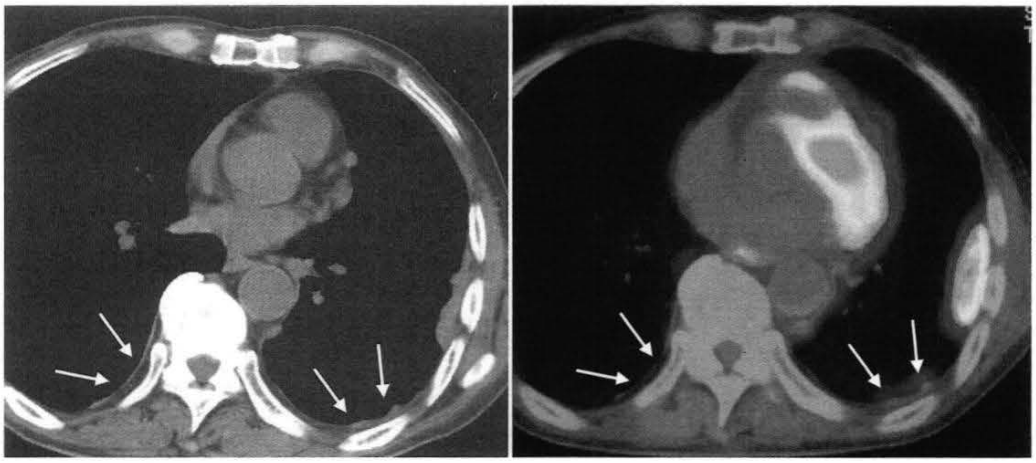


**図6　胸膜プラークを伴う中皮腫症例**

70歳代男性（単純CT，FDG-PET/CT）：左側胸部の中皮腫病変にFDG-PETの集積を認めるが，胸膜プラーク（→）には集積を認めない。

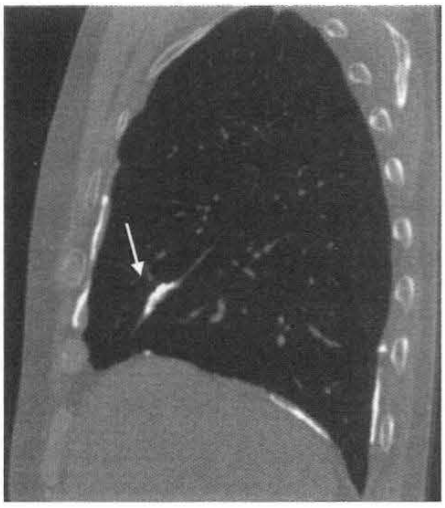


**図7　葉間胸膜上の胸膜プラーク**

80歳代男性（単純CT矢状断像）：横隔膜上や背側に石灰化胸膜プラークを認めるが，葉間胸膜にも同様の石灰化を伴う板状の形態を呈する胸膜プラークを認める。

られる程度の軽度胸膜肥厚所見しか示さなかったということで，画像診断の限界として常に念頭においておく必要がある。

これらのことを踏まえて，胸膜中皮腫の早期診断のポイントについて述べる。まず胸膜中皮腫早期例は原因不明の胸水で発見される場合が多いが，原因不明の胸水の鑑別診断としては，胸膜中皮腫以外に良性病変として，結核性胸膜炎，良性石綿胸水，膠原病関連胸水などが挙がる。悪性病変としては癌性胸膜炎が挙げられる。これらの鑑別にはまず，胸水穿刺を行い性状のチェックと細胞診を施行し，さらに胸水中のヒアルロン酸値，ADA値などを臨床的に検討することになるが，画像診断に主に求められるのは，良性と悪性の鑑別である。胸部CTにおいて胸膜病変の良悪性の鑑別点として挙げられている所見として，結節状や腫瘤状胸膜肥厚，環状胸膜肥厚（図10），1 cm以上の胸膜肥厚，縦隔胸膜肥厚（図11）がある。これらの所見が多く認められるほど悪性疾患が疑われるが，悪性病変であっても半数程度でしかこれらの所見は認められないともしている[11]。上述のわれわれの検討でも胸膜中皮腫症例の22%程度は，診断確定時のCT所見で良性胸膜病変と鑑別困難であった。これに関して，臨床的によく遭遇する肺癌胸膜播種などの癌性胸膜炎を例に考えるとわかりやすい。癌性胸膜炎でも中皮腫と同様に，その画像診断に際しては胸膜に播種を疑う不整像があるかどうかに注目するが，画像的に胸膜不整を認めないが，胸水穿刺で悪性細胞を認めたり，手術時に肉眼的に播種病変を認めるという経験は，それほどまれなことで

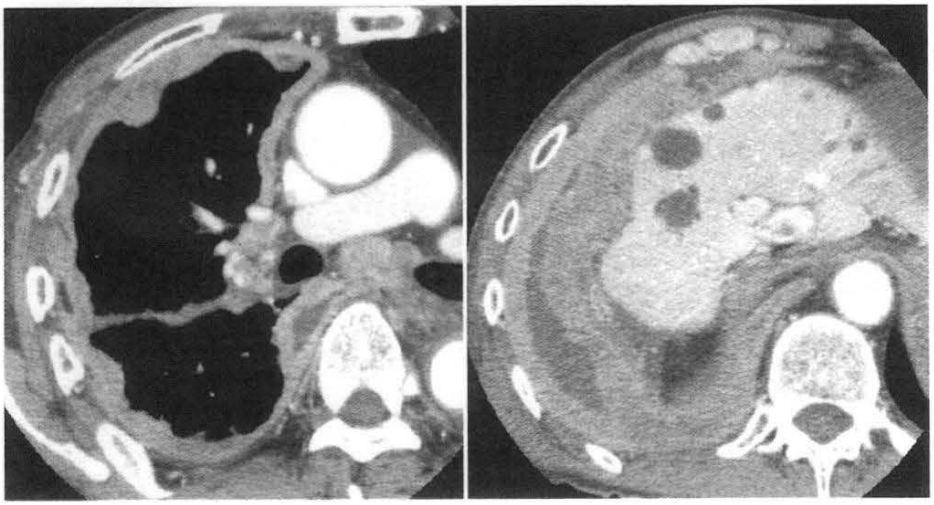


**図8　胸膜中皮腫典型例**

60歳代男性（造影CT）：胸部CTで胸水とびまん性全周性の不整胸膜肥厚像を認め，葉間胸膜にも病変が及んでいる。典型的胸膜中皮腫の所見である。

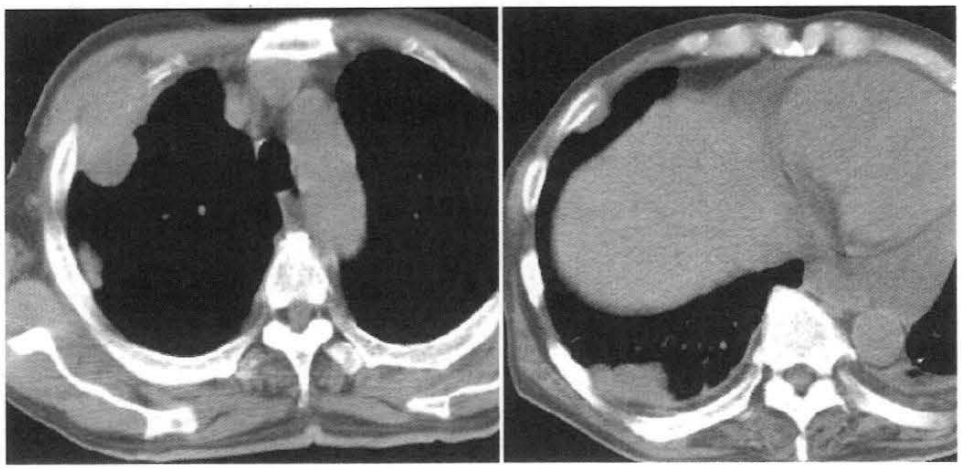


**図9　多発腫瘤を形成した中皮腫症例**

80歳代男性（単純CT）：右前胸壁に限局的に腫瘤形成を認め，胸壁に深く浸潤している。このほかにも多発胸膜腫瘤を形成しており，右肺底部の腫瘤も胸壁に軽度浸潤している。

はない。胸膜中皮腫も同様で，前述の検討でも示したように，進行すればCT上で明らかに悪性を疑う高度の胸膜不整像を認めるが，初期像は非特異的なごく軽度の胸膜不整であり，時には画像上明らかな不整を認めない症例も存在する。

このように画像上不整所見を捉えることができないという状況は画像診断の限界であるが，その後の治療ということを考えると，早期診断が大切なのはいうまでもない。早期診断するには，この悪性所見がない段階で悪性胸膜病変と診断することが重要である。すなわち，原因不明の胸水が続く症例では，画像での悪性所見の有無にとらわれすぎないことが大切である。まず胸水細胞診での良悪の鑑別を目指し，さらに胸水の性状，ADA値，ヒアルロン酸値なども含め，その他，胸水中腫瘍マーカーも参考にする。これらを総合的に判断し，悪性が除外できない

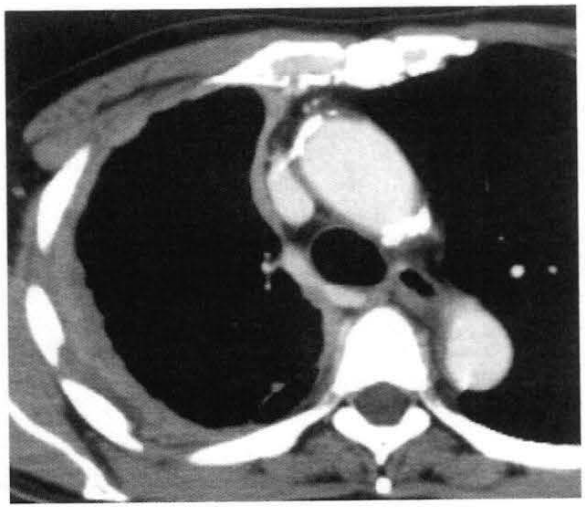


図 10　**中皮腫における環状胸膜肥厚**

60 歳代男性（造影 CT）：右胸腔を環状に取り巻くような不整胸膜肥厚を認め，中皮腫に典型的な所見である。

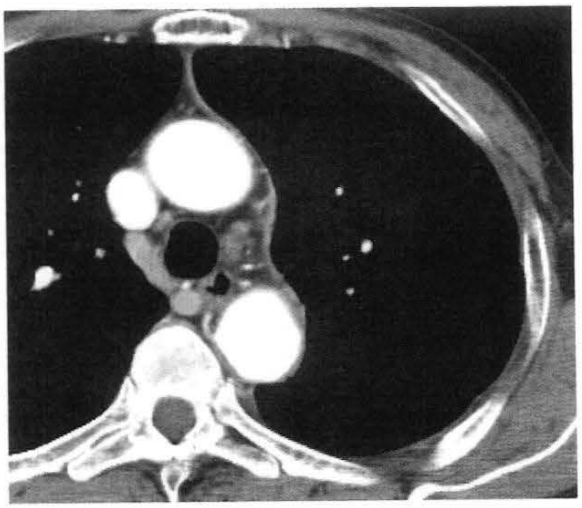


図 11　**中皮腫における縦隔側胸膜不整**

60 歳代男性（造影 CT）：縦隔側胸膜に広範囲肥厚所見を認める。凹凸不整は目立たないが，良性胸膜病変ではあまりみられない所見で，悪性胸膜病変，特に中皮腫を疑う所見である。

ということであれば，積極的に胸腔鏡下の観察・生検も検討する必要がある。CT を主とした画像診断で悪性所見を認めないことを悪性ではない根拠として経過観察され，かなり悪性病変が進行し，診断時の病期が上がったという症例を時にみることがあり注意を要する。かといって胸水症例全例に胸腔鏡を行うと中皮腫ほか悪性胸膜病変以外の症例に対し，必要のない侵襲を加える頻度が高くなるというジレンマに陥るわけであるが，少しでもより悪性病変の可能性が高い症例に対し生検を施行するために，画像的にはより胸膜の状態を正確に評価する必要がある。それには造影 CT を試行し，多撮像断面を用いて，軽度不整に注意して読影するということは最低限行われるべきである。石綿曝露の指標となる胸膜プラークの有無に注意することも挙げられる。

## 良性石綿胸水

良性石綿胸水とは，石綿曝露に関連して胸水貯留を来す疾患である。Epler らによる診断基準は，① 石綿曝露歴があること，② 胸部 X 線写真あるいは胸水穿刺で胸水の存在が確認されること，③ 石綿曝露以外に胸水の原因がないこと，④ 胸水確認後 3 年以内に悪性腫瘍を認めないこと，という 4 項目を満たすことである[12]。よって診断は通常除外診断により，確定診断には 3 年間の経過観察が必要ということになるが，Hillerdal[13] らは，胸部 CT などの画像診断で詳細な臨床経過を観察した場合には，発症後 1 年の経過観察でもよいとしている。

### 良性石綿胸水の画像所見

良性石綿胸水の画像所見については，まとまった報告がないのが現状であるが，われわれは 36 例の良性石綿胸水症例と IMIG 分類における T1–2 相当の早期中皮腫症例 66 例の CT 所見を対比検討している[14]。石綿関連肺胸膜病変の有所見率（括弧内の％は早期中皮腫群での検討）は石綿肺 17%（2%），胸膜プラーク 92%（35%），円形無気肺 44%（0%），びまん性胸膜

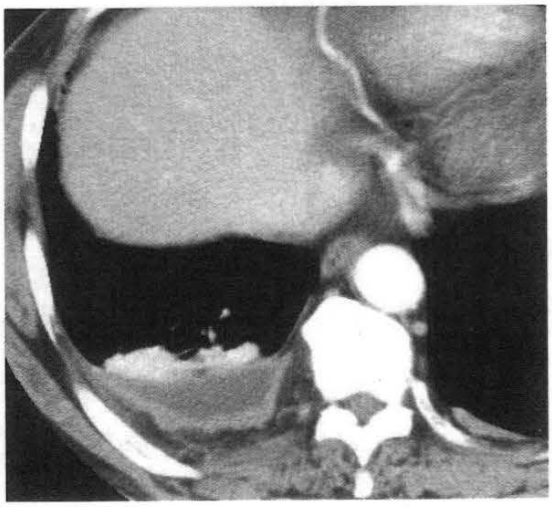


図 12　**良性石綿胸水（胸膜高度不整例）**

70 歳代男性（造影 CT）：背側の胸膜は厚みが 1 cm 近くに肥厚し，凹凸不整が目立っている。臓側胸膜に接して無気肺像を伴っている。悪性胸膜病変も考える所見であるが，胸腔鏡下生検で線維性胸膜炎の診断で，その後の経過観察でも増悪を認めなかった。

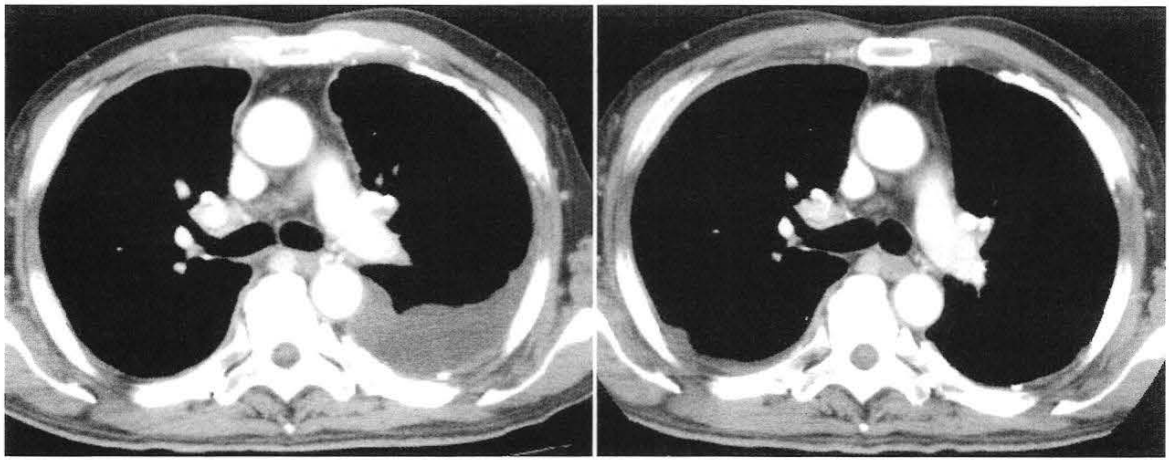
肥厚 25%（2%）と，従来の中皮腫症例における石綿関連肺胸膜病変の有所見率に比しかなり高率であり，中皮腫症例に比し，胸水以外の石綿関連肺胸膜病変を高頻度に合併している。また中皮腫との鑑別に際し問題となる胸膜不整所見についても検討しており，胸膜の厚さについては，5 mm 未満の症例が 56%と過半数を占めたが，5 mm 以上の症例も半数近くあり，1 cm を超える症例も存在した（図 12）。また中皮腫に比較的特徴的とされる縦隔側胸膜肥厚の所見は，早期中皮腫例の 76%で認めたが，良性石綿胸水症例でも 22%に認めた。しかし，この良性石綿胸水群での胸膜肥厚は軽度で，経過観察時の CT でほとんどの症例で消退しており，増強した症例は認めなかった（図 13）。縦隔側胸膜にある程度の不整を有して，徐々に肥厚が増強するような症例では良性石綿胸水よりも中皮腫を念頭におくべきと考えられる。

## びまん性胸膜肥厚[15)]

良性石綿胸水に引き続いて起こることが多いが，やはり石綿曝露以外のさまざまな胸膜炎後に生じる可能性がある。びまん性胸膜肥厚の画像上の定義は次のごとくで，広範囲で肺の 1 葉以上を巻き込むような胸膜の線維化（臓側胸膜の病変で，壁側胸膜との癒着を来す）であり，範囲が 1 側の場合は胸郭全体の 1/2 以上，両側の場合は 1/4 を超えるものをさす。また石綿曝露以外でも発生するため，石綿曝露歴が明確であることを必要とするとされている。以前は，胸部単純 X 線写真での胸膜の厚さが一部で 5 mm 以上必要とされていたが，2012（平成 24）年に認定要件が改正され厚さに関する項目はなくなった。この画像での定義を満たし，石綿曝露作業への従事期間が 3 年以上あり，著しい呼吸障害を伴う場合，労災や救済法での保障対象となる。

### びまん性胸膜肥厚の画像所見（図 14）

胸部単純 X 線写真では，胸膜肥厚は側胸壁内側の比較的滑らかな厚みのある濃度上昇としてとらえられ，大多数において肋骨横隔膜角の鈍化が見られる。胸膜プラークのみの症例ではこの肋骨横隔膜角が保たれることが多く，この鈍化を認めた場合，びまん性胸膜肥厚合併を疑う所見となる。ただし単純 X 線写真のみでは胸膜肥厚の正確な範囲を同定することは難しく，診断には胸部 CT 所見を参考にすべきである。胸部 CT ではプラークのように限局した板状の胸膜肥厚ではなく，連続した広範な胸膜肥厚像を認め，同時に末梢肺との癒着性変化を伴っている。Schwartz ら[16)]は，びまん性胸膜肥厚群は胸膜プラーク群に比較して，有意に%肺活量，%



4月　　7月

**図 13　良性石綿胸水（縦隔側胸膜肥厚例）**

60 歳代男性（造影 CT）：4 月の胸部造影 CT では左胸水貯留を認め，左側には石灰化を伴う胸膜プラークを認める。縦隔側の胸膜は両側で軽度肥厚している。次に撮像された 7 月の造影 CT では，依然胸膜プラークを認めるが，左胸水は消失し，右胸水を少量認めている。縦隔側胸膜肥厚の所見はほぼ消失している。

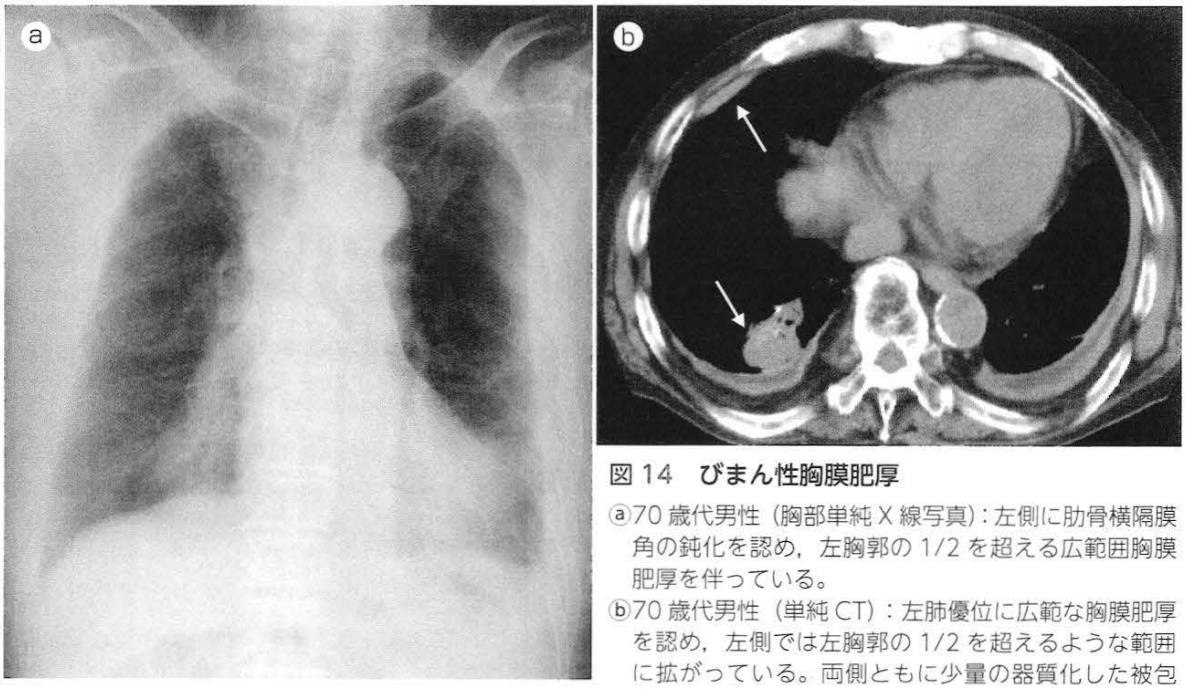



**図 14　びまん性胸膜肥厚**

ⓐ70 歳代男性（胸部単純 X 線写真）：左側に肋骨横隔膜角の鈍化を認め，左胸郭の 1/2 を超える広範囲胸膜肥厚を伴っている。

ⓑ70 歳代男性（単純 CT）：左肺優位に広範な胸膜肥厚を認め，左側では左胸郭の 1/2 を超えるような範囲に拡がっている。両側ともに少量の器質化した被包化胸水を伴っている。この胸水は 1 年以上の経過観察で，ほぼ変化を認めなかった。胸膜はかなり厚いが凹凸不整は認めない。右側では円形無気肺と胸膜プラークも認める（→）。

1 秒量の低下を認めたが，1 秒率は変わらなかったとしているが，びまん性胸膜肥厚による呼吸機能低下の主体は拘束性換気障害であり，広範な末梢肺と臓側胸膜の癒着が主たる原因になっていると考えられる。

## 胸膜肥厚・腫瘍の鑑別はどこでするのか?

ここまで石綿関連胸膜病変に関する鑑別を主に述べてきたが，改めて本稿でのこの問いに関して，これまで述べてきてことを踏まえて注意すべき点を挙げる。

① 胸膜不整の有無の適切な評価が基本

まずは，胸膜がはっきりした不整所見を有するかどうかが，腫瘍性肥厚と炎症性肥厚の基本となる鑑別点である。高度の凹凸を示すような胸膜不整所見やはっきりとした腫瘤形成が確認できれば，悪性胸膜病変の診断は画像のみで可能である。その際に注意しておくべきなのは，画像で診断できるのは悪性胸膜病変ということまでであり，肺野病変がないことのみを根拠に中皮腫とまでは診断できないということである。例えば偽中皮腫様腺癌といわれる一見胸膜病変のみの肺腺癌が存在しており，その除外には免疫染色を含めた組織診断が必要となる。

また胸膜不整の詳細な検討にはCTやMRIにおいて造影検査の追加が必須である。悪性胸膜病変ではよく胸水を伴うが，多くの場合は血性胸水であり，単純のみでは胸膜と胸水とのコントラストがつきにくい場合が多い。そのため，詳細な胸膜不整についての検討には造影が不可欠である。

② 縦隔側胸膜肥厚や葉間胸膜肥厚には特に要注意

縦隔側胸膜肥厚は悪性胸膜病変，その中でも胸膜中皮腫の初期病変で高頻度に認められ，中皮腫を疑う根拠となることが多い所見である。われわれのT1–2相当の初期中皮腫66例と良性石綿胸水36例の検討[14]でも，74%と高率に縦隔側胸膜に軽度不整所見を認めた。縦隔側でより胸膜肥厚が捉えやすい理由としては，胸壁側では壁側胸膜と連続して肋骨や肋間筋などの構造物が存在し胸膜の軽微な変化が捉えづらいのに対して，縦隔側には縦隔側胸膜に連続するのは縦隔脂肪のみであり，軽度の肥厚も描出されやすいということが考えられる。また通常陳旧性胸膜炎症例を思い出してもらえばよいが，胸壁側，その中でも背側優位に胸膜肥厚所見を認めることはよくあるが，縦隔側にまで及ぶような胸膜肥厚所見を認めることはほとんどない。

ただ良性石綿胸水の画像所見の解説中で示したが，良性石綿胸水でも22%に同様の所見を認めており，縦隔側胸膜肥厚を認めると即悪性疑いというわけにはいかない。しかし良性石綿胸水での胸膜肥厚は浮腫状で，それほど厚くないことが多い。さらに経過観察で，ほとんどの症例で肥厚は改善し，改善しない症例でもはっきりした増悪は認めない。すなわち縦隔側胸膜肥厚を認めた場合，かなり厚い場合や不整を伴う場合は，即生検を含めた精査の必要があり，軽微な場合には経過観察が重要である。さらに肥厚が増悪するようであれば，積極的に胸腔鏡下生検を施行すべきであると考える。また前述のわれわれの検討[14]において葉間胸膜肥厚の所見は55%程度にしか認めなかったが，良性石綿胸水では1例も認められず，悪性所見としての信頼度が高かった。癌性胸膜炎の診断においても同様であるが，葉間胸膜の不整所見には特に注意しておく必要がある。

③ 胸水のみ症例の取り扱い

造影検査も施行し良好な画像を得て，縦隔側胸膜や葉間胸膜に注意して詳細に画像を検討したとしても，まったく胸膜不整所見を認めない症例，すなわち胸水のみの症例がある。その場合良性胸膜病変である可能性が高まるわけであるが，常に念頭においておかねばならないのが，早期悪性胸膜病変である。われわれが行った中皮腫診断時CT所見の検討で，19%の症例ははっきりとした悪性所見を呈していなかったと示したが，画像所見のみで悪性胸膜病変を除

外し，良性と診断することは現状では不可能である。しかし一方，画像で不整を認めない初期中皮腫症例こそ，外科的手術を含めた積極的治療のよい適応であり，実際はっきりと不整を呈する症例よりも予後が良い。したがって，特に中皮腫など悪性病変の診断がつけば，外科的治療を含め積極的治療が行えるような比較的若年者でPSの良い症例においては，画像で悪性所見を認めないから，まずは経過観察とするのではなく，原因がはっきりしない胸水についてはまず胸水の性状はチェックして，さらに胸水穿刺の結果をふまえ，石綿曝露歴など総合的に判断して積極的に胸腔鏡下生検を行うべきであると考える。

## おわりに

「胸膜肥厚・腫瘤の鑑別はどこでするのか」という題をいただいたが，筆者が本音で語るだけの経験を最も持ち合わせている石綿関連疾患の鑑別を中心とした内容にしてしまい，編者の意図と少し異なったかもしれない点をお詫びさせていただく。ただ，石綿関連疾患での鑑別は，その他疾患での鑑別点と重なるところも多い。また，悪性胸膜病変の中で転移性腫瘍は外科的治療の適応がなく，まれな疾患ではあるが，中皮腫の場合，早期診断が積極的外科治療も含めた根治治療の可能性もあるという点からは悪性胸膜病変として中皮腫を主に考えるのは意味があると思われる。今回はほとんど触れていないが，中皮腫やびまん性胸膜肥厚など石綿関連疾患は，労災や救済の対象となり，その認定の中で胸膜プラークの有無など画像が果たす役割が大きいことからも，これらの所見に精通して，胸膜病変の良悪の鑑別を進めて行くことは重要であると考える。

### 鑑別診断のポイント

1. 胸膜プラークは石綿曝露に特異的所見であり，石綿関連疾患の診断における石綿曝露の医学的指標として用いられており，その特徴に精通しておく必要がある。
2. 胸膜病変の良悪の鑑別に際し，詳細に胸膜不整を評価するにはCT/MRI検査施行時に造影検査が必須である。
3. 胸膜中皮腫初期には，縦隔側胸膜のみに軽微な肥厚所見を認める場合があり，注意が必要である。
4. 胸水のみで胸膜不整を伴わない場合にも悪性胸膜病変初期像の可能性があり，胸水細胞診やヒアルロン酸など腫瘍マーカや石綿曝露歴も参考にしつつ，積極的な胸腔鏡下胸膜生検も念頭において診療する必要がある。

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*Original Article*

# Pilot Analysis of Asbestos-induced Diffuse Pleural Thickening with Respiratory Compromise

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**We investigated the clinical features of asbestos-induced diffuse pleural thickening (DPT) with severe respiratory compromise. We conducted a retrospective study of consecutive subjects with asbestos-induced DPT. Medical data such as initial symptoms, radiological findings, respiratory function test results, and clinical course were collected and analyzed. There were 24 patients between 2003 and 2012. All were men, and the median age at the development of DPT was 74 years. The top occupational category associated with asbestos exposure was dockyard workers. The median duration of asbestos exposure was 35.0 years, and the median latency from first exposure to the onset of DPT was 49.0 years. There were no significant differences in respiratory function test results between the higher and lower Brinkman index groups or between unilateral and bilateral DPT. Thirteen patients had a history of benign asbestos pleural effusion (BAPE), and the median duration from pleural fluid accumulation to DPT with severe respiratory compromise was 28.4 months. DPT with severe respiratory compromise can develop after a long latency following occupational asbestos exposure and a history of BAPE.**

**Key words:** asbestos, pleural thickening, MRC dyspnea scale, respiratory function test, costophrenic angle

Asbestos-related health problems remain a major public health concern. Asbestos-related pleural diseases include malignant pleural mesothelioma (MPM), benign asbestos pleural effusion (BAPE), and diffuse pleural thickening (DPT) [1]; cases of these diseases will continue to be seen in the next several decades due to past industrial asbestos use. Asbestos-induced DPT is considered to be a consequence of asbestos-induced inflammation of the visceral pleura, which leads to adhesion to the parietal pleura. However, the actual pathogenesis is still unknown and the radiological definition of DPT is ambiguous. McLoud *et al.* described DPT on chest X-rays as a smooth, uninterrupted pleural density extending over at least one-quarter of the chest wall, with or without involvement of the costophrenic angle (CPA) [2]. Yates *et al.* also proposed a definition of DPT based on dimensional criteria, in which DPT is characterized by pleural thickening of ≥ 5 mm that extends over more than one-quarter of the chest wall, with or without obliteration of the CPA [3].

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Several studies have examined the characteristics of DPT [2, 4–7]. A major limitation of these earlier studies is that their definitions of DPT varied; as such, some studies may have included patients with pleural plaques, BAPE, and MPM, mainly due to the difficulty of confirming a diagnosis of DPT based on chest X-ray without computed tomography (CT) images.

DPT often induces significant impairment of lung function [8]. In Japan, patients with DPT are provided worker's compensation or given financial relief by the Act on Asbestos Health Damage Relief if they present severe respiratory compromise. We recently retrospectively analyzed the clinical features and radiological findings of DPT, and we reported that some of the radiological findings, such as the involvement of CPA and pleural thickness and the craniocaudal and horizontal extension of pleural thickening (as determined by chest CT) were correlated with impaired respiratory function in patients with DPT [9]. There are few reports concerning the features of DPT in patients with severe respiratory compromise, however.

The objectives of the present retrospective analysis, which was conducted in a single region of Japan, were to clarify the clinical features of DPT patients with severe respiratory compromise, including the clinical course. We focused on the association between BAPE and DPT. We also investigated clinical issues associated with the diagnosis, evaluation, and handling of compensation for DPT.

## Materials and Methods

***Subjects.*** The consecutive subjects diagnosed as having asbestos-induced DPT in Okayama Rosai Hospital (Okayama, Japan) between 2003 and 2012 were identified. The inclusion criteria were a history of occupational asbestos exposure, pleural thickening $>5$ mm on chest X-ray extending for more than one-half of the lateral thoracic wall (LTW) in patients with unilateral DPT or more than one-quarter of the LTW in patients with bilateral DPT, and impaired respiratory function (defined below). The subjects had to have been followed up for at least 6 months after the diagnosis of DPT. Medical data from these patients were collected and analyzed retrospectively. The medical information included age, gender, initial symptoms, modified Medical Research Council (mMRC) dyspnea grade, smoking history, radiological findings, respiratory function test results, and the clinical course. Former smoker was defined as those quitted smoking for more than 6 months. Information about the history of asbestos exposure was also collected. In some patients who changed to local hospitals or clinics, we made inquiries about their information at outcome at the relevant medical institutions.

This study was done according to Ethical Guidelines for Epidemiological Research issued by the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japanese Ministry of Health, Labour and Welfare. This study was approved by the ethics committee of the Japan Labour Health and Welfare Organization and the institutional review board of Okayama Rosai Hospital.

***Respiratory function test.*** Respiratory function tests were performed in clinical settings based on the guidelines set forth in the Official Statement of the American Thoracic Society [10]. The data obtained included the percentage of vital capacity (%VC) and the forced expiratory volume percentage in 1 sec (FEV1). Blood gas data such as $PaO_2$ (the partial pressure of $O_2$ in arterial blood) and $PaCO_2$ (the partial pressure of carbon dioxide in arterial blood) were also extracted. The data obtained closest in time to when the chest CT was performed were used for the analyses. Impaired respiratory function was defined as (1) %VC $<60\%$ or (2) %VC 60–80%, FEV1 $\leq$ 70%, and FEV1/forced vital capacity (FVC) $<50\%$ or $PaO_2$ on arterial blood gas test $\leq 60$ Torr.

***Statistical analysis.*** Comparisons between groups were performed using a nonparametric analysis with the Wilcoxon rank-sum test. The latency period of DPT was calculated as the time between the first exposure to asbestos and the onset of DPT. In the patients with a history of BAPE, the period from the detection of pleural effusion to the onset of DPT and the development of severe respiratory impairment were calculated. Correlations were examined in a regression analysis. The software package used for the statistical analyses was JMP 10.0.2 (SAS Institute, Cary, NC, USA).

## Results

***Patient characteristics.*** A total of 24 patients were analyzed retrospectively. The patients' demo-

graphic details are listed in Table 1. All of the patients were men, and the median age at the diagnosis of DPT was 74 years. More than 90% of the patients smoked, and the Brinkman index was ≥ 600 in 14 (58.3%) of the patients. Fifteen patients had visited a clinic or a hospital with symptoms, 5 had an abnormal shadow on a chest X-ray at a medical checkup, and 4 were diagnosed with DPT while receiving medical treatment for other diseases. The mMRC dyspnea grade at the diagnosis of DPT was 1 in 3 patients, 2 in 15 patients, 3 in 3 patients, and 4 in 3 patients.

***Occupational asbestos exposure history.*** Occupational categories associated with asbestos exposure are listed in Table 2 and included dockyard, construction, and heating trade work and more. The median (range) duration of asbestos exposure was 35.0 (3.0–50.0) years. The median (range) latency period since the first exposure to the onset of DPT was 49.0 (37.0–64.0) years.

***Radiological findings.*** Unilateral DPT was found in the thorax in 6 cases and bilateral DPT was found in 18 cases at the time of diagnosis. In the 6 cases with unilateral DPT, DPT was found in the right thorax in 4 cases and in the left thorax in 2 cases. Radiographic findings associated with DPT are listed in Table 3. All of the cases showed CPA involvement on chest X-ray. Pleural plaques were detected in most of the patients. Pulmonary asbestosis was diagnosed in 3 (12.5%) cases, and the profusion rate according to the International Labour Organization criteria [11] was 1 in 2 cases and 3 in one case. Rounded atelectasis was detected in 18 (75.0%) cases on chest CT. Crow's feet signs, defined as fibrous strands with accompanying circumscribed pleural thickening, was detected in all cases on chest CT.

***Respiratory function test.*** The median (range) value of %VC was 51.4% (31.2–70.7%); the %VC was < 60% in 22 (91.7%) patients. The median (range) value for FEV1 was 82.9% (47.7–100%). Eighteen (75.0%) patients showed restrictive ventilatory impairment, and 6 (25.0%) showed combined restrictive and obstructive ventilatory impairment. Blood gas data were available in 23 cases, and the median (range) values for $PaO_2$ and $PaCO_2$ were 78.9 (53–86) Torr and 43.3 (35.8–56.5) Torr, respectively.

We then examined the association between respiratory function and smoking history. First, we compared %VC and FEV1 between lower (< 600) and higher (≥ 600) Brinkman index groups ($n = 10$ and 14, respectively). Never-smokers were included in lower Brinkman index group. The median values for %VC in the lower (< 600) and higher (≥ 600) Brinkman index groups were 50.9% and 51.4%, respectively. The median values for FEV1 in the lower (< 600) and higher (≥ 600) Brinkman index groups were 71.7% and 85.4%, respectively. There

**Table 1** Demographics of the study population ($n = 24$)

| Characteristics | | No. of patients (%) |
|---|---|---|
| Median age (range) | | 74 (63–92) years |
| Gender | Male | 24 (100.0%) |
| Smoking | Never | 6 (25.0%) |
| | Former | 16 (66.7%) |
| | Present | 2 (8.3%) |
| Initial symptom | Cough | 14 (56.0%) |
| | Chest pain | 3 (12.0%) |
| | Sputum | 1 (4.0%) |
| mMRC* grade | 0/1/2/3/4 | 0/3/15/3/3 |

*modified Medical Research Council.

**Table 2** Occupational category of asbestos exposure ($n = 24$)

| Occupation | No. of patients |
|---|---|
| Dockyards | 7 |
| Construction | 4 |
| Heating trade | 3 |
| Demolition work | 2 |
| Asbestos product industry | 2 |
| Furnace installation | 1 |
| Electric work | 1 |
| Painting | 1 |
| Plumbing | 1 |
| Welding | 1 |
| Shipman | 1 |

**Table 3** Radiographic findings associated with DPT

| Findings | No. of patients (%) |
|---|---|
| Asbestosis | 3 (12.5%) |
| Pleural plaques (X-ray) | 14 (58.3%) |
| Pleural plaque (CT*) | 22 (91.7%) |
| Rounded atelectasis | 18 (75.0%) |
| Crow's feet sign | 24 (100.0%) |
| Costophrenic angle involvement | 24 (100.0%) |

*Computed tomography. DPT: diffuse pleural thickening.

were no significant differences in %VC ($p = 0.5780$) and FEV1 ($p = 0.5387$) between the 2 Brinkman index groups (Fig. 1). We also compared the %VC and FEV1 values between lower (< 400) and higher (≥ 400) Brinkman index groups ($n = 6$ and 18, respectively). The median values for %VC in the lower (< 400) and higher (≥ 400) Brinkman index groups were 56.3% and 50.1%, respectively. The median values for FEV1 in the lower (< 400) and higher (≥ 400) Brinkman index groups were 68.1% and 86.4%, respectively. There were no significant differences in %VC ($p = 0.5709$) and FEV1 ($p = 0.0773$) between these 2 Brinkman index groups.

We next examined the association between respiratory function and unilateral or bilateral DPT. The median values for %VC in the unilateral ($n = 6$) and bilateral ($n = 18$) DPT cases were 53.3% and 51.4%, respectively. The median values for FEV1 in the unilateral and bilateral DPT cases were 67.7% and 84.4%, respectively. There were no significant differences in %VC ($p = 0.7642$) or FEV1 ($p = 0.3014$) between the cases of unilateral and bilateral DPT.

We compared respiratory function between 2 groups: those with longer asbestos exposure (≥ 30 years: $n = 16$) and those with shorter asbestos exposure (<30 years: $n = 8$). The median VC values in the longer- and shorter-exposure groups were 48.4% and 54.2%, respectively. The median FEV1 values in the longer- and shorter-exposure groups were 76.4% (41.3–70.7%) and 85.4%, respectively. The longer-exposure group showed more impaired respiratory function, although the difference was not significant ($p = 0.3123$ for %VC and $p = 0.4813$ for FEV1).

***Clinical course of DPT.*** Thirteen patients had a medical history of BAPE that preceded the diagnosis of DPT. The date of the accumulation of pleural effusion was identified based on their medical records, except in one case. The median (range) duration from the accumulation of pleural effusion to the development of DPT was 28.4 (8.9–255.3) months. The median duration from the accumulation of pleural effusion to the development of impaired respiratory function was 35.1 (2.8–255.3) months. We examined the correlation between the duration from the fluid accumulation to the onset of DPT and the duration of asbestos exposure using a regression analysis, but there was no significant correlation ($r = 0.09$). In 2 of the 6 cases with unilateral DPT, the DPT had progressed to the other side of the thorax in 3.2 and 18.7 months, respectively. At the time of the analysis, 15 patients were alive and 9 patients had died. Of the 9 patients who had died, 4 died of respiratory failure, and 1 died of lung cancer. The other 4 patients died of unknown causes. There was no patient who developed MPM.

## Discussion

We retrospectively analyzed the characteristic features of asbestos-induced DPT. We focused in particular on DPT cases with severe respiratory compromise. The criteria that we applied are those used for worker's compensation and the Act on Asbestos Health Damage Relief in Japan. The top

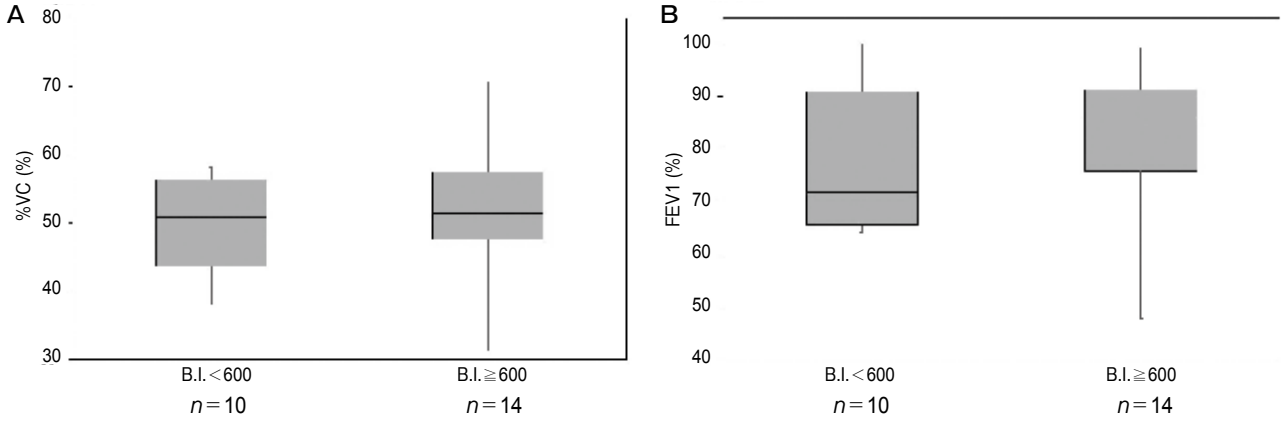


Fig. 1 Comparison of the percentage of vital capacity (%VC) (**A**) and the forced expiratory volume percentage in 1 sec (FEV1) (**B**), between the groups with lower and higher Brinkman index values.

occupational category in which the patients had been exposed to asbestos was dockyard workers, as there is a dockyard facility in the suburbs of the Okayama area. The median duration of asbestos exposure was 35 years, and the median latency period for the development of DPT from the first asbestos exposure was 49.0 years. These results are similar to those of our previous study [9] and those from other groups [7, 12]. The results of the present study confirm that DPT can develop after long latency periods following occupational asbestos exposure.

Making a diagnosis of DPT is challenging. In the present study, we defined DPT as pleural thickening of > 5 mm on a posteroanterior chest X-ray, extending for more than one-half of the LTW in the patients with unilateral DPT, or over more than one-quarter of the LTW in the patients with bilateral DPT. These definitions were made based on dimensional criteria [3], because these are the criteria that are applied when making a diagnosis for the certification of worker's compensation or the Act on Asbestos Health Damage Relief in Japan. However, these criteria are based on chest X-rays and are ambiguous and subjective.

Ameille *et al.* reported that obliteration of the CPA was a far more reliable sign than dimensional criteria in the characterization of DPT [13]. Accordingly, the revised International Labor Office (ILO) Classification of Radiographs of Pneumoconiosis provided clearer criteria, in which involvement of the CPA had to be demonstrated for DPT [11]. We recently reported that the involvement of the CPA was negatively correlated with %VC [9]. This finding supports the use of CPA involvement not only for making a diagnosis of DPT, but also for assessing the severity of DPT. There is still room for discussion regarding the most relevant radiological diagnostic criteria for DPT.

Our present findings demonstrated that breathlessness, cough, and chest pain were the most frequent symptoms of DPT; this finding was similar to that of Yates *et al.* [3]. Moreover, 75.0% of the patients showed restrictive ventilatory impairment, and 25.0% of the patients showed combined ventilatory impairment. The characteristic features of DPT with respect to respiratory function testing are a restrictive ventilator defect, decreased compliance, a reduction in total lung capacity, and impairment of gas transfer [8]. We suggest that the obstructive ventilatory impairment demonstrated in some of the patients in our study may be due to a history of smoking, although we could find no clear association between respiratory function and smoking history.

Unilateral DPT has been reported to cause less severe ventilatory impairment than bilateral DPT [8]. However, there were no differences in ventilatory impairment between the patients with unilateral or bilateral DPT in the present study and in our previous study [9]. However, caution should be used when drawing conclusions from these results, since the sample size was small. In the present study, FEV1 in unilateral DPT was lower than that in bilateral DPT. In fact, among 6 cases with unilateral DPT, there were 3 cases with mild pulmonary emphysema, 1 case with asbestosis, and 1 case with pulmonary emphysema and bronchial asthma. We consider that these concomitant diseases contributed to lower FEV1 in unilateral DPT.

Severe respiratory compromise is the key factor for approval for worker's compensation, and thus the most appropriate method for assessing respiratory impairment associated with DPT should be investigated further.

In the present study, we focused on the sequence of DPT and BAPE (also known as asbestos pleuritis). Epler *et al.* advocated the following diagnostic criteria for BAPE: (1) previous asbestos exposure, (2) determination of pleural effusion by chest X-ray or thoracentesis, and (3) absence of other causes of effusion [4]. We examined the patients' history of pleural fluid accumulation based on the medical information available and their subsequent clinical courses. We found that 54.2% of the 24 DPT cases had a history of pleural fluid accumulation, and they subsequently developed DPT at a median of 28.4 months. In addition, these patients developed severe respiratory compromise at a median of 35.1 months following the episode of pleural fluid accumulation. In a previous report, 40% of DPT cases were preceded by the development of pleural effusion [7]. However, in the present study, there were some cases in which it was difficult to determine the radiological change from BAPE to DPT, especially in patients with BAPE in the organizing stage. Diagnostic criteria for differentiating between BAPE in the organizing stage and DPT would be of critical importance with respect to

worker's compensation in Japan.

Our present findings demonstrated that the main cause of death was respiratory failure. Karjalainen *et al.* reported that asbestos-induced benign pleural disease raised the risk of MPM, but the risk of lung cancer was only slightly elevated [14]. In our study, only one of the 24 patients died of lung cancer and there were no patients with MPM. Associations between DPT and MPM or lung cancer should be clarified in a large-scale, long-term study.

In conclusion, our results indicate that DPT can develop after a long latency period following occupational asbestos exposure and a history of BAPE. Radiological diagnostic criteria that are suitable for practical use and appropriate methods for assessing respiratory impairment due to DPT should be established. Clinical, pathological, and temporal alterations that occur in the transition from BAPE to DPT should be clarified, and the association between DPT and MPM or lung cancer should be examined in large-scale, long-term prospective studies.

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# Prognostic significance of the lymphocyte-to-monocyte ratio in patients with malignant pleural mesothelioma☆

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ABSTRACT

*Objectives:* Chronic inflammation plays a key role in the pathogenesis of malignant pleural mesothelioma (MPM) as a result of asbestos exposure. Several inflammation-based prognostic scores including the lymphocyte-to-monocyte ratio (LMR), Glasgow Prognostic Score (GPS), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) reportedly predict survival in many malignancies, while the role of LMR in MPM remains unclear. The aim of this study was to evaluate the clinical value of LMR and to compare the prognostic value of these inflammation-based scores in predicting overall survival (OS) in MPM.

*Materials and methods:* One hundred and fifty patients with histologically proven MPM were included in this retrospective study. Kaplan–Meier curves and multivariate Cox-regression analyses were calculated for OS. The area under the receiver operating characteristics curve (AUC) was calculated to compare the discriminatory ability of each scoring system.

*Results:* An elevated LMR was significantly associated with prolonged OS. Patients with LMR <2.74 had significantly poor survival compared with LMR ≥2.74 (median, 5.0 versus 14.0 months; $p = 0.000$). The LMR consistently had a higher AUC value at 6 months (0.722), 12 months (0.712), and 24 months (0.670), compared with other scores. Multivariate analysis showed that the LMR was independently associated with OS.

*Conclusions:* The LMR is an independent prognostic marker for OS in patients with MPM and is superior to other inflammation-based prognostic scores with respect to prognostic ability.



## 1. Introduction

Malignant pleural mesothelioma (MPM) is a rare but aggressive primary pleural malignancy that is associated with asbestos exposure [1]. Its prognosis remains poor, as most patients present with unresectable disease at diagnosis or are deemed inoperable owing to age or medical comorbidities. Although several treatment options have been delivered to patients with MPM, the median survival time is approximately 12 months [2]. Despite a very poor prognosis, some selected patients live with the disease for a considerable period of time; median survival is 12.8–46.9 months when treated using a multimodality therapy that contains either (1) neoadjuvant chemotherapy and surgery with or without radiation therapy, or (2) adjuvant chemotherapy [3].

The best-known clinical prognostic scoring systems for MPM have originated from the European Organization for Research and Treatment of Cancer (EORTC) [4] and the Cancer and Leukemia Group B (CALGB) [5], and take into account a combination of biological and clinical factors. However, these scoring systems are not routinely used in MPM prognosis because they are time-consuming to perform.

It is well known that systemic inflammatory response plays an important role in cancer progression [6]. Although the mechanisms

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of carcinogenesis in MPM are incompletely understood, chronic inflammation is critically involved in the pathogenesis of MPM as a result of asbestos exposure. In addition to its involvement in the pathogenesis of cancer-related cachexia, systemic inflammation can predict clinically meaningful outcomes, such as overall survival (OS) and response to systemic treatment [7]. Several studies have shown that inflammation-based prognostic scores that include a combination of serum C-reactive protein (CRP) and albumin (ALB) (e.g., the Glasgow Prognostic Score [GPS] and modified GPS [mGPS]), a combination of neutrophil and lymphocyte counts (e.g., the neutrophil-to-lymphocyte ratio, NLR), and a combination of platelet (PLT) and lymphocyte counts (e.g., the platelet-to-lymphocyte ratio, PLR) are associated with survival in patients with various cancers, including MPM [8,9]. Lymphocytes act as tumor suppressors by inducing cytotoxic cell death and inhibiting tumor cell proliferation and migration [10]. The important role of monocytes and macrophages in cancer, including thoracic malignancies, has recently been uncovered [11]. More recently, some studies demonstrated that lymphocyte-to-monocyte ratio (LMR) is associated with prognosis in several cancers, including hematological malignancy [12] and some solid tumors. However, to the best of our knowledge, there is no evidence determining the association between LMR and the survival of MPM patients.

The aim of this study was to assess the prognostic role of inflammation-based prognostic scores such as the GPS, mGPS, NLR, PLR, and LMR in patients with MPM.

## 2. Materials and methods

### 2.1. Patient population

The study included 150 consecutive patients with histologically proven MPM between July 1993 and October 2014, at Okayama Rosai Hospital. No patients showed obvious clinical evidence of infection or other inflammatory conditions such as rheumatoid arthritis at the diagnosis of MPM. Baseline prognostic clinical and laboratory variables were collected retrospectively from patients' medical records. These included age, gender, histological subtype, stage, and Eastern Cooperative Oncology Group (ECOG) performance status (PS). Staging was determined according to the International Mesothelioma Interest Group (IMIG) staging system [13], based on enhanced CT of whole body, magnetic resonance imaging of the brain, and bone scintigraphy. Positron-emission tomography was available since 2012 and substituted to bone scintigraphy in 20 cases.

This study followed REMARK guidelines [14] and was conducted according to The Ethical Guidelines for Epidemiological Research by Japanese Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labour and Welfare. This study was approved by the Japan Labour, Health and Welfare Organization and the institutional review boards of Okayama Rosai Hospital. Patient confidentiality was strictly maintained.

### 2.2. Inflammation-based prognostic scores and other variables

Blood samples were obtained at the time of diagnosis of MPM for measurement of white blood cell count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), absolute monocyte count (AMC), PLT, CRP, and ALB. The LMR was defined as the ALC divided by the AMC. The NLR was calculated by dividing the ANC by the ALC; we applied a cutoff value of $\leq 5$ versus >5 in accordance with the first report of NLR in MPM [15]. The same calculation was applied to derive the PLR; the cutoff for positivity was 150 [16].

The GPS was calculated as described in previous studies [17]. Briefly, patients with both elevated CRP (>1.0 mg/dL) and low ALB (<3.5 g/dL) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1. Patients in whom neither abnormality was present were allocated a score of 0. Regarding mGPS, patients with both elevated CRP (>1.0 mg/dL) and low ALB (<3.5 g/dL) were allocated a score of 2, patients in whom only CRP was elevated (>1.0 mg/dL) were allocated a score of 1, and those with normal CRP were allocated a score of 0 [18]. The EORTC Prognostic Score (EPS) was devised to subcategorize patients into low-risk or high-risk groups based on age, gender, histology, probability of diagnosis, and leukocyte count [4]. The CALGB score incorporates the presence of non-epithelial histology, weight loss, or chest pain; high platelet (PLT) and WBC; low hemoglobin; high serum lactate dehydrogenase; advanced age; and PS [5]. The CALGB groups were combined into three prognostic groups (e.g., groups 1/2, 3/4, or 5/6), because of the small numbers in the even numbered groups [19].

### 2.3. Statistical analysis

Receiver operating characteristic (ROC) curve analysis was performed to select the most appropriate cut-off point for LMR, AMC, and ALC to predict poor prognosis in patients with MPM. The score with the maximum sensitivity and specificity was selected as the best cut-off value. Survival outcomes were dichotomized by survival (alive versus death) in the ROC analysis. The relationships between the LMR and clinico-pathological features were evaluated using the chi-squared test. The primary objective of this study was to assess the association of inflammation-based scores and OS, which was defined as the time from diagnosis until death from any cause. OS was obtained by using Kaplan–Meier methods, and between-group differences were compared using the log-rank test. Univariate and multivariate analyses were performed regarding the potential prognostic factors using the Cox proportional hazard model. A stepwise backward procedure was used to derive a final model of the variables. Age (<70 versus $\geq$70 years), gender (male versus female), ECOG PS ($\leq$1 versus >1), histological subtype (epithelioid versus non-epithelioid), IMIG stage (I/II versus III/IV), EPS (high-risk versus low-risk), CALGB scoring system (group 1/2 versus group 3/4 versus group 5/6), surgical intervention (yes versus no), baseline WBC ($\leq 8.3 \times 10^9$/L versus $>8.3 \times 10^9$/L) and PLT ($\leq 400 \times 10^9$/L versus $>400 \times 10^9$/L), ALB ($\geq$3.5 g/dL versus <3.5 g/dL), and difference in hemoglobin (<10 g/L versus $\geq$10 g/L) entered the calculations. An ROC curve was also generated and the area under the curve (AUC) was calculated to evaluate the discriminatory ability of each scoring system. A two-tailed $p$-value <0.05 was considered statistically significant. All statistical analysis was performed using STATA software (version 12.1; StataCorp, College Station, Texas).

## 3. Results

### 3.1. Patient characteristics

The patient and tumor characteristics at baseline are shown in Table 1. Median age at the time of diagnosis was 70 (range: 38–92) years. The majority of study participants were male (93.3%) and had a diagnosis of stage III/IV (64.0%). Surgery was performed in 44 (29.3%) patients including 38 extrapleural pneumonectomy, 3 pleural decortication, and 3 tumor excision. Systemic chemotherapy and radiotherapy were delivered as initial treatment to 86 (57.3%) and 3 (2.0%) patients, respectively. The remaining 17 (11.3%) patients received best supportive care. The median follow-up duration was 11 (range: 1.0–150.0) months. At the time of analysis, 116 (77.3%) patients had died, and the median OS was 13.0 [95% confidence interval (CI), 10.0–16.0] months.

**Table 1**
Patient characteristics ($n = 150$).

| | No. of patients | % |
|---|---|---|
| Median age at diagnosis (range) | 70 (38–92) | |
| Gender | | |
| Male | 140 | 93.3 |
| Female | 10 | 6.7 |
| ECOG PS | | |
| ≤1 | 129 | 86.0 |
| >1 | 21 | 14.0 |
| Histological subtype | | |
| Epithelioid | 97 | 64.7 |
| Non-epithelioid | 53 | 36.3 |
| IMIG stage at diagnosis | | |
| I/II | 54 | 36.0 |
| III/IV | 96 | 64.0 |
| Treatment modalities | | |
| Surgery (including multimodal treatment) | 44 | 29.3 |
| Systemic chemotherapy only | 86 | 57.3 |
| Radiotherapy only | 3 | 2.0 |
| Best supportive care only | 17 | 11.3 |
| CALGB group | | |
| 1–2 | 49 | 32.7 |
| 3–4 | 68 | 45.3 |
| 5–6 | 33 | 22.0 |
| EORTC prognostic score | | |
| Low risk | 91 | 60.7 |
| High risk | 59 | 39.3 |
| Platelet counts, mean ± SD ($\times 10^9$/L) | 299.5 ± 102.1 | |
| ≤400 | 130 | 86.7 |
| >400 | 20 | 13.3 |
| Hemoglobin difference (g/L) | | |
| <10 | 18 | 12.0 |
| ≥10 | 132 | 88.0 |
| Albumin (g/dL) | | |
| ≥3.5 | 76 | 50.7 |
| <3.5 | 74 | 49.3 |
| White cell count, mean ± SD ($\times 10^9$/L) | 8.32 ± 5.25 | |
| ≤8.3 | 102 | 68.0 |
| >8.3 | 48 | 32.0 |
| Neutrophil count, mean ± SD ($\times 10^9$/L) | 6.01 ± 5.00 | |
| Lymphocyte count, mean ± SD ($\times 10^9$/L) | 1.50 ± 0.57 | |
| Monocyte count, mean ± SD ($\times 10^9$/L) | 0.57 ± 0.42 | |
| Neutrophil-to-lymphocyte ratio, mean ± SD | 5.63 ± 10.99 | |
| ≤5 | 112 | 74.7 |
| >5 | 38 | 25.3 |
| Platelet-to-lymphocyte ratio, mean ± SD | 228.40 ± 114.18 | |
| <150 | 44 | 29.3 |
| ≥150 | 106 | 70.7 |
| Lymphocyte-to-monocyte ratio, mean ± SD | 3.36 ± 2.26 | |
| ≥2.74 | 109 | 72.7 |
| <2.74 | 41 | 27.3 |
| The Glasgow Prognostic Score | | |
| 0 | 45 | 30.0 |
| 1 | 37 | 24.7 |
| 2 | 68 | 45.3 |
| The modified Glasgow Prognostic Score | | |
| 0 | 51 | 34.0 |
| 1 | 31 | 20.7 |
| 2 | 68 | 45.3 |

ECOG: Eastern Cooperative Oncology Group; PS: performance status; IMIG: International Mesothelioma Interest Group; CALGB: Cancer and Leukemia Group B; EORTC, EORTC: European Organisation for Research and Treatment of Cancer.

### 3.2. The cutoff value for ALC, AMC, and LMR at diagnosis for survival analysis

The mean (±SD) lymphocyte count was 1.50 (±0.57) × $10^9$/L, and the mean monocyte count was 0.57(±0.42) × $10^9$/L. The mean (±SD) LMR level was 3.36 (±2.26). Applying ROC analysis, the optimal LMR cut-off was 2.74 (AUC, 0.736; 95% CI, 0.647–0.824) for OS. All patients were categorized as either high-LMR (≥2.74) or low-LMR (<2.74). Overall, there were 41 (27.3%) patients with LMR <2.74 and 109 (72.7%) patients with LMR ≥2.74. Similarly, AMC of 0.28 × $10^9$/L (AUC, 0.741; 95% CI, 0.648–0.834) and ALC of 1.73 × $10^9$/L (AUC, 0.532; 95% CI, 0.408–0.656) were selected as the optimal cut-off values.

### 3.3. Prognostic factor analysis for OS

Univariate analysis variables that predicted poor OS included male gender ($p = 0.022$), PS > 1 ($p = 0.001$), non-epithelioid histologic subtype ($p = 0.000$), stages III–IV ($p = 0.000$), no surgical intervention ($p = 0.000$), baseline WBC >8.30 × $10^9$/L ($p = 0.038$), baseline ALB <3.5 g/L ($p = 0.031$), high CALGB ($p = 0.010$), high-risk EPS ($p = 0.014$, Fig. 1A), NLR >5 ($p = 0.002$, Fig. 1B), PLR >150 ($p = 0.014$, Fig. 1C), LMR <2.74 ($p = 0.000$, Fig. 1D), high GPS ($p = 0.006$), high mGPS ($p = 0.014$), AMC ≥0.28 × $10^9$/L ($p = 0.011$, Fig. 1E), and ALC <1.73 × $10^9$/L ($p = 0.009$, Fig. 1F). Multivariate analysis revealed PS > 1 (hazard ratio [HR], 1.84; 95% CI, 1.07–3.16; $p = 0.027$), non-epithelioid histologic subtype (HR, 2.24; 95% CI, 1.51–3.33; $p = 0.000$), stage III/IV (HR, 1.70; 95% CI, 1.08–2.69; $p = 0.022$), no surgical intervention (HR, 2.41; 95% CI, 1.43–4.04; $p = 0.001$), LMR <2.74 (HR, 2.34; 95% CI, 1.58–3.47; $p = 0.000$) as independent prognostic factors of OS (Table 3).

ROC curves were constructed for survival status at 6, 12, and 24 months of follow-up, and the areas under the ROC curves were compared to assess the discrimination ability of each inflammation scoring system. The LMR consistently had higher AUC value at 6 months (0.772), 12 months (0.712), and 24 months (0.670) in comparison with other inflammation-based prognostic scores (Fig. 2).

An elevated LMR was significantly associated with prolonged OS. Patients with an LMR of <2.74 exhibited a median OS of 5.0 (95% CI, 4.0–10.0) months, while patients with an LMR ≥2.74 had a median OS of 14.0 (95% CI, 13.0–23.0) months ($p = 0.000$). Subgroup analyses were performed regarding histological subtype (epithelioid versus non-epithelioid), stage (I/II versus III/IV), and surgical intervention (yes versus no). In univariate analysis, the prognostic value of LMR was significant in patients with epithelioid subtype (HR, 2.76; 95% CI, 1.61–4.71; $p = 0.000$), patients with non-epithelioid subtype (HR, 3.38; 95% CI, 1.67–6.87; $p = 0.001$), and patients designated stage I/II (HR, 2.85; 95% CI, 1.21–6.71; $p = 0.016$) or stage III/IV (HR, 2.71; 95% CI, 1.69–4.35; $p = 0.000$). In addition, the predictive value of LMR was significantly stratified by surgical intervention (HR, 2.90; 95% CI, 1.20–6.98; $p = 0.017$) and by no surgical intervention (HR, 3.15; 95% CI, 1.98–5.05; $p = 0.000$). Kaplan–Meier curves revealed that decreased LMR (<2.74) about these factors was significantly associated with decreased OS (Fig. 3).

This study demonstrated that the LMR was associated with ECOG PS ($p = 0.001$), histological subtype ($p = 0.035$), stage ($p = 0.010$), surgical intervention ($p = 0.043$), CALGB ($p = 0.022$), EPS ($p = 0.001$), and baseline ALC ($p = 0.000$) and AMC ($p = 0.000$) (Table 2).

## 4. Discussion

In the present study, we demonstrated that elevated LMR, an inflammation-based prognostic score, was a favorable prognosis factor for OS in MPM. To the best of our knowledge, this study is the first to demonstrate that the LMR is an independent marker of prognosis in patients with MPM and features prognostic ability superior to that of the NLR, PLR, GPS, and mGPS.

Accumulating studies have demonstrated a strong link between systemic inflammatory responses (including neutrophils, lymphocytes, and monocytes) and cancer, and those responses have been

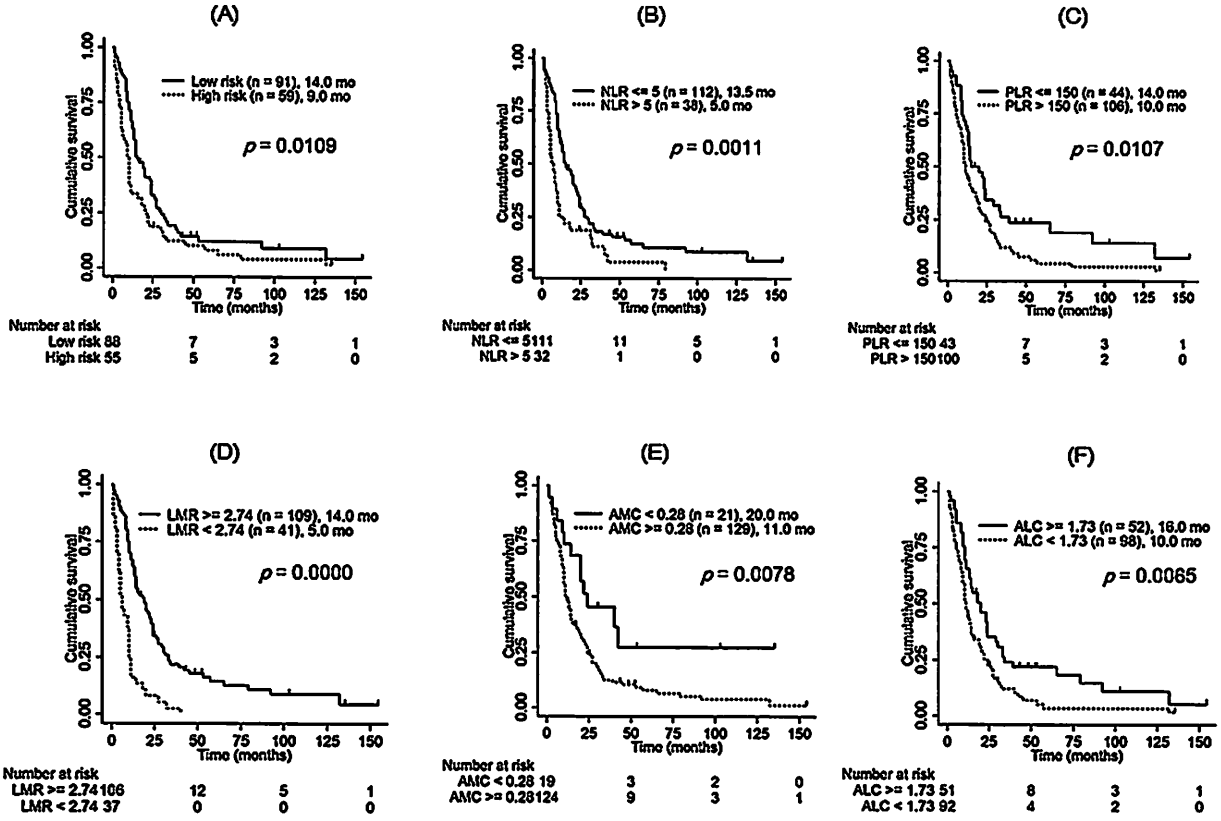


**Fig. 1.** Kaplan–Meier survival curves for patients affected by malignant pleural mesothelioma, stratified by European Organisation for Research and Treatment of Cancer prognostic score (A), neutrophil-to-lymphocyte ratio (NLR) (B), platelet-to-lymphocyte ratio (PLR) (C), lymphocyte-to-monocyte ratio (LMR) (D), absolute monocyte count (AMC) (E), and absolute lymphocyte count (ALC) (F).

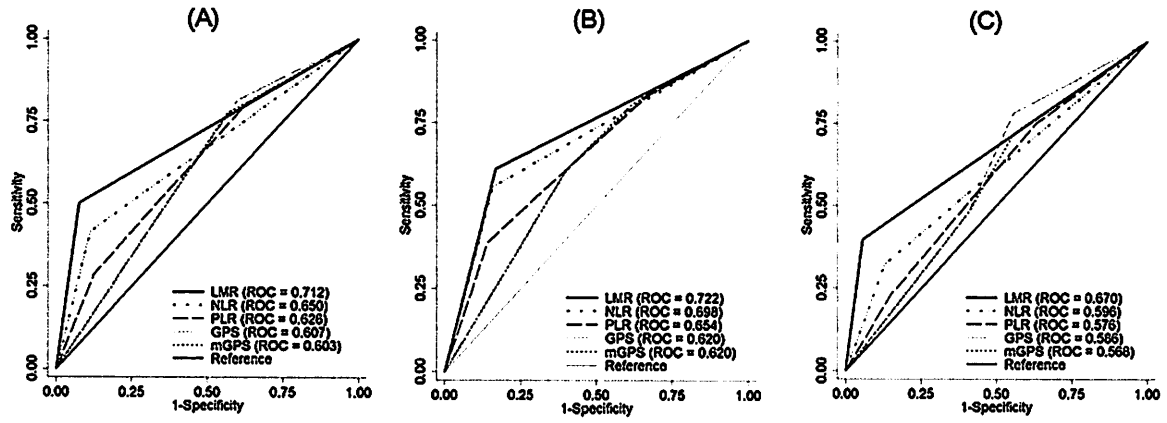


**Fig. 2.** Comparison of the areas under the receiver operating characteristic curves, for outcome prediction between the inflammation-based prognostic scores at (A) 6 months, (B) 12 months, and (C) 24 months in patients with malignant pleural mesothelioma. LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; GPS, Glasgow Prognostic Score; mGPS, modified Glasgow Prognostic Score.

significantly associated with clinical outcomes in cancer patients [20,21]. Among some inflammation-based scores, GPS is the most extensively validated [22]. However, recent years have seen a wealth of information about the prognostic value of ALC and AMC, together with their ratio, LMR.

Lymphocytes are components of the host immunity; they are important in the destruction of residual tumor cells and related micrometastases [23], and infiltrating lymphocytes can activate an effective antitumor cellular immune response [24]. Over the last few decades, a vast amount of evidence has highlighted the importance of monocytes and macrophages in cancer. Macrophages are derived from circulating monocytes and myeloid progenitor cells when entering tissues. Macrophages in tumors, which are usually referred to as tumor-associated macrophages (TAM), promote tumor cell invasion, migration, tumor-associated angiogenesis [25], and the suppression of antitumor immune reactions [26]. The role of TAMs in tumor biology and their prognostic value in various cancers, including thoracic malignancies, has become a major topic of interest [11]. The peripheral monocytes may reflect an increased production of tissue macrophage as a surrogate marker of high tumor burden. Burt et al. reported that higher numbers of circulating monocytes are associated with poor survival in all patients with MPM and higher densities of tumor-infiltrating macrophages are associated with poor survival in patients with the non-epithelioid

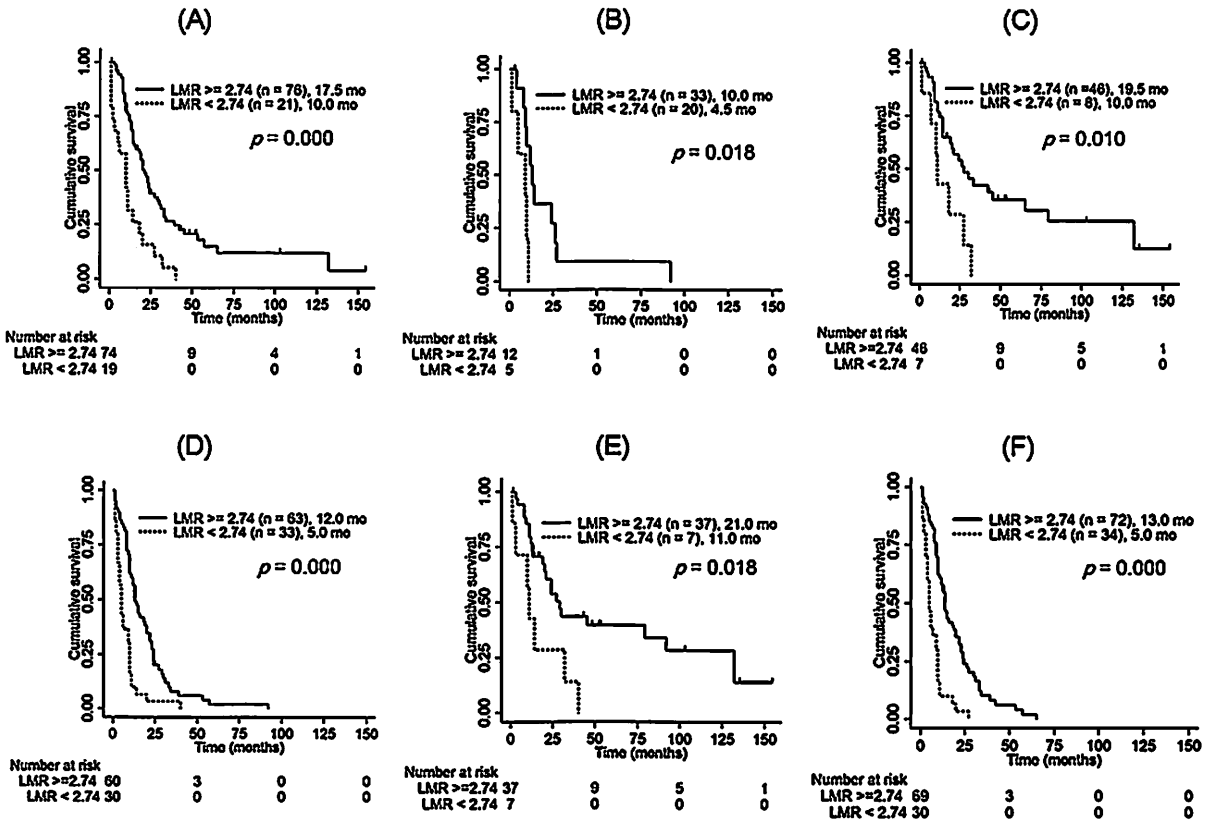


**Fig. 3.** Kaplan–Meier survival curves, stratified by lymphocyte-to-monocyte ratio (LMR) in patients with epithelioid subtype (A), with non-epithelioid subtype (B), with stage I/II disease (C), with stage III/IV disease (D), with surgical intervention (E), and without surgical intervention (F).

**Table 2**
Association of LMR with baseline clinical characteristics.

| Variable | LMR ≥ 2.74 *N* = 109 (%) | LMR < 2.74 *N* = 41 (%) | *p* |
|---|---|---|---|
| Age, years | | | 0.828 |
| <70 | 51 (47) | 20 (49) | |
| ≥70 | 58 (53) | 21 (51) | |
| Gender | | | 0.352 |
| Male | 103 (95) | 37 (90) | |
| Female | 6 (5) | 4 (10) | |
| ECOG PS | | | 0.001 |
| ≤1 | 100 (92) | 29 (70) | |
| >1 | 9 (8) | 12 (30) | |
| Histological subtype | | | 0.035 |
| Epithelioid | 76 (70) | 21 (51) | |
| Non-epithelioid | 33 (30) | 20 (49) | |
| IMIG stage | | | 0.010 |
| I/II | 46 (42) | 8 (20) | |
| III/IV | 63 (58) | 33 (80) | |
| Surgery | | | 0.043 |
| Yes | 72 (66) | 34 (83) | |
| No | 37 (34) | 7 (17) | |
| CALGB group | | | 0.022 |
| 1–2 | 40 (37) | 9 (22) | |
| 3–4 | 51 (47) | 17 (41) | |
| 5–6 | 18 (16) | 15 (37) | |
| EORTC prognostic score | | | 0.001 |
| Low risk | 75 (69) | 16 (39) | |
| High risk | 34 (31) | 25 (61) | |
| Lymphocyte count, mean ± SD ($\times 10^9$/L) | 1.63 ± 0.54 | 1.13 ± 0.48 | 0.000 |
| Monocyte count, mean ± SD ($\times 10^9$/L) | 0.44 ± 0.17 | 0.93 ± 0.62 | 0.000 |

LMR: lymphocyte-to-monocyte ratio; ECOG: Eastern Cooperative Oncology Group; PS: performance status; IMIG: International Mesothelioma Interest Group; CALGB: Cancer and Leukemia Group B; EORTC: European Organisation for Research and Treatment of Cancer.

subtype [27]. These findings might explain how decreasing LMR confers a negative prognosis in cancer patients.

Historically, the prognostic value of LMR was investigated mainly in hematological malignancies [12,28], and data were sparse in the context of solid tumors. In recent years, LMR has also been documented as an independent predictor of survival in a variety of patients with solid tumors, including colorectal tumor [29], breast cancer [30], lung cancer [31,32], pancreatic cancer [33], bladder cancer [34], and nasopharyngeal carcinoma [35]. However, until now, LMR data has not been reported in patients with MPM.

MPM is strongly associated with exposure to airborne asbestos fibers. Inhaled asbestos fibers present in the lung cause circulating macrophages to infiltrate the pleural space, where they attempt to phagocytose the inhaled foreign bodies [36]. As the macrophages are unable to eliminate the asbestos fibers, a state of chronic inflammation occurs, during which the secretion of free radicals causes genotoxic damage, which in turn facilitates the transformation of normal mesothelial cells to malignant mesothelioma [37].

In the present study, we initially evaluated the usefulness of LMR for predicting OS in patients with MPM. This study demonstrated that the ALC ($\geq 1.73 \times 10^9$/L) was a favorable prognosis factor and the AMC ($\geq 0.28 \times 10^9$/L) was an inferior prognostic factor for MPM patients. Multivariate analysis showed that LMR, ECOG PS, histological subtype, stage, and surgical intervention were significantly associated with OS. Moreover, AUC analysis has shown that the LMR was superior to other inflammation-based prognostic scores regarding predictive accuracy. In our study cohort, an elevated LMR (≥2.74) was significantly associated with increased OS. Similar results were observed in Hodgkin's lymphoma [38], diffuse large B-cell lymphoma [28], and nasopharyngeal carcinoma [35]. We also focused on the relationship between LMR and prognosis according to subtype. Our results showed that decreased

**Table 3**
Analysis of prognostic factors regarding overall survival.

| Variable | OS (univariate analysis) | | OS (multivariate analysis) | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | p | Hazard ratio (95% CI) | p |
| Age (<70/≥70 years) | 1.00 (0.69–1.43) | 0.993 | NI | |
| Gender (male/female) | 2.36 (1.13–4.93) | 0.022 | NI | |
| ECOG PS (≤1/>1) | 2.39 (1.44–3.98) | 0.001 | 1.84 (1.07–3.16) | 0.027 |
| Histological subtype (epithelioid/non-epithelioid) | 1.95 (1.34–2.84) | 0 | 2.24 (1.51–3.33) | 0 |
| IMIG stage (I/II vs. III/IV) | 2.73 (1.81–4.12) | 0 | 1.70 (1.08–2.69) | 0.022 |
| Surgery (yes/no) | 0.35 (0.22–0.55) | 0 | 2.41 (1.43–4.04) | 0.001 |
| Hemoglobin difference (g/L) (<10/≥10) | 1.33 (0.76–2.33) | 0.31 | NI | |
| White cell count ($\times 10^9$/L) (≤8.30/>8.30) | 1.49 (1.02–2.17) | 0.038 | NI | |
| Platelet count ($\times 10^9$/L) (≤400/>400) | 1.17 (0.72–1.90) | 0.517 | NI | |
| Albumin (g/dL) (≥3.5/<3.5) | 1.48 (1.03–2.12) | 0.031 | NI | |
| CALGB group (1–2/3–4/5–6) | 1.43 (1.09–1.89) | 0.01 | NI | |
| EPS (low risk/high risk) | 1.57 (1.09–2.26) | 0.014 | NI | |
| NLR (≤5/>5) | 1.93 (1.28–2.92) | 0.002 | NI | |
| PLR (≤150/>150) | 1.65 (1.10–2.47) | 0.014 | NI | |
| LMR (≥2.74/<2.74) | 3.10 (2.07–4.66) | 0 | 2.34 (1.58–3.47) | 0 |
| GPS (0/1/2) | 1.32 (1.08–1.63) | 0.006 | NI | |
| mGPS (0/1/2) | 1.28 (1.05–1.56) | 0.014 | NI | |
| AMC ($\times 10^9$/L) (<0.28/≥0.28) | 2.16 (1.19–3.93) | 0.011 | NI | |
| ALC ($\times 10^9$/L) (≥1.73/<1.73) | 1.66 (1.13–2.44) | 0.009 | NI | |

ECOG: Eastern Cooperative Oncology Group; PS: performance status; IMIG: International Mesothelioma Interest Group; CALGB: Cancer and Leukemia Group B; EPS: European Organisation for Research and Treatment of Cancer prognostic score; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio; GPS: Glasgow Prognostic Score; mGPS: modified Glasgow Prognostic Score; AMC: absolute monocyte count; ALC: absolute lymphocyte count; NI: Not included.

LMR was significantly associated with poor prognosis for patients with the epithelioid subtype, as well as for those with the non-epithelioid subtype. In addition, the predictive value of LMR was also significant in patients with stage I/II and stage III/IV disease, and in patients with or without surgical intervention. Some studies reported that the inflammation-based prognostic scores are associated with responses to treatment (e.g., chemotherapy and radiotherapy) and perioperative outcomes (e.g., post-operative mortality and complications) [17,21]. Additional work is required to define the clinical utility of LMR within the individual therapeutic process.

Although the roles of monocytes and macrophage in tumor biology and their prognostic value in cancer have become major topics of interest, and much recent research has been dedicated to targeting these cells, there is currently no available clinical therapy to target these cells in patients with MPM. Conventional treatment strategies have met with limited success for patients with MPM. The combination strategy, which targets cancer cells directly, as well as macrophages and monocytes, may present a potentially new therapeutic approach [39]. Additional research is warranted regarding the use of immune-modulatory therapies to treat this malignancy.

One potential limitation of this study is that this is a retrospective and single-centre study. In addition, the study did not assess potential confounding factors (e.g., local or systemic infection, ischemia, acute coronary syndrome, metabolic syndrome, diabetes mellitus, and renal or hepatic dysfunction) that might affect the lymphocyte and monocyte counts. A large-scale prospective validation study is needed to confirm the results.

## 5. Conclusion

Our study is the first to demonstrate that the LMR is an independent marker of prognosis in patients with MPM and is superior to the other inflammation-based scores regarding prognostic ability. LMR is easily assessed using a simple complete blood count test, and is both technically and financially feasible to predict patients' clinical outcomes in routine practice. The identification of simple and valuable prognostic markers for MPM will enable clinicians to select patients who are most likely to benefit from intensive therapy, and avoid subjecting unsuitable candidates to futile treatment.

## Conflict of interest

The authors declare that they have no competing interests.

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# DNA copy number gains in malignant pleural mesothelioma

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**Abstract.** Malignant pleural mesothelioma (MPM) is a highly aggressive tumor with an extremely poor prognosis. The incidence of MPM is increasing as a result of widespread exposure to asbestos. The molecular pathogenesis of MPM remains unclear. The present study analyzed the frequency of various genomic copy number gains (CNGs) in MPM using reverse transcription-quantitative polymerase chain reaction. A total of 83 primary MPMs and 53 primary lung adenocarcinomas were analyzed to compare the CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2*. In MPM, the CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2* were detected in 12 (14.5%), 8 (9.6%), 5 (6.0%), 4 (4.8%) and 1 (1.2%) of the samples, respectively. In lung adenocarcinomas, the CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2* were detected in 21 (39.6%), 12 (22.6%), 5 (9.4%), 10 (18.9%) and 0 (0.0%) of the samples, respectively. The CNGs of *EGFR*, *KRAS* and *FGFR1* were significantly less frequent in the MPMs compared with the lung adenocarcinomas (P=0.0018, 0.048 and 0.018, respectively). Overall, the MPMs exhibited these CNGs less frequently compared with the lung adenocarcinomas (P=0.0002). The differences in CNGs between the two tumor types suggested that they are genetically different.

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*Key words:* malignant pleural mesothelioma, lung adenocarcinoma, copy number, reverse transcription-quantitative polymerase chain reaction

## Introduction

Malignant pleural mesothelioma (MPM) is a tumor derived from the mesothelial cells lining the pleural spaces. MPM has highly invasive and aggressive clinical characteristics. Approximately 80% of MPM patients have a history of occupational asbestos exposure, which is considered to be a risk factor for the development of the disease (1). The molecular pathogenesis of MPM is not well understood. The most common mutations in MPMs are losses in 9p21, 1p36, 14q32 and 22q12, and gains in 5p, 7p and 8q24, which have been detected by comparative genomic hybridization analysis (2,3). Homozygous deletion of the 9p21 locus encoding two critical cyclin-dependent kinase inhibitors, $p16^{INK4a}$ and $p15^{INK4b}$, have been reported in up to 80% of MPMs, and this mutation may be of diagnostic utility (4,5). The tumor suppressor neurofibromin 2 is encoded by the *NF2* gene, located on chromosome 22q12. Mutations in *NF2* are found in ~40% of MPMs, and heterozygous loss of *NF2* is identified in ~74% of MPMs (6,7). Mutations are rare in the *TP53* and *RAS* genes, which are frequently present in epithelial solid tumors (8,9). Epigenetic alterations, such as DNA methylation, have been found in MPMs, which have a different profile compared with lung cancer (10-12). MPMs, particularly of the epithelioid subtype, may be hard to differentiate from adenocarcinoma arising in the lung periphery, and epidemiological evidence indicates that asbestos and smoking are shared risk factors for these diseases (2,13,14). Currently, the differential diagnosis of MM is based on a range of morphological analyses, including a combination of histological and immunohistochemical staining, and electron microscopy (13,15,16).

Cytogenetic studies have been performed on MPMs and adenocarcinomas arising in the lung periphery, however, no chromosomal aberrations specific to either of the tumor types have been identified (2,14).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is a method for evaluating DNA copy number

changes, including losses, gains and amplifications of DNA sequences (17-19). Copy number gains (CNGs) of *EGFR* and *KRAS* have been observed in lung cancer, particularly in adenocarcinoma (18,20). Furthermore, CNGs of *FGFR1* and *SOX2* have been observed in lung cancer, particularly in squamous cell carcinoma (21-25). c-Met was recently reported to be activated in MPM by overexpression or mutations in *MET* (26), and *MET* amplification is a known cause of resistance to *EGFR*-tyrosine kinase inhibitor (TKI) treatment in lung cancer (27). RT-qPCR was used in the present study on 83 primary MPM and 53 primary lung adenocarcinomas to compare the CNGs of *EGFR*, *KRAS*, *MET, FGFR1* and *SOX2*.

## Materials and methods

*Tumor samples*. Surgically resected specimens of 53 lung adenocarcinomas and 83 MPMs (57 epithelioid, 8 sarcomatoid, 15 biphasic, 2 desmoplastic and 1 lymphohistiocytic) were obtained. All the lung adenocarcinomas and 11 of the MPM samples were obtained from Okayama University Hospital (Okayama, Japan). Another 18 MPMs were obtained from Yamaguchi-Ube Medical Center (Ube, Japan), 2 were obtained from Okayama Rosai Hospital (Okayama, Japan) and the remaining 52 were obtained from Karmanos Cancer Center (Detroit, MI, USA). All Japanese samples were collected between March 2002 and September 2011, and all samples from the USA were collected >10 years ago. Resected tumors were stored at -80˚C until DNA extraction. Permission from the Institutional Review Board and informed consent were obtained at each collection site.

*DNA extraction*. Genomic DNA was obtained from primary tumors by standard phenol:chloroform (1:1) extraction, followed by ethanol precipitation, or using a DNeasy Tissue kit (Qiagen, Inc., Valencia, CA, USA).

*RT-qPCR for copy number evaluation*. CNGs of *EGFR*, *KRAS, MET, FGFR1* and *SOX2* genes were determined by RT-qPCR assays using Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), as previously described (18,19). Briefly, samples of 1 $\mu$l were analyzed per assay using with StepOne Plus Real-Time PCR System (Themo Fisher Scientific). PCR conditions were initial denaturation at 95˚C for 10 min followed by 40 cycles of amplification at 95˚C for 15 sec and 60˚C for 60 sec. The samples were analyzed in triplicate using StepOne Plus RT PCR software (version 2.0; Themo Fisher Scientific) and the *LINE1* gene was used as a reference gene for all copy number analyses, as this is the most abundant autonomous retrotransposon in the human genome, constituting 17%. Each amplification reaction was checked for the absence of non-specific PCR products by performing a melting curve analysis. The copy number calculation was conducted using the comparative cycle threshold (Ct) method following validation of the PCR reaction efficiency of *EGFR, KRAS, MET, FGFR1, SOX2* and *LINE1*. The PCR primer sequences for *EGFR, KRAS, MET* and *LINE1* primers have previously been described (17-19). The PCR primer sequences for *FGFR1* and *SOX2* were designed by Primer 3 plus software and by modification of the sequences. The PCR primer sequences were as follows: *FGFR1* forward, 5'-AGC CAC CAC ATG GCA TAC TT-3' and reverse, 5'-GGT GAC AAG GCT CCA CAT CT-3'; and *SOX2* forward, 5'-CGT CAC ATG GAT GGT TGT CT-3' and reverse, 5'-GCC GCC GAT GAT TGT TAT TA-3'. The relative copy number of each sample was determined by comparing the ratio of the target gene to *LINE1* in each sample with the ratio of these genes in normal human genomic DNA (EMD Biosciences, Darmstadt, Germany) prepared from a mixture of human blood cells from 6-8 donors, as a diploid control. Our previous study defined a copy number of ≥4 as a gene gain in cell lines (17,18). However, considering the contamination by non-malignant cells in primary samples (estimated mean per tumor, 50% tumor cells and 50% non-malignant cells), the cut-off value of 3 copy numbers rather than 4 was used for primary tumors in this study (17).

*Detection of EGFR mutations*. The *EGFR* mutational status was determined using a PCR-based length polymorphism and restriction fragment length polymorphism assay, as previously described (28). Briefly, the common deletions of exon 19 were distinguished from the wild-type based on PCR product length polymorphisms using 12% polyacrylamide gel electrophoresis (PAGE) and ethidium bromide staining. For the exon 21 L858R mutation, *Sau*96I digestion, which specifically digests the mutant type, was performed prior to 12% PAGE.

*Statistical analyses*. Differences between the two groups were assessed using the $\chi^2$ test or Fisher's exact test as required. All data were analyzed using JMP software version 9.0.0 (SAS Institute Inc., Cary, NC, USA). For all analyses, $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*CNGs in MPMs and lung adenocarcinomas*. In the 83 MPM samples, the CNGs of *EGFR*, *KRAS, MET, FGFR1* and *SOX2* were detected in 12 (14.5%), 8 (9.6%), 5 (6.0%), 4 (4.8%), and 1 (1.2%) of the samples, respectively. In the epithelioid subtype of MPM (n=57), the CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2* were detected in 7 (12.3%), 5 (8.8%), 3 (5.3%), 4 (7.0%) and 0 (0.0%) of the samples, respectively. In the other subtypes of MPMs (n=26), the CNGs of *EGFR*, *KRAS*, *MET*, FGFR and *SOX2* were detected in 5 (19.2%), 3 (11.5%), 2 (7.7%), 0 (0%) and 1 (3.8%) of the samples, respectively. In the 53 lung adenocarcinomas, the CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2* were detected in 21 (39.6%), 12 (22.6%), 5 (9.4%), 10 (18.9%) and 0 (0.0%) of the samples, respectively (Table I; Fig. 1). Three cases of MPMs were demonstrated to have numerous CNGs of *EGFR* (269, 62 and 14, respectively). The CNGs of *EGFR*, *KRAS* and *FGFR1* were significantly less frequent in the MPMs compared with the lung adenocarcinomas (P=0.0018, 0.048 and 0.018, respectively). In the epithelioid subtype of MPMs, the CNGs of *EGFR* were significantly less frequent than those in the lung adenocarcinomas (P=0.0018), and in other subtypes of MPMs, the CNGs of *FGFR1* were significantly less frequent compared with those of the lung adenocarcinomas (P=0.026). In the MPMs, an absence and presence of CNGs were observed in 64 (77.1%) and 19 (22.9%) of the 83 cases, respectively. In the epithelioid MPMs, absent/present CNGs were observed in 47 (82.5%) and 10 (17.5%) of the 57 cases, respectively. In the other subtypes of

Table I. CNGs of *EGFR*, *KRAS*, *MET*, *FGFR1* and *SOX2* in MPMs and lung adenocarcinomas.

| | MPMs (n=83) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | All (n=83) | | Epithelioid subtype (n=57) | | Other subtypes (n=26) | | Lung adenocarcinoma (n=53) | |
| Genes | No. | % | No. | % | No. | % | No. | % |
| *EGFR* | 12[a] | 14.5 | 7[a] | 12.3 | 5 | 19.2 | 21 | 39.6 |
| *KRAS* | 8[b] | 9.6 | 5 | 8.8 | 3 | 11.5 | 12 | 22.6 |
| *MET* | 5 | 6.0 | 3 | 5.3 | 2 | 7.7 | 5 | 9.4 |
| *FGFR1* | 4[b] | 4.8 | 4 | 7.0 | 0[b] | 0.0 | 10 | 18.9 |
| *SOX2* | 1 | 1.2 | 0 | 0.0 | 1 | 3.8 | 0 | 0.0 |

CNGs of *FGFR1* and *KRAS* were significantly less frequent in MPMs compared with lung adenocarcinomas. In epithelioid MPMs, CNGs of *EGFR* were found to be significantly less frequent compared with lung adenocarcinomas. In other types of MPMs, CNGs of *FGFR1* were found to be significantly less frequent compared with lung adenocarcinomas ([a]P<0.05; [b]P<0.01). CNGs, copy number gains; MPMs, malignant pleural mesotheliomas.

Table II. Frequency of the absence or presence of CNGs in MPMs and lung adenocarcinomas.

| | Absence of CNGs | | Presence of CNGs | |
|---|---|---|---|---|
| Cancer type | No. | % | No. | % |
| Malignant pleural mesothelioma (n=83)[a] | 64[a] | 77.1 | 19 | 22.9 |
| Epithelioid subtype (n=57)[a] | 47[a] | 82.5 | 10 | 17.5 |
| Other subtypes (n=26) | 17 | 65.4 | 9 | 34.6 |
| Lung adenocarcinoma (n=53) | 24 | 45.3 | 29 | 54.7 |

Frequency of none of CNGs in MPMs and epithelioid MPMs was significantly higher compared with lung adenocarcinomas ([a]P<0.01). CNGs, copy number gains; MPM, malignant pleural mesothelioma.

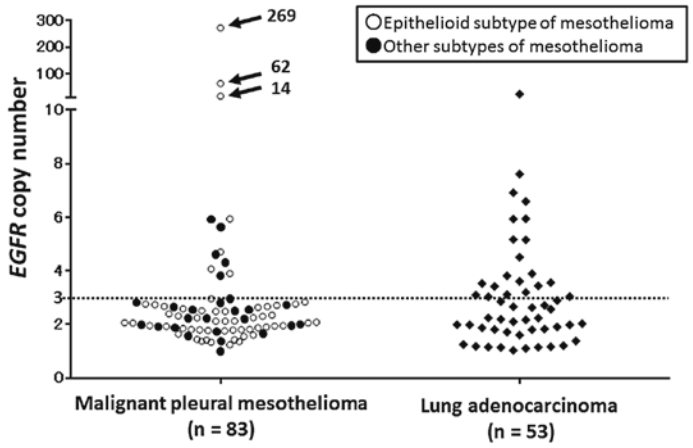


Figure 1. *EGFR* gene copy number, determined by reverse transcription-quantitative polymerase chain reaction in malignant pleural mesotheliomas (MPMs) and lung adenocarcinomas. Copy numbers >3 were considered as copy number gain (CNG). Three cases of MPMs were shown to have high CNGs of *EGFR* (269, 62 and 14, respectively).

the MPMs, the absence and presence of CNGs were observed in 17 (65.4%) and 9 (34.6%) of the 26 cases, respectively. In the lung adenocarcinomas, the absence and presence of CNGs were observed in 24 (45.3%) and 29 (54.7%) of the 53 cases, respectively (Table II). The MPMs and the epithelioid subtypes of the MPMs had less frequent CNGs than the lung adenocarcinomas (P=0.0002 and P=0.0001, respectively).

*EGFR mutations*. No *EGFR* mutation was detected in the 83 MPMs. In the lung adenocarcinomas, *EGFR* mutations

were detected in 21 (39.6%) cases; 14 cases exhibited an exon 19 deletion and 7 cases exhibited an exon 21 mutation (L858R).

## Discussion

The main finding of the present study is that the pattern of DNA CNGs of MPM is different from that in lung adenocarcinoma. MPMs exhibited less CNGs of the genes examined in compared with the lung adenocarcinomas. The epithelioid subtype of MPM, which is often difficult to distinguish from lung adenocarcinoma, similarly exhibited these CNGs less frequently compared with the lung adenocarcinomas. To the best of our knowledge, only a limited number of studies have previously analyzed the presence and frequency of *EGFR* CNGs in MPMs (2,29-32), and no studies have focused on CNGs of *KRAS*, *MET*, *FGFR1* or *SOX2* in MPM. A large number of samples (n=83) were screened in the present study, whereas the previous studies were based on smaller sample sizes and may have underestimated the true frequency of such CNGs.

Although CNGs of *SOX2* were seldom observed in the MPMs and lung adenocarcinomas, the CNGs of the remaining four genes were detected in the MPM samples to a certain extent. The fact that the CNGs of four genes in the MPMs were less frequent in comparison to the lung adenocarcinomas suggested that CNG may not be a pivotal mechanism for the activation of oncogenes in MPMs, and that different mechanisms may be of greater importance. It has been previously reported that *EGFR* is overexpressed in 60-70% of MPM tissue specimens; however, it is not overexpressed in the normal mesothelium (29,33). Furthermore, exposure to asbestos fibers is known to cause *EGFR* aggregation (34). In the present study, *EGFR,* located at 7p12-p13, was the most frequent gene to exhibit CNGs (12 out of 83 MPMs and 20 out of 53 lung adenocarcinomas). Bjorkqvist *et al* (2) reported similar results, such as gains of genetic material in 5p, 6p and 7p between MPMs and lung adenocarcinomas. The study detected a gain in 7p in 7 out of 34 MPMs and 11 out of 30 lung adenocarcinomas (2). MPMs rarely harbor *EGFR* mutations (31,35-37). There were no *EGFR* mutations detected in MPMs in the present study, as expected. Upon analysis, three cases of MPMs exhibited high *EGFR* gene amplification (CNG>10), and these cases were all epithelioid MPMs, which was consistent with the previous studies by Okuda *et al* (29) and Enomoto *et al* (31). It remains unclear whether high-level amplification of *EGFR* is more prominent in MPMs compared with lung adenocarcinomas, although the frequency of CNGs for *EGFR* is lower in MPMs compared with lung adenocarcinomas. In MPMs with *EGFR* amplification, the inhibition of *EGFR* pathways should exert an antitumor effect. In lung cancer, the results of two randomized phase III trials that compared a placebo to erlotinib or gefitinib treatment indicated that *EGFR* copy number detected by fluorescence *in situ* hybridization was the best predictor of survival (38). Patients with colorectal cancer who responded to anti-*EGFR* treatment with cetuximab or panitumumab exhibited an increased *EGFR* copy number (39). Although two phase II studies of single-agent EGFR-TKI therapy to treat MPMs failed to demonstrate their clinical efficacy, in the gefitinib trial, 2 of 43 MPM patients responded to gefitinib (40,41). These data suggest that a small proportion of patients (with *EGFR* gene amplification) may be candidates for anti-*EGFR* treatment (29).

In conclusion, the present study detected novel CNGs in genes other than *EGFR*. MPM samples exhibited these CNGs less frequently compared with lung adenocarcinomas. The differences in DNA CNG between the two tumor types suggested that they are genetically different.

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*Research Article*

# Clinical Investigation of Benign Asbestos Pleural Effusion

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There is no detailed information about benign asbestos pleural effusion (BAPE). The aim of the study was to clarify the clinical features of BAPE. The criteria of enrolled patients were as follows: (1) history of asbestos exposure; (2) presence of pleural effusion determined by chest X-ray, CT, and thoracentesis; and (3) the absence of other causes of effusion. Clinical information was retrospectively analysed and the radiological images were reviewed. There were 110 BAPE patients between 1991 and 2012. All were males and the median age at diagnosis was 74 years. The median duration of asbestos exposure and period of latency for disease onset of BAPE were 31 and 48 years, respectively. Mean values of hyaluronic acid, adenosine deaminase, and carcinoembryonic antigen in the pleural fluid were 39,840 ng/mL, 23.9 IU/L, and 1.8 ng/mL, respectively. Pleural plaques were detected in 98 cases (89.1%). Asbestosis was present in 6 (5.5%) cases, rounded atelectasis was detected in 41 (37.3%) cases, and diffuse pleural thickening (DPT) was detected in 30 (27.3%) cases. One case developed lung cancer (LC) before and after BAPE. None of the cases developed malignant pleural mesothelioma (MPM) during the follow-up.

## 1. Introduction

Asbestos-related pathological changes of the pleura include pleural plaques, malignant pleural mesothelioma (MPM), diffuse pleural thickening (DPT), and benign asbestos pleural effusion (BAPE). BAPE is a nonmalignant pleural disease initially described in 1964 [1]. It is also termed asbestos pleuritis. Once a patient is diagnosed with BAPE, he or she is compensated by workers' compensation in Japan. Epler et al. [2] advocated diagnostic criteria for BAPE, which include (1) previous asbestos exposure, (2) determination of pleural effusion by chest X-ray or thoracentesis, and (3) the absence of other causes of effusion. They also stated that follow-up assessments for at least 3 years were essential to confirm the diagnosis and to exclude the development of malignant diseases such as MPM or lung carcinomatous pleuritis. Later, Hillerdal and Ozesmi [3] described that a 1-year follow-up would be sufficient based on a detailed exploration including computed tomographic (CT) scanning. Most of the previous studies included small numbers of

patients and were undertaken in the 1980s, so no detailed information is available about the disease.

In the current study, we retrospectively analysed the clinical features of BAPE in patients in Japan. The aim of the study was to clarify the clinical features of BAPE and to suggest more practical diagnostic standard for the disease.

## 2. Patients and Methods

*2.1. Subjects.* Enrolled patients were referred to Rosai Hospital and affiliated hospitals in Japan for an examination for pleural effusion and were finally diagnosed with BAPE. The criteria of enrolled patients were as follows: (1) previous history of asbestos exposure obtained by an in-person questionnaire or interview; (2) presence of pleural effusion determined by chest X-ray, CT, and thoracentesis; and (3) the absence of other causes of effusion. The pleural fluid was collected by thoracentesis or thoracoscopy, and information on cell classification, cytological analysis, and the biochemical examination was extracted from the medical records. Hyaluronic acid (HA), adenosine deaminase (ADA), and carcinoembryonic antigen (CEA) were included among the clinical laboratory tests. The HA concentration was determined using a latex agglutination turbidimetric immunoassay. ADA was measured using an enzymatic technique. CEA was measured using a chemiluminescent immunoassay.

*2.2. Data Collection and Analysis.* Clinical and demographic information was obtained from the medical records at each facility. The information included age, gender, smoking status, initial symptoms, and results of laboratory testing of the pleural effusion. The work histories, those of the family members, and residential histories were investigated to assess the patient's history of asbestos exposure.

The radiological images were sent to Okayama Rosai Hospital for review. Characteristic radiological findings associated with asbestos exposure were assessed as the presence of pleural effusion, asbestosis, rounded atelectasis, pleural plaques, and DPT. Asbestosis was classified on chest X-rays according to perfusion rate (PR) based on the International Labour Organization (ILO) criteria [4]. DPT was defined as pleural thickening of more than 5 mm on chest X-rays, extending for more than half of the lateral thoracic wall (LTW) in cases of unilateral DPT or more than quarter of the LTW in cases of bilateral DPT [5]. The presence of pleural effusion, rounded atelectasis, and pleural plaques was assessed on chest CT.

Survival data were determined from the day pleural effusion was detected to the day of death or last follow-up and analysed using the Kaplan-Meier method with SPSS 11.0 software (SPSS, Inc., Chicago, IL, USA).

This study was done according to the Ethical Guidelines for Epidemiological Research by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labour, and Welfare. This study was approved by Japan Labour Health and Welfare Organization and the institutional review boards of each institution. Patient confidentiality was strictly maintained. This study was carried out according to the principles set out in the Declaration of Helsinki.

Table 1: Patient characteristics.

| | |
|---|---|
| Age ($n$ = 110) | |
| Median (range) | 74 (36–90) |
| Gender ($n$ = 110) | |
| Male/female | 110/0 |
| Smoking history ($n$ = 63) | |
| Ever/current | 56 |
| Never | 7 |
| Symptoms ($n$ = 65, multiple answers) | |
| Dyspnea | 34 |
| Cough | 15 |
| Chest pain | 13 |
| Fever | 3 |
| Palpitation | 2 |
| Sputum | 1 |
| Wheezing | 1 |
| Back pain | 1 |
| Weight loss | 1 |
| Fatigue | 1 |

## 3. Results

*3.1. Patient Characteristics.* One hundred ten patients from 9 institutions fulfilled the enrolled criteria based on the descriptions in their medical records and review of the radiographs between 1991 and 2012. Characteristics of the patients are shown in Table 1. Smoking history was obtained in 63 cases including 56 ever/current smokers and 7 never smokers, with the median (range) pack-years of 34.5 (0–112). Pleural effusion was found in 56 cases in the right, 25 in the left, and 27 in both thoracis. Sixty-five patients visited the clinic for subjective symptoms, and pleural effusion was detected at the regular medical check-up in 35 cases without any symptoms. Pleural effusion was detected during the treatment of other diseases in another 15 cases. Thoracentesis was performed in all patients to collect pleural fluid. Thoracoscopic exploration was done in 78 patients to exclude carcinomatous pleuritis or MPM and to confirm the diagnosis of BAPE.

*3.2. Asbestos Exposure History.* A history of asbestos exposure was reported by 109 patients, with one patient whose detailed information of asbestos exposure was not obtained. Among the 109 patients, 108 patients had a history of occupational asbestos exposure and one patient had a history of environmental asbestos exposure. The occupational categories associated with asbestos exposure are shown in Table 2. The median (range) age of the first exposure to asbestos was 21.5 (14–58) years. The median (range) duration of asbestos exposure was 31 (0.75–50) years and the median (range) period of latency for disease onset of BAPE was 48 (17–76) years.

Table 2: Occupational category related to asbestos exposure.

| | |
|---|---|
| Shipbuilding | 25 |
| Construction | 20 |
| Chemical facility | 10 |
| Asbestos products manufacturing | 8 |
| Electrical work | 8 |
| Plumbing | 7 |
| Asbestos transportation | 5 |
| Moisturizing work | 4 |
| Asbestos spraying | 3 |
| Steel production | 3 |
| Demolition work | 2 |
| Automobile manufacturing | 2 |
| Heat insulation | 2 |
| Firebrick manufacturing | 2 |
| Glasswork | 1 |
| Metallic product manufacture | 1 |
| Furnace installation | 1 |
| Coating industry | 1 |
| Shipman | 1 |
| Others | 2 |
| Total | 108 |

*3.3. Characteristics of the Pleural Effusion.* Information regarding the pleural effusion was obtained in 104 cases. The gross impression of the pleural fluid was bloody in 75 cases, light yellow in 27, and light brown and dark red in 1 case each. The effusions were exudative in all cases. A cellular classification of the fluid was obtained in 57 cases and the median proportions of lymphocytes, macrophages, neutrophils, and eosinophils were 77.7%, 9.7%, 8.0%, and 8.0%, respectively. The HA concentration was determined in 106 cases and the mean (standard deviation) concentration was 39,840 (40,228) ng/mL. Mean (standard deviation) values of ADA and CEA were 23.9 (24.9) IU/L and 1.8 (1.3) ng/mL, respectively.

*3.4. Concomitant Asbestos-Related Findings.* As shown in Table 3, pleural plaques were detected in 98 cases (89.1%), among which 76 cases were calcified. Asbestosis was present in 6 cases, rounded atelectasis was detected in 41 cases (37.3%), and DPT was detected in 30 cases (27.3%). One of the cases developed lung cancer (LC) before and after diagnosis of BAPE. The patient had undergone right upper lobectomy for LC two years before his BAPE diagnosis and left partial lobectomy for another LC two years after his BAPE diagnosis.

*3.5. Clinical Course.* In most of the cases, thoracentesis and/or thoracotomy were done to collect the fluid and drain the pleural effusion. Oral steroids were prescribed in 5 cases and one of them demonstrated temporal decrease of the effusion. Survival data was obtained in 70 cases from Okayama Rosai Hospital. As shown in Figure 1, median overall survival was 104.2 months (95% confidence interval (CI), 67.3–141.0 months) after a median observation period of 73.0 months (95% CI, 16.2–268.2 months). There were 17 dead cases out of 70 cases at the analysis. The causes of death were determined in 11 cases including 7 respiratory failure cases and each 1 of renal failure, suicide, septic shock due to urinary tract infection, and death of old age. There were 9 cases that developed DPT out of the 17 cases, including the 7 dead cases of respiratory failure. At the time of the analysis, none of the cases had developed MPM.

Table 3: Concomitant asbestos-related radiological findings.

| Findings | | *n* | % |
|---|---|---|---|
| Pleural plaques | | 98 | 89.1 |
| Calcified | | 76 | |
| Asbestosis | | 6 | 5.7 |
| PR[†] | 1 | 3 | |
| | 2 | 2 | |
| | 3 | 1 | |
| Rounded atelectasis | | 41 | 37.3 |
| DPT[‡] | | 30 | 27.3 |

[†]Perfusion rate, [‡]diffuse pleural thickening.

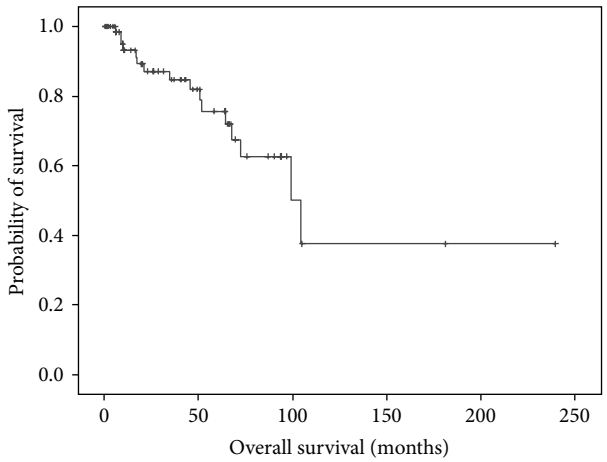


Figure 1: Overall survival of patients with benign asbestos-related pleural effusions at Okayama Rosai Hospital.

## 4. Discussion

In the current study, we examined the clinical features of BAPE and demonstrated that BAPE developed after long-term asbestos exposure. In a previous report, BAPE occurred 15–20 years after exposure and was more common in younger patients aged 21–40 years [6]. In another report, the interval between asbestos exposure and presentation of BAPE varied between 5 and more than 30 years, and early onset was correlated with higher asbestos exposure [7]. Wagner reported that BAPEs were usually unilateral, and the most common manifestation of asbestos-related pleural disease occurred 10 to 20 years after exposure [8]. A limitation of these earlier studies is that the diagnosis criteria of BAPE were ambiguous in the studies. The median latency period between asbestos exposure and BAPE development in the current study was

Table 4: Proposed diagnostic criteria of benign asbestos pleural effusion.

| Diagnostic criteria |
|---|
| (1) Asbestos exposure history. |
| (2) Exudative effusion. |
| (3) Exclusion of other pleuritides such as lung cancer, MPM[†], and tuberculous pleuritis by radiological examination and pleural biopsy via thoracoscopy. |
| **Additional diagnostic information** |
| (1) In cases thoracoscopy could not be undergone, the diagnosis should be discussed based on the bacteriological examination and biochemical markers below. |
| (a) Elevated carcinoembryonic antigen (>5 ng/mL) suggests carcinomatous pleuritis. |
| (b) Elevated adenosine deaminase (>35 IU/L) suggests tuberculous pleuritis. |
| (c) Elevated hyaluronic acid (>100,000 ng/dL) suggests MPM. |
| (2) In cases with some concomitant medical problem such as autoimmune diseases, the activity of the disease should be carefully evaluated. |

[†]Malignant pleural mesothelioma.

48 years, which was similar to that observed for MPM (41 years), LC (47 years), and asbestos-induced DPT (46 years) in our previous reports [4, 9, 10]. We consider that BAPE develops after a long latency period in those with a history of asbestos exposure. There is one point, however, that most of the patients of BAPE in the current study have associated with other asbestos-related lesions such as rounded atelectasis and/or diffuse pleural thickening. It is possible that BAPE might have been developed earlier in these cases, and this could be an explanation of the longer latency of BAPE than previously published. The current study suggests that BAPE can develop after moderate-to-high levels of exposure to asbestos, because the occupational category of the subjects in the current study included those of relatively high levels of asbestos exposure such as asbestos product manufacturing, construction, and shipbuilding, although the correlation between the exposure amount and development of BAPE is unclear. The subjects in the current study included substantial portion of those with smoking history. To our knowledge, the correlation between BAPE and smoking history has not been reported.

The diagnosis of BAPE should be based on a history of asbestos exposure and an exclusion of other causes of effusion such as tuberculous pleuritis, bacterial pleuritis, collagen diseases, heart failure, and malignant conditions such as MPM and LC. In our analysis, the gross impression of the pleural fluid was bloody in 72% of the cases, and cellular classification of the fluid demonstrated lymphocyte dominancy. These results are similar to those of a previous report showing that the effusion was exudative and could be hemorrhagic, as well as predominantly eosinophilic [11].

In cases of LC, tumor cells are detected in the fluid in more than 60% of cases [12]. In cases with MPM, tumor cells can be detected in the pleural fluid, but the detection rate has been reported as less than 30% [13]. Tuberculosis pleuritis or bacterial pleuritis could be diagnosed by staining for acid-fast bacteria, polymerase chain reaction detection, or bacterial culture, although the detection rate is usually low. These analyses may not always determine the diagnosis but should be undergone to exclude MPM, LC, and tuberculosis or bacterial pleuritis and to make the diagnosis of BAPE.

In addition, we analysed some markers such as HA concentration, ADA, and CEA. Recently, we reported the clinical usefulness of HA for the differential diagnosis of MPM and BAPE [14]. In cases with tuberculosis pleuritis, elevated values of ADA could help in the diagnosis [15]. However, elevated ADA may not be limited to tuberculous pleuritis, as it is also present in LC or MPM [16]. In cases with elevated CEA values, carcinomatous pleuritis is strongly suggested [17]. These markers should be determined to exclude these conditions and to confirm a diagnosis of BAPE. However, the differential diagnosis of MPM and BAPE is especially difficult, even when based on these markers. Especially in cases with exudative pleural effusions, thoracoscopic exploration and pleural biopsy should be performed to exclude MPM and confirm the diagnosis of BAPE [18].

Based on the findings in the current study and previous reports, we propose more practical diagnostic standard for the diagnosis of BAPE including (1) asbestos exposure history, (2) exudative effusion, and (3) exclusion of other pleuritides such as LC, MPM, and tuberculous pleuritis by radiological examination and pleural biopsy via thoracoscopy. Additional diagnostic information is as follows: (1) in cases thoracoscopy could not be undergone, the diagnosis should be discussed based on the bacteriological examination and biochemical markers such as CEA, ADA, and HA; in cases with elevated CEA (>5 ng/mL), ADA (>35 IU/L), or HA (>100,000 ng/dL), carcinomatous pleuritis, tuberculous pleuritis, or MPM is more likely, respectively; and (2) in cases with some concomitant medical problem such as autoimmune diseases, the activity of the disease should be carefully evaluated, because autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis could involve the pleura and cause pleural effusion (Table 4).

"Benign" is meant to refer to a nonmalignant process, but these effusions can be associated with significant morbidity [19]. The effusion generally takes a long time to resolve. It may resolve spontaneously or be followed by DPT, which causes extrapulmonary restriction and may thereby ultimately become disabling. Previous studies reported that a considerable number of patients with BAPE subsequently developed DPT [2, 3]. Actually, in our previous study, half

the patients with asbestos-induced DPT had a history of BAPE [4]. Furthermore, in the current study one patient developed LC before and after being diagnosed with BAPE. The risks of developing MPM or LC in patients with BAPE are increased compared with those of the general population because of their past history of asbestos exposure. Particular attention should be paid to the management of patients with BAPE.

There are a few limitations to the current study. First, this was a retrospective study. Second, pathological analyses including immunohistochemistry were not reviewed. In addition, there are recent reports that increased uptake of fluorodeoxyglucose (FDG) by positron emission tomography (PET) may be a useful marker to distinguish MPM from benign pleural disease [20, 21]. In addition, recent reports revealed that biomarkers such as soluble mesothelin-related peptides (SMRP) are selectively elevated in patients with MPM [22, 23]. A clinical study to evaluate the utility of PET and/or SMRP for the differentiation between MPM and BAPE is warranted.

## 5. Conclusions

BAPE develops after a long latency period after past asbestos exposure. The diagnosis of BAPE should be based on the exclusion of other pleural diseases. A thorough evaluation, including diagnostic thoracentesis and cytological and bacterial analysis, must be performed. Clinical markers such as HA, ADA, and CEA might help with the differential diagnosis. However, thoracoscopic exploration and pleural biopsy should be performed to confirm a diagnosis of BAPE.

## Disclosure

This study is a part of "the research and development and the dissemination projects related to the 9 fields of occupational injuries and illnesses" of Japan Labour Health and Welfare Organization. This organization had no involvement in the study design, collection, analysis, and interpretation of the data, writing of the paper, or decision to submit the paper for publication.

## Conflict of Interests

The authors declare that there is no conflict of interests in their submitted paper.

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RESEARCH PAPER

# Brain metastases in malignant pleural mesothelioma

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**Abstract** The brain is a rare site of metastasis in malignant pleural mesothelioma (MPM), and its clinical features and prognosis remain unclear. The aim of this study was to investigate the incidence, prognosis, and risk factors for brain metastases (BM) in MPM patients. Between July 1993 and October 2014, 150 patients with histologically proven MPM were included in this retrospective study. The cumulative incidence of BM was estimated with the Kaplan–Meier method, and differences between groups were analyzed by the log-rank test. Multivariate logistic regression analysis was applied to assess risk factors for BM. The median follow-up time was 11 months (range 0–154.0 months). A total of eight patients (5.3 %) developed BM during the course of their illness. Multivariate analysis identified age <65 years (odds ratio [OR] = 5.83, $p = 0.038$) and International Mesothelioma Interest Group stage IV (OR = 1.69, $p = 0.040$) as independent factors related to increased risk of developing BM. The 1-and 2-year cumulative rates of BM were 4.0 % (95 % confidence intervals [CI] 1.4–8.5 %) and 5.3 % (95 % CI 2.3–10.2 %), respectively. Our study showed that the overall survival (OS) of patients with BM was worse than that of patients without BM (median OS 6.5 vs. 11.0 months, $p = 0.037$). The prognosis for BM in MPM patients is poor. Clinicians should perform careful screening for BM, especially in patients with risk factors.

**Keywords** Asbestos · Malignant pleural mesothelioma · Brain metastases · Central nervous system · Brain

**Abbreviations**

| | |
|---|---|
| MPM | Malignant pleural mesothelioma |
| BM | Brain metastasis |
| ECOG | Eastern Cooperative Oncology Group |
| PS | Performance status |
| IMIG | International Mesothelioma Interest Group |
| EPS | European Organization for Research and Treatment of Cancer Prognostic Score |
| CALGB | Cancer and Leukemia Group B |
| MRI | Magnetic resonance imaging |
| CT | Computed tomography |
| OS | Overall survival |

## Introduction

Malignant pleural mesothelioma (MPM) is a rare but aggressive primary pleural neoplasm that is associated with asbestos exposure [1]. Its prognosis remains poor, as most patients have unresectable disease at the time of diagnosis. Although several treatment options are available to patients with MPM, the median survival time is approximately 12 months [2].

Brain metastasis (BM) is the most common type of intracerebral neoplasm and occurs in 10–30 % of adult

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patients with systemic cancer [3]. The incidence of BM may be increasing, because of improved imaging techniques to detect BM and more effective systemic treatment that can prolong the tumor-bearing period of time, permitting the cancer to disseminate to the brain. Lung cancer, breast cancer, and malignant melanoma are the neoplasms most frequentlyassociated with BM [4].

MPM typically spreads by local invasion or extension. Distant metastasis may occur; however, BM is quite rare and the majority of reported cases were identified by postmortem examination. The incidence of BM was reported to be only 3 % of MPM patients [5]. To date, there have been few reports on BM in patients with MPM. Recently, Chari et al. a case report and review of the literature on the topic [6], however, its clinical features and prognosis remain unclear. The aim of this single-institution retrospective study was to evaluate the incidence, risk factors, and survival outcome associated with BM inpatients with MPM.

## Materials and methods

### Patient population

The study included 150 consecutive patients with histologically proven MPM who were seen between July 1993 and October 2014 at Okayama Rosai Hospital, Japan. Baseline demographic and clinicopathological variables were collected retrospectively from patients' medical records. These included age at initial diagnosis, gender, histological subtype, clinical stage, and baseline Eastern Cooperative Oncology Group (ECOG) performance status (PS).Clinical staging was determined according to the International Mesothelioma Interest Group (IMIG) staging system [7]. The European Organization for Research and Treatment of Cancer Prognostic Score (EPS) was used to subcategorize patients into low- or high-risk groups based on age, gender, histology, probability of diagnosis, and leukocyte count [8]. We also used the Cancer and Leukemia Group B (CALGB) score, which incorporates the presence of nonepithelial histology, weight loss, chest pain, high platelets and WBC, low hemoglobin, high serum lactate dehydrogenase, advanced age, and PS [9]. The patients were divided based on their CALGB score into three groups (i.e., groups 1/2, 3/4, or 5/6), because of the small numbers in the even-numbered groups [10].

Diagnosis of BM in MPM was based on magnetic resonance imaging (MRI) or contrast-enhanced computed tomography (CT) scans. Routine brain imaging was performed at the diagnosis but not during the follow-up period, unless BM was suspected. Leptomeningeal metastases were not included in the actuarial incidence of BM in this study.

This study was approved by the Japan Labour, Health and Welfare Organization and the institutional review board of Okayama Rosai Hospital. Patient confidentiality was strictly maintained.

### Statistical analysis

A comparison of patient characteristics at diagnosis was performed by Fisher's exact test or Student's $t$ test, as appropriate. Overall survival (OS) was calculated from the date of initial diagnosis to the date of last follow-up or death from any cause. OS was obtained by using Kaplan–Meier methods, and between-group differences were compared with the log-rank test. To determine risk factors associated with the development of BM, univariate and multivariate logistic regression models with a stepwise selection procedure were generated. A two-tailed $p$ value of $<0.05$ was considered statistically significant. All statistical analysis was performed with STATA software (version 12.1; StataCorp, College Station, TX, USA).

## Results

### Patient characteristics

The median follow-up time for the 150 study participants was 11.0 months (range 0–154.0 months). Eight of the patients developed BM: three at diagnosis of MPM and five during the clinical course of MPM. Thus, the crude incidence rate of BM was 2.0 % (95 % confidence interval [CI] 0.4–5.7 %) at diagnosis and 5.3 % (95 % CI 2.3–10.2 %) overall. The median time from initial diagnosis to development of BM was 4.5 months (range 0–24.0 months); the 1-and 2-year cumulative rates for BM were 4.0 % (95 % CI 1.4–8.5 %) and 5.3 % (95 % CI 2.3–10.2 %), respectively.

In the cohort of patients with BM ($n = 8$), the median ($\pm$SD) age was 61.3 ($\pm$6.3) years and all patients were men. The majority of the histological types were epithelioid (62.5 %) followed by sarcomatous (37.5 %). Three patients (37.5 %) had stage III disease, and five patients (62.5 %) had stage IV disease. Four patients (50 %) had symptoms of BM, such as ataxia ($n = 2$), headache ($n = 1$), and sensitivity disorders ($n = 1$). BM were in various locations including 5 in parietal lobe (62.5 %), 3 in frontal lobe (37.5 %), and each 2 in temporal lobe (25.0 %) and the cerebellum (25.0 %). The size of BM was 2 cm or less in all cases. At the time of detection of BM, four

**Table 1** Patients with brain metastases

| Age/ gender | Histology | IMIG stage | Treatment for primary tumor | Interval to BM (months) | Metastatic site | Presence of other metastases | Treatment for BM | Clinical response to treatment for BM | Survival post BM (months) | OS (months) |
|---|---|---|---|---|---|---|---|---|---|---|
| 64/M | Epithelioid | T3N0M0, stage III | Chemotherapy | 24.0 | Multiple | Lung, adrenals, lymph node | WBRT | No | 3.0 | 27.0 |
| 70/M | Epithelioid | T3N0M1, stage IV | Chemotherapy | 0.0 | Right parietal lobe | – | Chemotherapy | No | 2.0 | 2.0 |
| 63/M | Sarcomatous | T3N1M0, stage III | Surgery | 2.0 | Cerebellum | Lung, adrenals, liver, muscle, lymph node | Chemotherapy | No | 1.0 | 3.0 |
| 54/M | Epithelioid | T2N0M1, stage IV | Chemotherapy | 7.0 | Multiple | Adrenals, bone | WBRT | No | 1.0 | 8.0 |
| 64/M | Sarcomatous | T3N0M1, stage IV | Best supportive care | 0.0 | Multiple | Bone | WBRT | No | 5.0 | 5.0 |
| 64/M | Epithelioid | T4N2M0, stage IV | Chemotherapy | 15.0 | Multiple | – | SRT + chemotherapy | Yes | 5.0 | 20.0 |
| 49/M | Epithelioid | T3N0M0, stage III | Chemotherapy | 7.0 | Right frontal lobe | Adrenals, liver | SRT | No | 5.0 | 12.0 |
| 66/M | Sarcomatous | T2N0M1, stage IV | Chemotherapy | 0.0 | Right parietal lobe | – | SRT | No | 4.0 | 4.0 |

*BM* brain metastases, *OS* overall survival, *IMIG* International Mesothelioma Interest Group, *WBRT* whole brain radiation therapy, *SRT* stereotactic radiotherapy

patients (50 %) had developed other distant metastases, including adrenal gland ($n = 3$), lung ($n = 2$), lymph node ($n = 2$), liver ($n = 2$), muscle/soft tissue ($n = 1$), and bone ($n = 1$). The patients' baseline characteristics and therapies are presented in Table 1.

The clinicopathological characteristics of the patients with BM were compared with those of patients without BM (non-BM) (Table 2). Age at diagnosis was younger in BM than in non-BM patients ($p = 0.034$), and there were more patients with pleural effusion at initial diagnosis in BM compared to non-BM patients ($p = 0.044$). There was no difference between the two groups with respect to histological subtype.

## Treatment and survival

Of the three patients who presented BM at initial diagnosis, one patient underwent whole brain radiotherapy and one underwent radiosurgery. The remaining patient had asymptomatic BM and received systemic chemotherapy. Of the four patients who developed BM after initial systemic chemotherapy, two were treated with whole brain radiotherapy and two underwent radiosurgery. The final patient developed asymptomatic BM during the observation period after initial surgery and received systemic chemotherapy.

The median OS of the non-BM and BM groups was 11.0 months (range 0–154.0 months) and 6.5 months (range 2.0–27.0 months), respectively ($p = 0.037$) (Fig. 1). All patients died of progressive disease, with a median survival of 3.5 months (range 1.0–5.0 months) after the development of BM.

## Risk factors

Univariate analysis revealed that age <65 years (odds ratio [OR] 6.06, 95 % CI 1.17–31.19, $p = 0.031$), absence of pleural effusion (OR 4.56; 95 % CI 1.03–20.01, $p = 0.044$), and IMIG stage IV (OR 1.72, 95 % CI 1.05–2.81, $p = 0.031$) were potential risk factors for BM. In the multivariate analysis, age < 65 years (OR 5.83; 95 % CI 1.10–30.73, $p = 0.038$) and IMIG stage IV (OR 1.69 95 % CI 1.02–2.80, $p = 0.040$) remained associated with a high risk of BM (Table 3).Based on the results of multivariate analysis, we analyzed the effect of these two factors on the incidence of BM. For patients age ≥65 years and stage I–III, age <65 years and stage I–III, age ≥65 years and stage IV, and age <65 years and stage IV, the cumulative incidence rates of BM were 0, 8.1, 8.3, and 18.7 %, respectively ($p = 0.003$, Fisher's exact test) (Fig. 2).

**Table 2** Patient characteristics

| Characteristic | Brain metastases ($n = 8$) | No brain metastases ($n = 142$) | $p$ value |
|---|---|---|---|
| Median age, years (range) | 61.3 ± 6.7 | 68.9 ± 9.9 | 0.034 |
| Men | 8 (100 %) | 132 (93 %) | 0.568 |
| ECOG PS > 2 | 2 (25 %) | 19 (13 %) | 0.311 |
| Pleural effusion at diagnosis | | | 0.044 |
| Yes | 3 (38 %) | 104 (73 %) | |
| No | 5 (62 %) | 38 (27 %) | |
| Histological subtype | | | 0.584 |
| Epithelioid subtype | 5 (63 %) | 92 (65 %) | |
| Nonepithelioid subtype | 3 (37 %) | 50 (35 %) | |
| Calretinin | | | 0.07 |
| Positive | 5 (63 %) | 121 (85 %) | |
| Negative | 2 (25 %) | 9 (6 %) | |
| Missing | 1 (12 %) | 12 (8 %) | |
| IMIG stage | | | 0.051 |
| I–II | 0 (0 %) | 54 (38 %) | |
| III–IV | 8 (100 %) | 88 (62 %) | |
| EORTC prognostic score | | | 0.712 |
| High risk | 4 (50 %) | 87 (61 %) | |
| Low risk | 4 (50 %) | 55 (39 %) | |
| CALGB score | | | 0.076 |
| 1–2 | 0 (0 %) | 49 (35 %) | |
| 3–4 | 5 (63 %) | 63 (44 %) | |
| 5–6 | 3 (37 %) | 30 (21 %) | |
| Treatment modality | | | 0.630 |
| Surgery | 1 (12 %) | 43 (30 %) | |
| Systemic chemotherapy alone | 6 (75 %) | 80 (56 %) | |
| Radiotherapy | 0 (0 %) | 3 (2 %) | |
| Best supportive care | 1 (13 %) | 16 (11 %) | |

*ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *IMIG* International Mesothelioma Interest Group, *CALGB* Cancer and Leukemia Group B, *EORTC* European Organisation for Research and Treatment of Cancer

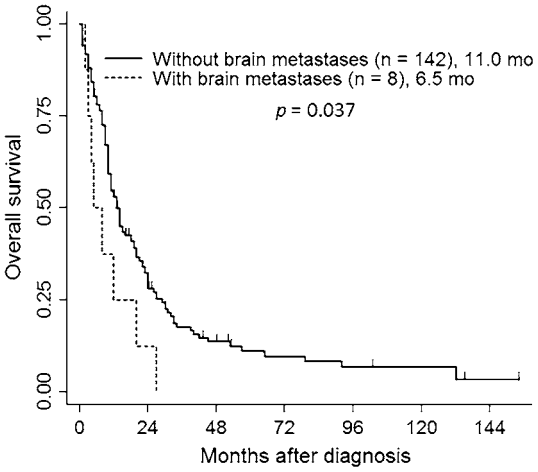


**Fig. 1** Overall survival in malignant pleural mesothelioma patients with and without brain metastases

## Discussion

MPM is an uncommon tumor of the thorax; typically, there is pernicious local invasion and extension into the pleural space and surrounding organs. The brain is a rare site for metastasis from MPM, and the majority of reported cases were identified in postmortem studies. To the best of our knowledge, this is the first report to have focused on the incidence, risk factors, and survival outcome associated with BM in patients with MPM.

In our patient cohort, eight out of 150 patients (5.3 %) developed BM. Falconieri et al. reviewed 171 cases of MPM at postmortem examination and discovered that 54 % of patients had distant metastases. The most frequently involved organs were the liver (55.9 %), adrenal glands (31.1 %), kidney (30.1 %), and contralateral lung (26.8 %). Intracranial (brain and meningeal) metastases

**Table 3** Cox regression model for factors associated with incidence of brain metastases

| Variable | Univariate | | Multivariate | |
|---|---|---|---|---|
| | Odds ratio (95 % Cl) | *p* value | Odds ratio (95 % CI) | *p* value |
| Age at initial diagnosis | | | | |
| ≥65 years | 1 | | 1 | |
| <65 years | 6.06 (1.17–31.19) | 0.031 | 5.83 (1.10–30.73) | 0.038 |
| ECOG PS | | | | |
| ≤1 | 1 | | NI | |
| >1 | 2.15 (0.40–11.48) | 0.367 | | |
| Pleural effusion at diagnosis | | | | |
| Yes | 1 | | NI | |
| No | 4.56 (1.03–20.01) | 0.044 | | |
| Histological subtype | | | | |
| Epithelioid | 1 | | NI | |
| Nonepithelioid | 1.10 (0.25–4.81) | 0.895 | | |
| Calretinin | | | | |
| Positive | 1 | | NI | |
| Negative | 5.02 (0.85–29.62) | 0.075 | | |
| IMIG stage | | | | |
| I–III | 1 | | 1 | |
| IV | 1.72 (1.05–2.81) | 0.031 | 1.69 (1.02–2.80) | 0.040 |
| Surgery | | | | |
| Yes | 1 | | NI | |
| No | 0.32 (0.03–2.75) | 0.305 | | |
| CALGB score | | | | |
| 1–3 | 1 | | NI | |
| 4–6 | 2.22 (0.53–9.31) | 0.273 | | |
| EORTC prognostic score | | | | |
| Low risk | 1 | | NI | |
| High risk | 1.58 (0.37–6.58) | 0.529 | | |

*ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *IMIG* International Mesothelioma Interest Group; *CALGB* Cancer and Leukemia Group B, *EORTC* European Organisation for Research and Treatment of Cancer; *NI* not included

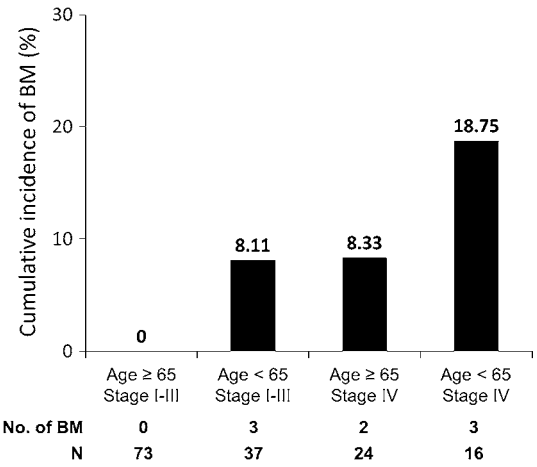


**Fig. 2** Cumulative incidence of brain metastases in malignant pleural mesothelioma patients characterized by identified risk factors. *BM* brain metastases

were found in only 3 % of cases [5]. Furthermore, pooling analysis of seven postmortem studies consisting of 655 patients revealed that the rate of intracranial metastases from malignant mesothelioma (including those of pleural, pericardial, and tunica vaginalis testis origin) was 2.7 % [11]. Our study demonstrated that the incidence of BM was approximately 5 %, which is higher than that in previous studies. The previous studies were limited to autopsy cases, and not all MPM patients were included. We believe that the true incidence of BM may be higher.

In our study, multivariate analysis showed that age <65 years and IMIG stage IV were independently associated with BM. An increased risk for BM in younger patients was reported in several studies on other malignancies [12–15]; however, the reason why younger patients are at a higher risk of BM is not well understood. Further

investigations are needed to determine the biological factors associated with BM in younger patients.

Among the clinicopathological features of MPM, histological subtype is one of the most important factors influencing survival and sarcomatous histology has the worst prognosis [16]. Miller et al. showed that the sarcomatous subtype predominates in patients with BM [11]; however, another study observed that the rate of extra thoracic dissemination does not differ among histology subtypes [17]. Our findings also demonstrated that histology was not a risk factor for BM.

The current study showed that outcomes for BM were equally poor regardless of treatment modalities. Surgery, stereotactic therapies, and systemic chemotherapy may play a role in selected situations; however, rapid recurrence after surgical excision [18] or after regression in response to systemic chemotherapy [19] has been reported. Clarification regarding the most appropriate treatment strategy for BM is urgently needed. The next best policy would be to identify patients with MPM who are at greater risk of developing BM. Based on our results, it may be reasonable to perform brain imaging on MPM patients with risk factors such as age <65 years and IMIG stage IV, even in the absence of neurological signs and symptoms.

There are a few limitations to the current study. First, this was a retrospective single-institution study. Second, due to the relatively small number of patients and events, statistical analysis was limited. Another limitation is that most BM were detected based on patients' symptoms. Therefore, asymptomatic BM may have been missed.

In conclusion, the prognosis for MPM patients with BM is poor. The incidence of MPM is predicted to reach a peak between 2015 and 2025 [20], and the development of BM has a severe effect on patients' quality of life and survival. Clinicians should perform careful screening for BM, especially in patients with risk factors.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interests.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study (retrospective), formal consent is not required.

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# Lymphohistiocytoid mesothelioma with a response to cisplatin plus pemetrexed: A case report

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**KEYWORDS**
Malignant pleural mesothelioma;
Lymphohistiocytoid mesothelioma;
Asbestos;
Systemic chemotherapy;
Pemetrexed

**Abstract**
We report the case of a patient with lymphohistiocytoid mesothelioma (LHM) with a response to systemic chemotherapy consisting of cisplatin and pemetrexed. A 72-year-old man was referred to our hospital because of abnormal shadows seen on chest X-rays. He had been exposed to asbestos at shipyards for 3 years. Computed tomography (CT) images of the chest showed multiple masses on the parietal pleura, diaphragm, and the interlobar pleura of the right. CT-guided percutaneous needle biopsy was performed and the biopsy specimen demonstrated fibrous thickening of the pleura with abundant lymphocyte infiltration. Immunohistochemical analyses revealed that the cells were positive for calretinin, WT-1, and CAM5.2, and negative for CEA, TTF-1, CK5/6, AE1/AE3, desmin, CD3, CD20, CD30, and CD68. Based on these findings, the diagnosis was confirmed as LHM. Systemic chemotherapy consisting of cisplatin (75 mg/m$^2$) and pemetrexed (500 mg/m$^2$) was delivered. After 6 courses of chemotherapy, multiple tumors had remarkably regressed, and the patient remains on maintenance treatment with pemetrexed. There are few reports of chemotherapy for LHM. The combination of cisplatin and pemetrexed could be a good treatment option for LHM.


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## 1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from the mesothelial cells lining the pleura [1] and is generally associated with a history of asbestos exposure [2]. Lymphohistiocytoid mesothelioma (LHM) is a rare subtype of MPM and there are few reports on the efficacy of systemic chemotherapy for this subtype. We report the case of a patient with LHM who had a response to systemic chemotherapy consisting of cisplatin and pemetrexed.

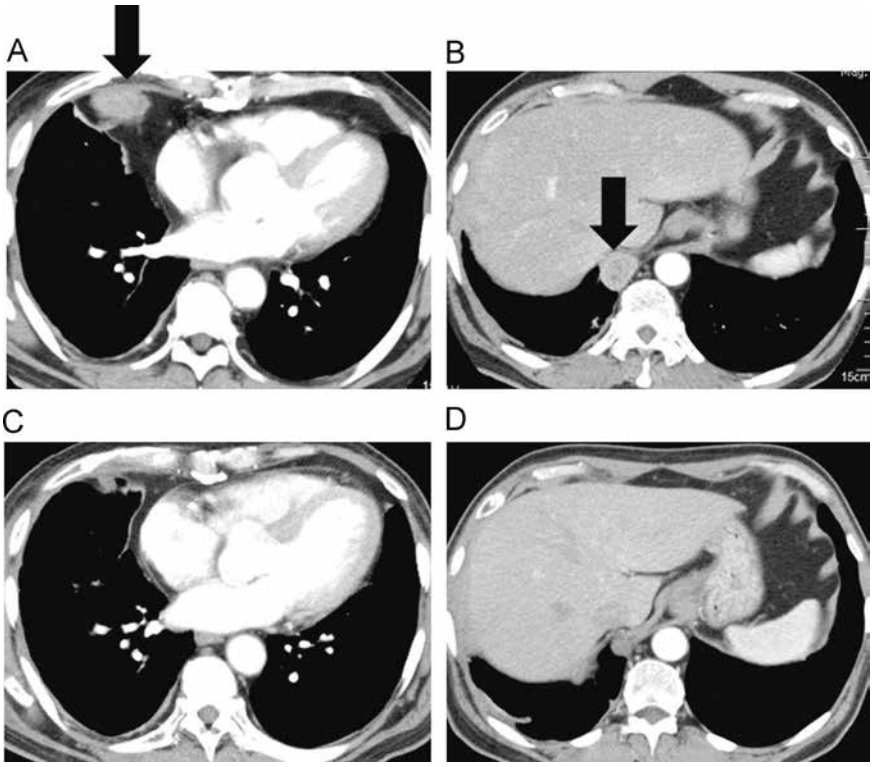


**Figure 1** Computed tomography images at diagnosis (A, B) and after the 6 cycles of systemic chemotherapy consisting of cisplatin and pemetrexed (C, D). Black arrows indicate the tumors on the pleura.

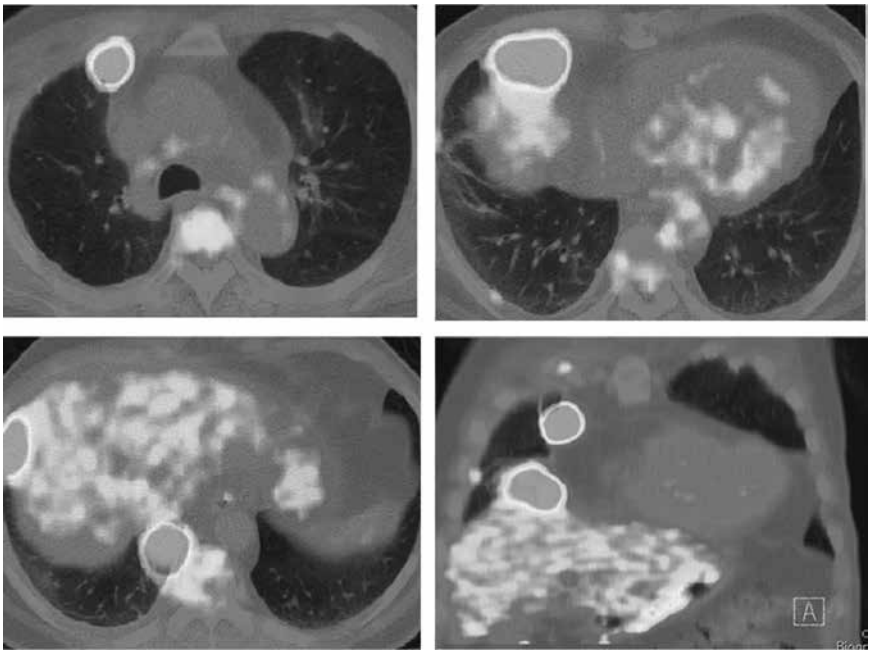

**Figure 2** Whole-body fluorine-18 2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography and CT images demonstrating abnormal FDG uptake (shown in red) in multiple masses on the pleura.

## 2. Case report

A 72-year-old man was referred to our hospital because of abnormal shadows seen on his chest X-ray determined at a regular medical checkup. He had a history of appendicitis and been diagnosed with type II diabetes mellitus. He had smoked between the ages of 18 and 35 years, and as a teenager had been exposed to asbestos at shipyards for 3 years. His physical examination revealed nothing specific and no superficial lymph nodes were palpable. Blood tests revealed a slight elevation in C-reactive protein and glucose levels. There were no increases in tumor markers. A chest X-ray revealed permeability decay in the right lower lung field, and computed tomography (CT) images of the chest showed multiple masses on the parietal pleura, diaphragm, and the interlobar pleura of the right (Figure 1A and B). Multiple masses on the pleura showed a contrast effect from the early stage of the arterial phase on enhanced CT images. Anterior mediastinal and gastric cardia lymphadenopathy were also detected and suspected to be lymph node metastases. Whole-body fluorine-18 2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography and CT revealed abnormal FDG uptake in multiple masses and enlarged lymph nodes (Figure 2). CT-guided percutaneous needle biopsy was performed and the biopsy specimen demonstrated fibrous thickening of the pleura with an abundant lymphocyte infiltration (Figure 3). Among them were scattered polygonal cells with a nucleus containing clear oval nucleoli. Immunohistochemical analyses revealed that the cells were positive for calretinin, WT-1, and CAM5.2, and negative for CEA, TTF-1, CK5/6, AE1/AE3, desmin, CD3, CD20, CD30, and CD68 (Figure 4). Based on these findings, the diagnosis was confirmed as LHM. The patient's disease was diagnosed as stage IV (T1bN2M1) based on the International Mesothelioma Study Group staging system [3].

Systemic chemotherapy consisting of cisplatin (75 mg/m$^2$) and pemetrexed (500 mg/m$^2$) was delivered. After 6 courses of the chemotherapy, multiple tumors had remarkably regressed (Figure 1C and D). Mediastinal and intraperitoneal lymph nodes were also significantly diminished. The patient has been on maintenance treatment with pemetrexed and his disease remains stable.

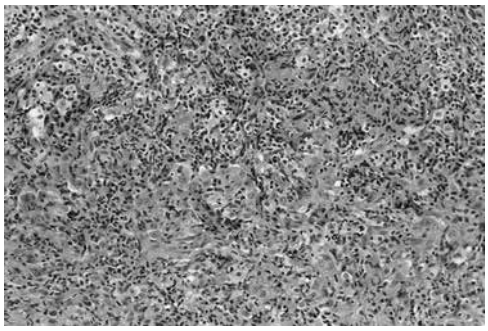

**Figure 3** Percutaneous needle biopsy specimen demonstrating fibrous thickening of the pleura with abundant lymphocyte infiltration.

## 3. Discussion

We report a case of LHM, which is a rare variant of MPM, in a patient who had a remarkable response to systemic chemotherapy consisting of cisplatin and pemetrexed. LHM was first identified in 1988 by Henderson et al. and characterized as having neoplastic cells with a histiocytoid appearance that are surrounded by a marked lymphocytic infiltration. LHM comprises less than 1% of mesotheliomas [4] and was categorized previously as a sarcomatous subtype of MPM [5]. However, in a recent report Galateau-Salle

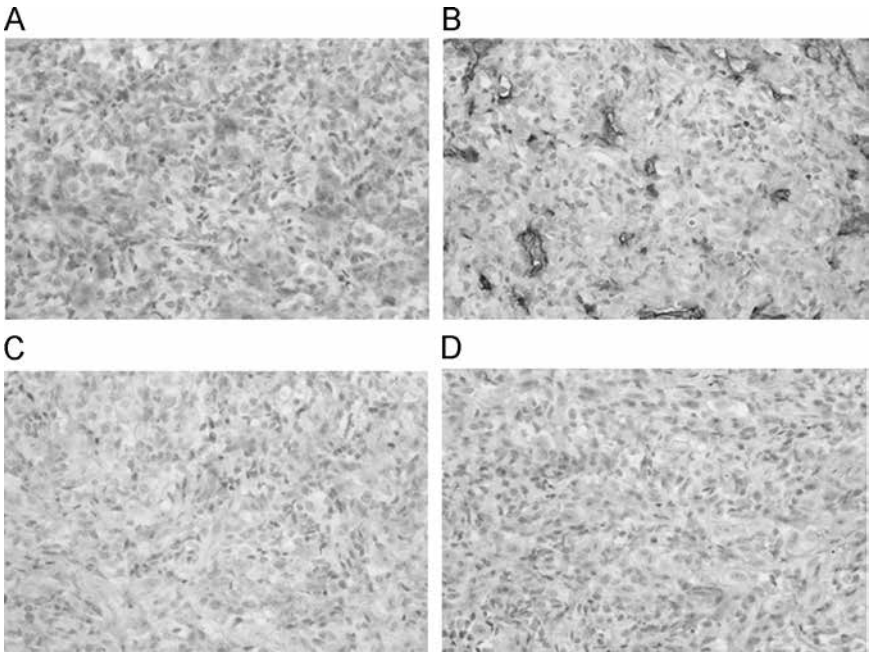


**Figure 4** Immunohistochemical analyses of the biopsy specimen revealed that the cells were positive for (A) calretinin and (B) D2-40 and negative for (C) CEA and (D) TTF-1.

**Table 1** Literature review of reported cases of lymphohistiocytoid mesothelioma.

| Author | Year | Age/ sex | Asbestos exposure | Surgery | Radiotherapy | Chemotherapy | Pleurodesis | Survival (months) |
|---|---|---|---|---|---|---|---|---|
| Khalidi et al. [8] | 2000 | 60/M | Unlikely | − | + | − | − | 72 |
| Khalidi et al. [8] | 2000 | 74/F | Unknown | N.D. | N.D. | N.D. | N.D. | 10 |
| Khalidi et al. [8] | 2000 | 67/M | Unlikely | N.D. | N.D. | N.D. | N.D. | 3 |
| Gallateau S et al. [6] | 2007 | 56/F | Probably | − | + | − | + | 12 |
| Gallateau S et al. [6] | 2007 | 78/M | Probably | − | − | − | + | 3 |
| Gallateau S et al. [6] | 2007 | 62/M | Possibly | Pleurectomy | + | − | − | 5 |
| Gallateau S et al. [6] | 2007 | 59/M | Probably | Pleurectomy | − | + | − | 4 |
| Gallateau S et al. [6] | 2007 | 78/M | Unlikely | Palliative surgery | + | − | + | 8 |
| Gallateau S et al. [6] | 2007 | 71/M | Unlikely | Palliative surgery | + | − | + | 40 |
| Gallateau S et al. [6] | 2007 | 80/F | Probably | − | + | − | + | 8 |
| Gallateau S et al. [6] | 2007 | 66/M | Probably | − | + | + | + | 11 |
| Gallateau S et al. [6] | 2007 | 78/F | Unlikely | Palliative surgery | − | − | − | 8 |
| Gallateau S et al. [6] | 2007 | 75/F | Unlikely | N.D. | N.D. | N.D. | N.D. | 11 |
| Gallateau S et al. [6] | 2007 | 77/F | Unlikely | − | + | − | + | 4 |
| Gallateau S et al. [6] | 2007 | 65/M | Probably | − | − | − | − | 37 |
| Gallateau S et al. [6] | 2007 | 74/F | Unlikely | − | − | + | − | 25 |
| Gallateau S et al. [6] | 2007 | 67/M | Possibly | Pleurectomy | − | − | − | 32 |
| Gallateau S et al. [6] | 2007 | 58/M | Probably | Palliative surgery | + | + | − | 7 |
| Gallateau S et al. [6] | 2007 | 82/M | Unknown | − | − | + | − | 8 |
| Gallateau S et al. [6] | 2007 | 76/M | Probably | − | + | − | + | 11 |
| Gallateau S et al. [6] | 2007 | 68/M | Probably | − | − | + | − | 20 |
| Gallateau S et al. [6] | 2007 | 70/M | Probably | − | − | + | − | 11 |
| Gallateau S et al. [6] | 2007 | 77/F | Probably | − | − | − | + | 6 |
| Gallateau S et al. [6] | 2007 | 50/M | Possibly | − | − | − | + | |
| Gallateau S et al. [6] | 2007 | 63/F | Probably | Palliative surgery | − | + | + | 8 |
| Kawai et al. [4] | 2010 | 59/F | Unknown | Thoracotomy | N.D. | N.D. | N.D. | 12 |
| Kawai et al. [4] | 2010 | 52/M | Unknown | Thoracotomy | N.D. | N.D. | N.D. | 36 |
| Kawai et al. [4] | 2010 | 61/M | Unknown | − | N.D. | N.D. | N.D. | 3 |
| Kawai et al. [4] | 2010 | 63/M | Probably | Extrapleural pneumonectomy | N.D. | N.D. | N.D. | 19(alive) |
| Present case | 2015 | 72/M | Probably | − | − | + | − | 14(alive) |

N.D.; not described.

et al. suggested that the survival of LHM was more similar to that of the epithelioid or biphasic subtype of MPM [6]. Whether LHM should be categorized as an epithelioid or sarcomatous subtype of MPM is still controversial.

Concerning chemotherapy for MPM, the combination of pemetrexed and cisplatin has shown significantly prolonged survival compared with cisplatin alone in a phase III study [7]. However, it was not clear whether or not LHM cases were included in the study. In fact, there are few reports of chemotherapy for LHM. We reviewed the reported cases of LHM particularly concerning the delivered treatment modalities in Table 1. These previous reports mainly discussed the pathological differentiation and there were few description of treatment. There were 8 cases those were delivered systemic chemotherapy, but the treatment regimen or the tumor responses to those chemotherapy was not described. To our knowledge, the current case might be the first case with a significant response to the treatment regimen. Cisplatin plus pemetrexed could therefore be a good treatment option for LHM.

In conclusion, we present a case of LHM, a rare variant of MPM, in a patient with a significant response to systemic chemotherapy.

## Conflict of interest statement

All authors have no conflict of interest to be declared.

## Acknowledgments

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# Endobronchial T-cell lymphoma in a patient with chronic pyothorax

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## Abstract

We report a very rare case of primary endobronchial peripheral T-cell lymphoma (PTCL) not otherwise specified (NOS), which presented as an endobronchial tumor obstructing the main airway. An 81-year-old man was referred to our hospital for a 1-month history of productive cough and wheeze. Computed tomography revealed chronic pyothorax with calcified foci in the right lung and a mass inside the bronchus intermedius. Flexible bronchoscopy identified an endobronchial tumor obstructing the bronchus intermedius. The biopsy specimen showed an infiltration composed predominantly of small atypical lymphocytes. Immunohistochemical analyses demonstrated that the proliferating cells were positive for CD3, CD4, and CD5 and negative for CD8 and CD20. Pathological tests confirmed that the case was PTCL-NOS. PTCL-NOS should be considered in the differential diagnosis of endobronchial tumors.

## Introduction

Peripheral T-cell lymphoma (PTCL) is one of the subtypes of T-cell lymphoma (TCL); it comprises a heterogeneous group of nodal and extranodal mature TCLs. Among the PTCLs, a subset is specifically defined as PTCL not otherwise specified (PTCL-NOS). PTCL-NOS do not correspond to any of the known T-cell entities. Primary pulmonary lymphoma (PPL) is a distinct entity that arises de novo in lung tissue [1]. We report an extremely rare case of primary endobronchial PTCL-NOS that presented as a tumor obstructing the main airway.

## Case Report

An 81-year-old Japanese man was referred to our hospital for a 1-month history of productive cough and wheeze. He was a smoker (approximately 56 packs per year) and had a medical history of pulmonary tuberculosis at the age of 17 years. He had been diagnosed with chronic pyothorax and treated with intravenous injection of antibiotics 1 year prior to being seen at our facility. Upon examination, his body temperature was 37.2°C and a physical examination revealed wheeze and reduced air entry in the right mid-lung. No superficial lymph nodes were palpated. Blood assays revealed leukocytosis (12,500 leukocytes per microliter) and normal lactate dehydrogenase values (223 IU/L). Carcinoembryonic antigen and soluble interleukin-2 receptor values were slightly elevated to 5.8 ng/mL and 575 U/mL, respectively. A chest X-ray showed hypolucency of the right middle lung field, which suggested fluid accumulation. Computed tomography (CT) revealed chronic pyothorax with calcified foci in the right-hand portion of the right lung and a mass inside the bronchus intermedius (Fig. 1A). No mediastinal lymphadenopathy was detected. Flexible bronchoscopy

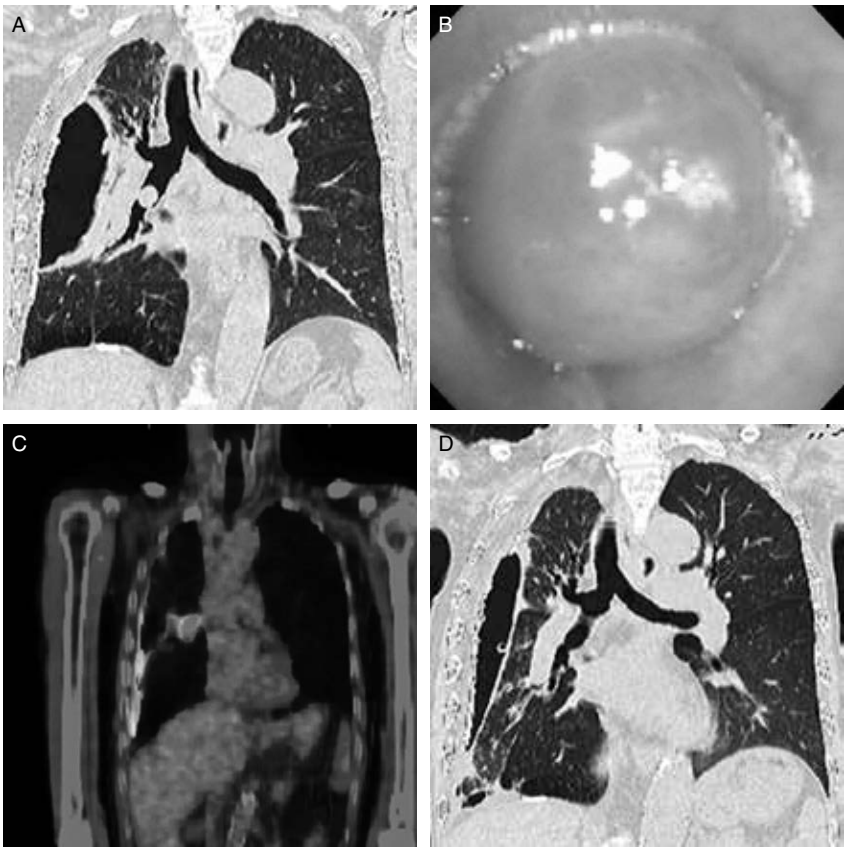


**Figure 1.** Imaging findings of the case. (A) Computed tomography (CT) revealed chronic pyothorax with calcified foci on the right and a mass inside the bronchus intermedius. (B) Flexible bronchoscopy identified an endobronchial tumor obstructing the bronchus intermedius. (C) Positron emission tomography with [18F] fluoro-2-deoxyglucose and CT revealed uptake at the endobronchial tumor. (D) CT after the chemotherapy demonstrated that the endobronchial tumor markedly diminished.

identified an endobronchial tumor obstructing the bronchus intermedius (Fig. 1B). The biopsy specimen showed an infiltration composed predominantly of small atypical lymphocytes (Fig. 2A). Immunohistochemical analyses demonstrated that the proliferating cells were positive for CD3 (Fig. 2B), CD4, CD5 (Fig. 2C), and CD7 and negative for CD8 (Fig. 2D), CD20 (Fig. 2E), and CD30. The Ki-67 labeling index was low. Based on these findings, we diagnosed the tumor as PTCL-NOS. Positron emission tomography (PET) with [18F] fluoro-2-deoxyglucose and CT revealed uptake at the endobronchial tumor, with a maximum standardized uptake value of 20.6 (Fig. 1C). There was no evidence of abnormal uptake at the pyothorax, mediastinal lymph node, or other extrathoracic organs. His disease was considered stage IE, and the patient was treated with systemic chemotherapy (pirarubicin, cyclophosphamide, vincristine, and prednisone). After the first course of the chemotherapy, the endobronchial tumor markedly diminished on chest CT (Fig. 1D) and bronchoscopy. However, because of complication with refractory pyothorax, fenestration surgery was undertaken in a definitive fashion. Systemic chemotherapy was not delivered thereafter; however, the patient is free of recurrence 1 year after diagnosis.

## Discussion

PPL is defined as lymphomas that affect the lungs (parenchyma, bronchi, and/or trachea) with no evidence of extrapulmonary extension in 3-month follow-up [2]. It is a rare condition that comprises only 3–4% of extranodal non-Hodgkin lymphomas (NHL), less than 1% of all NHLs [2], and 0.5–1% of primary pulmonary malignancies [3]. The majority of the reported cases have been of the B-cell type, so-called mucosa-associated or bronchus-associated lymphoid tissue lymphomas [4]. The current patient presented with a solitary endobronchial tumor, which is an extremely rare manifestation of PPL. Solomonov et al. reported that in a series of 441 patients with newly diagnosed NHL over a 7-year period, eight patients presented

 

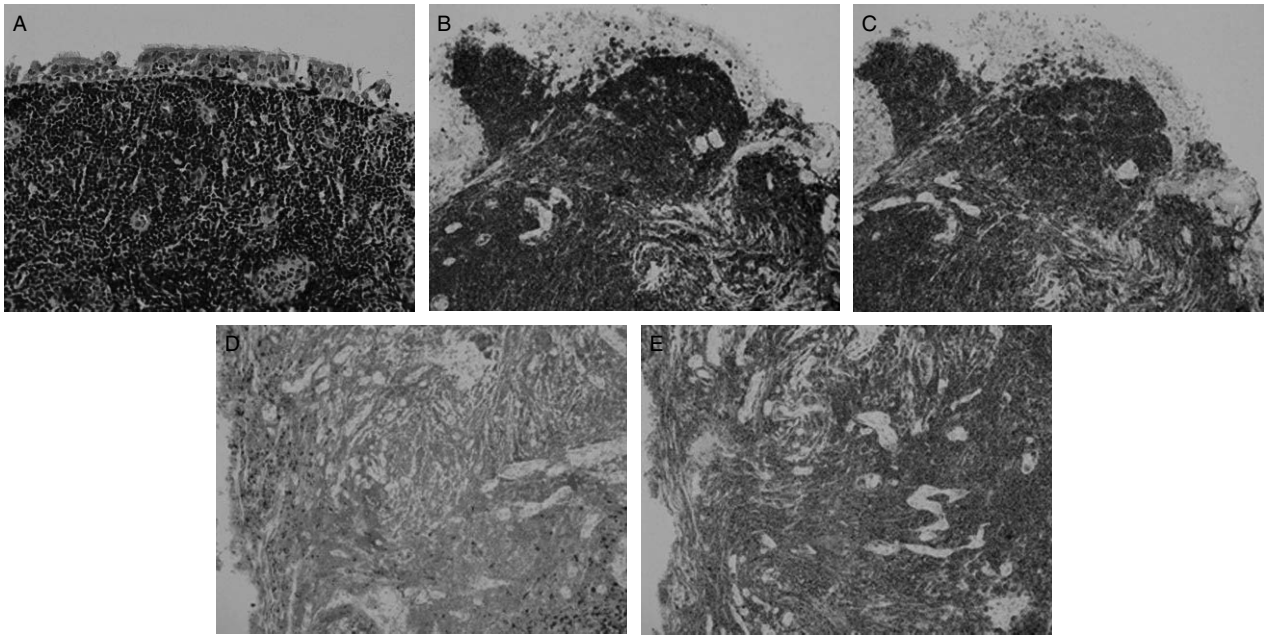


**Figure 2.** Biopsy specimen showed an infiltration comprised predominantly of small atypical lymphocytes (hematoxylin-eosin, 40×) (A). Immunohistochemical analyses demonstrated that the proliferating cells were positive for CD3 (B) and CD5 (C), and negative for CD8 (D) and CD20 (E).

with a primary endobronchial B-cell lymphoma [1]. To our knowledge, this is the first report of PTCL-NOS presenting as a primary endobronchial tumor. PTCL-NOS is a diagnosis made based on the results of a tissue biopsy that demonstrates evidence of a TCL that does not meet the criteria for other subtypes of TCL including anaplastic large-cell lymphoma, angioimmunoblastic TCL, extranodal NK/TCL, nasal type, subcutaneous panniculitis-like TCL, enteropathy-associated TCL, and hepatosplenic TCL. The differential diagnosis is based on histologic examination and immunophenotype evaluation such as immunohistochemical panel (CD20, CD3, CD10, BCL6, Ki-67, CD5, CD30, CD2, CD4, CD8, CD7, CD56, CD57, CD21, CD23, ALK) or cell surface marker analysis by flow cytometry (kappa/lambda, CD45, CD3, CD5, CD19, CD10, CD20, CD30, CD4, CD8, CD7, CD2), and in difficult or equivocal cases, polymerase chain reaction-based assay for molecular detection of a clonal T-cell receptor rearrangement. The limitation of the current case was that the pathological diagnosis was based on small specimen through flexible bronchoscopy, so not all these markers were examined. However, we are confident of the diagnosis based on multiple immunohistochemical analyses.

The current case was complicated by chronic pyothorax. Pyothorax-associated lymphoma is a category in which a lymphoproliferative disorder develops in the pleural cavity after a long-standing history of pyothorax; it represents an entity distinct from other malignant lymphomas [5]. In our case, PET/CT revealed a hypermetabolic mass at the endobronchial tumor, but there was no abnormal uptake at the pyothorax. Endoscopic examination showed a smooth-surfaced, round tumor with a stalk; these features do not correspond to an outer parietal invasion. However, the coronal CT showed dense scar-like tissue between the wall of pyothorax and bronchus intermedius. These findings might suggest the relationship between the lymphoma and pyothorax. We think that the chronic inflammation could have contributed to the development of the disease.

In conclusion, we have reported upon what is, to the best of our knowledge, the first case of PTCL-NOS, which was confined to the bronchus. PTCL-NOS should be considered in the differential diagnosis of endobronchial tumor.

## Disclosure Statements

No conflict of interest declared.

Appropriate written informed consent was obtained for publication of this case report and accompanying images.

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Case report

# Foreign body granuloma mimicking recurrence of malignant pleural mesothelioma

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ABSTRACT

A 72-year-old man visited our hospital due to right pleural effusion. He had worked as a welder at a shipbuilding company and had been exposed to asbestos. Cytological examination and thoracoscopic pleural biopsy yielded a diagnosis of epithelial malignant pleural mesothelioma (MPM); extrapleural pneumonectomy (EPP) was performed. Two years later, he became aware of right-back swelling that became a fist-sized mass over 2 months. Microscopy of a tissue specimen revealed no malignant cells, but did indicate foreign body granuloma. Subcutaneous lesions that develop after EPP do not necessarily result from the recurrence of MPM, but could have benign etiologies.



## 1. Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from the mesothelial cells lining the pleura and is generally associated with a history of asbestos exposure [1]. The clinical benefits of radical surgery for MPM remain controversial. Extrapleural pneumonectomy (EPP) has been performed for patients with earlier disease and good physical condition, but the disease often recurs at wide intervals. Here we report a case of foreign body granuloma (FBG) that mimicked a postoperative recurrence of MPM.

## 2. Case report

A 72-year-old man visited our hospital for examination of right pleural effusion. He had worked as a welder at a shipbuilding company and had been exposed to asbestos for 43 years. His pleural effusion had been detected at a medical checkup for subjects with an occupational history of past asbestos exposure. Cytological examination of the effusion revealed the aggregated atypical mesothelial cells with nuclear enlargement and nucleus irregularity. Immunohistochemical analyses demonstrated that these cells were positive for calretinin, CAM5.2, CK5/6, AE1/AE3, WT-1, and EMA, and negative for CEA and TTF-1. These findings gave the diagnosis of epithelial subtype of MPM, and thoracoscopic pleural biopsy confirmed the diagnosis. EPP was performed; the pericardium and the diaphragm were also removed because the tumor had invaded the diaphragm. The pericardium and the diaphragm were reconstructed with Gore-Tex® mesh (1 mm in thickness, 20 cm × 15 cm, and 2 mm in thickness, 24 cm × 15 cm, respectively). The disease was categorized as T2N0M0, stage II, based on the staging system of the International Mesothelioma Study Group [2]; adjuvant chemotherapy consisting of carboplatin and pemetrexed was delivered. Talc was not used to treat the effusion during the course.

Two years later, the patient became aware that his right back was swelling. This swelling became a fist-sized mass over 2 months. Computed tomography (CT) of the chest visualized a tumor of soft-tissue density that expanded from the right pleural cavity into the subcutaneous tissue (Fig. 1A). Fluorine-18 2-fluoro-2-deoxy-D-glucose (18F-FDG) positron emission tomography (PET)-CT

*Abbreviations:* MPM, malignant pleural mesothelioma; EPP, extrapleural pneumonectomy; FBG, foreign body granuloma; CT, computed tomography; 18-F-FDG, fuorine-18 2-fluoro-2-deoxy-D-glucose; PET, positron emission tomography.

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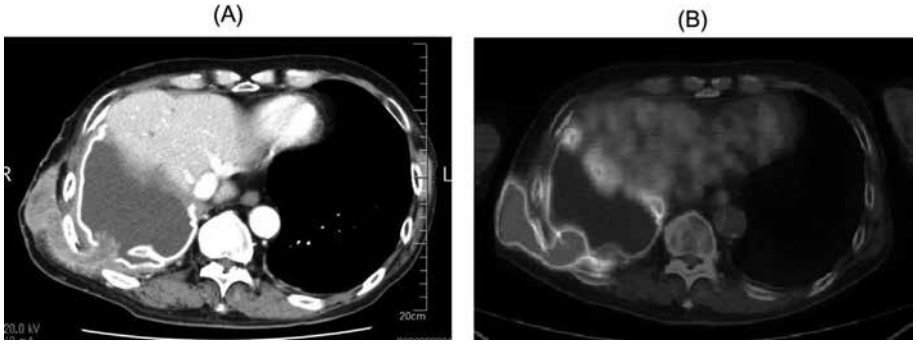


**Fig. 1.** Subcutaneous tumor on the right back of the patient. (A) CT of the chest revealed a tumor of soft-tissue density that expanded from the right pleural cavity into the subcutaneous tissue. (B) 18F-FDG PET-CT showed accumulation of 18F-FDG in the mass.

revealed an accumulation of 18F-FDG in the mass and along the pleura (Fig. 1B). It was near the chest tube site after the EPP. We suspected a recurrence of MPM, so we performed percutaneous needle biopsy. Microscopy of the tissue specimen showed no malignant cells; rather, we observed granulomas accompanied by giant cells, histiocytes, and inflammatory cells (Fig. 2). Immuno histochemical analyses revealed that these cells were negative for calretinin, CK AE1/3, or CAM5.2. Because the possibility of recurrence of MPM could not be ruled out with small specimen of needle biopsy, a tumorectomy was carried out. The tumor involved the Gore-Tex® mesh, so the mesh was removed with the tumor. Subsequent microscopy confirmed the diagnosis of FBG without evidence of MPM recurrence.

## 3. Discussion

A standard treatment for MPM has not yet been established. Patients exhibiting earlier stages of this disease have undergone EPP. However, a significant proportion of patients experience local recurrence as the first site of disease recurrence [3]. When patients who have undergone EPP display subcutaneous tumors, it is logical to suspect a recurrence of MPM.

FBG is a tumor-like mass or nodule of granulation tissue, with actively growing fibroblasts and capillary buds, consisting of a collection of modified macrophages resembling epithelial cells, surrounded by a rim of mononuclear cells, chiefly lymphocytes, and sometimes a center of giant multinucleate cells. It is due to a chronic inflammatory process associated with infectious disease or invasion by a foreign body such as surgical materials or pieces of stone or wood from a trauma. In the current case, we suspected that the causative foreign material was the Gore-Tex® mesh that was used to reconstruct the patient's diaphragm. It is quite rare that Gore-Tex mesh induced inflammatory reactions, however, in the current case, the Gore-Tex mash was involved in the tumor, so we considered that the granuloma was originated in the mesh.

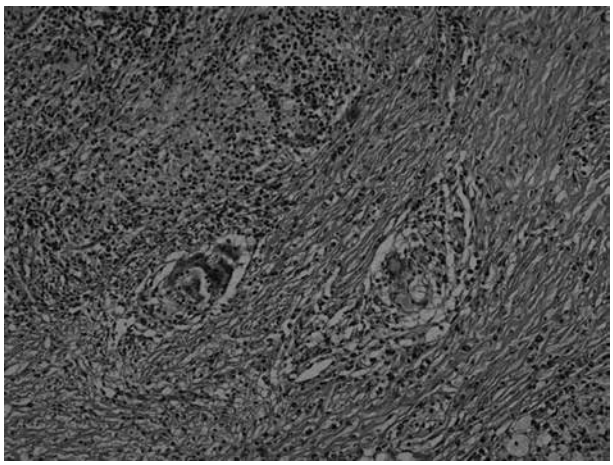

**Fig. 2.** Microscopy of the tissue specimen revealed a FBG accompanied by foreign-body giant cells, histiocytes, and inflammatory cells (×10).

Clinical diagnosis of FBG is challenging; it is difficult to identify FBG through physiological findings and CT. PET-CT is often applied to detect malignant lesions, but inflammatory lesions or granulomas (including FBG) would also accumulate 18F-FDG [4], rendering it difficult to diagnose FBG by imaging alone. The diagnosis should be confirmed through other means, such as percutaneous biopsy or surgery.

To our knowledge, this is the second report of FBG mimicking the recurrence of MPM; Shrestha et al. recently reported cases with pseudo-tumors that mimicked indwelling pleural catheter-tract metastases of MPM [5]. These cases remind physicians that subcutaneous lesions that develop after EPP do not necessarily result from the recurrence of MPM, but could have benign etiologies. Diagnostic procedures should be considered in such cases.

## Conflict of interest

I declare on behalf of my co-authors and myself that we do not have any conflict of interest to declare.

## Sources of funding

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Case report

# Lymphoproliferative disorder in pleural effusion in a subject with past asbestos exposure

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ABSTRACT

Primary effusion lymphoma (PEL) is a subtype of non-Hodgkin lymphoma that presents as serous effusions without detectable masses or organomegaly. Here we report a case of PEL-like lymphoma in a patient with past asbestos exposure. A 65-year-old man was referred to our hospital due to dyspnea upon exertion. He had been exposed to asbestos for three years in the construction industry. Chest X-ray and CT images demonstrated left pleural effusion. Cytological analysis of the pleural effusion revealed large atypical lymphocytes with distinct nuclear bodies and high nucleus-to-cytoplasm ratio. Immunohistochemical analyses showed that the cells were $CD20^{+}$, $CD3^{-}$, $CD5^{-}$, and $CD10^{-}$. These findings led to a diagnosis of diffuse large B-cell lymphoma. PEL or PEL-like lymphoma should be considered a potential cause of pleural effusion in subjects with past asbestos exposure.



## 1. Introduction

Primary effusion lymphoma (PEL) is a subtype of non-Hodgkin lymphoma that presents as serous effusions without detectable masses or organomegaly. PEL usually occurs in the setting of immunodeficiency and is associated with human herpesvirus (HHV)-8. Here we report a case of PEL-like lymphoma in a subject with a history of asbestos exposure.

## 2. Case report

A 65-year-old man was referred to our hospital due to dyspnea upon exertion. He was an occasional smoker and had been exposed to asbestos from 18 to 20 years old while employed in the construction industry, cutting asbestos-containing building material. Chest X-ray showed left pleural effusion (Fig. 1A), and computed tomographic (CT) images revealed left pleural effusion and calcified pleural plaques without pleural tumor (Fig. 1B). Thoracoscopic exploration revealed red–brown pleural effusion and plaques, but no tumor was detected on the pleura. Culture of the pleural effusion was negative, and cytological examination revealed a cellular distribution of 1.0% macrophages, 1.5% neutrophils, and 97.5% lymphocytes. Other examinations of the fluid showed nonspecific findings. Pleural biopsy showed no evidence of malignancy.

During follow-up, the patient repeatedly exhibited accumulation of left pleural effusion, which was treated each time with thoracentesis. Four years after the first admission, analysis of pleural effusion from the fourth thoracentesis revealed large atypical lymphocytes with distinct nuclear bodies and a high nucleus-to-cytoplasm ratio (Fig. 2). Malignant lymphoma was suspected and thoracoscopic pleural biopsy was performed again, and the biopsy specimen from the fibrin tissues attached to the parietal pleura showed several groups of small round atypical lymphocytes. All of acid-fast bacteria stain, PCR analysis of tuberculosis, and culture for acid fast bacillus were negative. A culture test for standard plate count bacteria was also negative. Immunohistochemical analyses revealed that these cells were $CD20^{+}$ (Fig. 3), $CD3^{-}$, $CD5^{-}$, and $CD10^{-}$. Based on these findings, the diagnosis was confirmed as diffuse large B-cell lymphoma (DLBCL). Contrast-enhanced CT imaging of whole body (neck to pelvis) revealed no lymphadenopathy or tumor. Serological tests for hepatitis-C virus, human T-cell lymphoma virus-1, and Epstein–Barr virus were negative, as was the

*Abbreviations:* PEL, primary effusion lymphoma; HHV, human herpesvirus; CT, computed tomography; DLBCL, diffuse large B-cell lymphoma.

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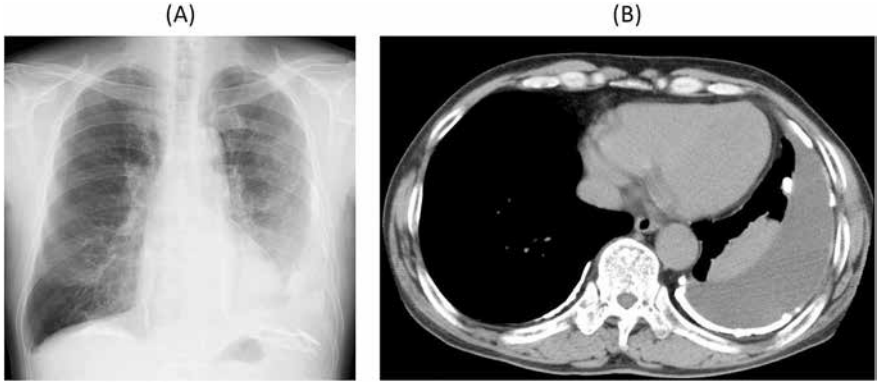


**Fig. 1.** Left pleural effusion and calcified pleural plaques visible on chest X-ray (A) and computed tomographic (B) images.

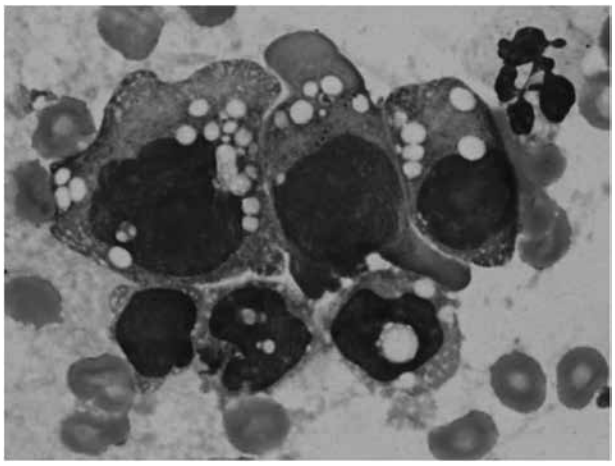

**Fig. 2.** Cytological analysis of pleural effusion revealed large atypical lymphocytes with distinct nuclear body and high nucleus-to-cytoplasm ratio (×40).

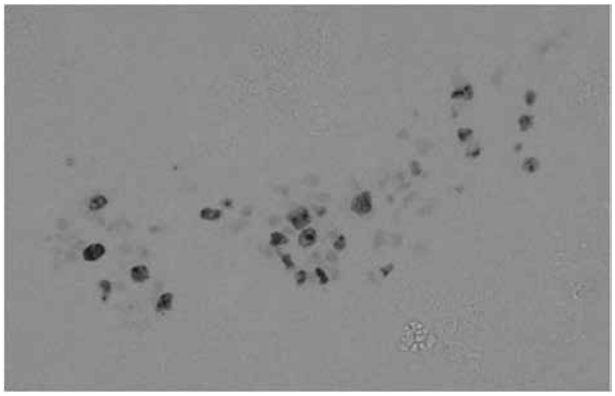

**Fig. 3.** Immunohistochemical analyses revealed that the cells were $CD20^{+}$ (×10).

immunohistochemical analysis for HHV8. The patient has been followed-up, and has shown no disease progression for 3 years.

## 3. Discussion

The 2001 World Health Organization classification defines PEL as a disease entity representing a part of diffuse large B-cell lymphoma [1]. PEL is commonly associated with HHV8 infection [2]; however, PEL-like disease processes have recently been reported in HHV8-negative patients. Those cases are considered a rare disease entity called HHV8-unrelated PEL-like lymphoma [3]. Advanced age [4] remains the only known risk factor for PEL-like lymphoma, and the pathogenic mechanisms remain unclear. It has been reported that patients with HHV8-unrelated PEL-like lymphoma show better outcomes than patients with PEL [5].

In our present case, the patient was elderly and had a past occupational history of asbestos exposure, but showed no immunological deterioration. To our knowledge, this is the first report of PEL-like lymphoma in a subject with past asbestos exposure. While no association has been established between lymphoproliferative disorder and asbestos exposure, it is possible that asbestos-induced chronic inflammation, in addition to advanced age, may have triggered lymphoproliferative disorder in the current case, though we cannot deny the possibility that the disease developed independently, considering relative short term of asbestos exposure.

In conclusion, here we report a rare manifestation of PEL-like lymphoma from a subject with past asbestos exposure. Subjects with past asbestos exposure sometimes develop pleural effusion, and suspected diagnoses include malignant pleural mesothelioma, lung cancer, benign asbestos pleural effusion, or tuberculous pleuritis. Our present case suggests that PEL or PEL-like lymphoma should also be considered as a potential cause of pleural effusion in subjects with past asbestos exposure.

## Conflict of interest

I declare on behalf of my co-authors and myself that we do not have any conflict of interest to declare.

## Source of funding

This study was supported by Research and Development and the Dissemination of Projects Related to the Nine Fields of Occupational Injuries and Illnesses of the Japan Labour Health and Welfare Organization. This work is also supported by grants-in-aid from the Ministry of Health, Labor and Welfare (grant number: 150401-02), Japan. These study sponsors had no involvement in study design, writing of the manuscript, the collection of data, and decision to submit the manuscript.

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*Case Report*

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# Angiosarcoma of the thoracic wall responded well to nanoparticle albumin-bound paclitaxel: A case report

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**Summary** **An 81-year-old woman visited a local clinic due to chest pain and a skin induration on the right precordia. She had a history of right breast cancer, and she had undergone a mastectomy and radiation therapy 10 years prior. Computed tomography (CT) imaging of the chest demonstrated a lobular mass that involved the right anterior thoracic wall and partially extruded from the thoracic cavity into the subcutaneous tissue. The tumor was surgically excised, and pathological analyses yielded a diagnosis of angiosarcoma. Five months after the operation, CT imaging showed multiple masses on the right pleura, indicating a local relapse and pleural dissemination of the angiosarcoma. Systemic chemotherapy composed of nanoparticle albumin-bound paclitaxel (nab-PTX) (80 mg/m$^2$) was delivered weekly. After 4 courses of chemotherapy, the tumors regressed remarkably. Nab-PTX may be an effective treatment option for recurrent or metastatic angiosarcoma.**

***Keywords:*** Angiosarcoma, paclitaxel, chemotherapy

## 1. Introduction

Angiosarcoma is an extremely rare malignant vessel tumor that comprises 1% of all soft tissue sarcomas (*1*). It develops in subcutaneous tissue at many sites in the body, and a previous medical history of trauma, breast cancer, and/or radiotherapy are considered risk factors for the disease. Localized tumors are treated with surgical removal. However, for recurrent and unresectable conditions, there is limited evidence to support chemotherapy regimens. Here, we describe a patient with angiosarcoma that developed in the thoracic wall, which responded well to systemic chemotherapy composed of nanoparticle albumin-bound paclitaxel (nab-PTX).

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## 2. Case report

An 81-year-old woman was referred to our hospital for an examination due to right chest pain. She had a history of right breast cancer and had undergone a mastectomy and adjuvant radiotherapy 10 years prior. Upon examination, a skin induration with tenderness was found on the right precordia. Computed tomography (CT) imaging of the chest demonstrated right pleural effusion and a lobular mass that involved the right anterior thoracic wall; this mass had partially extruded from the thoracic cavity into the subcutaneous tissue (Figure 1A). On enhanced CT images, the mass showed a contrast effect in the early stages of the arterial phase. The tumor was surgically excised. Pathological analyses of the tumor showed disarrayed growth of hyperchromatic and vasoformative mesenchymal tumor cells with abnormal mitosis (Figure 2A). Immunohistochemical analyses revealed that the cells were positive for CD31 (Figure 2B) and CD34 (Figure 2C), but negative for epithelial markers, S-100 (Figure 2D) and D2-40 (Figure 2E). Based on these findings, the diagnosis was confirmed as angiosarcoma. Five months after the operation, CT images showed

multiple masses on the right pleura, indicating a local relapse and pleural dissemination of the angiosarcoma (Figure 1B). Systemic chemotherapy composed of nab-PTX (80 mg/m$^2$) was delivered weekly. After 4 courses of chemotherapy, the masses in the pleura regressed remarkably (Figure 1C). The only adverse event was alopecia, no myelosuppression or neurotoxicity was observed. After a total of 14 courses of chemotherapy, multiple tumors reappeared, and the patient died at 18 months after the initial diagnosis. Autopsy was not allowed.

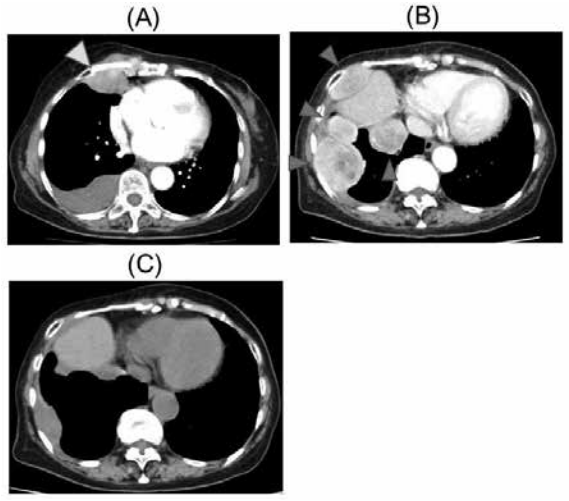


**Figure 1. Computed tomography images of the chest. (A)** Right pleural effusion and a lobular mass (white arrowhead) were observed at the initial examination. **(B)** Multiple masses on the right pleura (red arrowheads) appeared 5 months after the operation. **(C)** Regressed masses on the pleura after 4 courses of chemotherapy.

## 3. Discussion

Angiosarcoma is an uncommon malignant vessel tumor. Angiosarcoma can develop in the subcutaneous tissue in almost all parts of the body, but the most common sites are the head and neck, followed by the breast and liver (*2*). Angiosarcoma of the pleura is extremely rare (*3*). A history of breast cancer and radiation therapy are known risk factors for this disease (*4,5*), and both these factors were present in the current case study. There is limited evidence to support chemotherapy regimens for unresectable and recurrent angiosarcomas; however, a few reports have suggested that anthracyclines, ifosfamide, and taxanes are potential treatment options. A retrospective study showed that, when paclitaxel was used to treat unresectable angiosarcomas, progression-free survival was achieved for 6.8 months for scalp angiosarcoma and 2.8 months for sites below the clavicle (*6*). Nab-PTX is a novel, soluble, polyoxyethylated, castor oil-free, biologically interactive form of paclitaxel, which allows shorter infusion times and requires no premedication for hypersensitive reactions. Nab-PTX has been approved for breast cancer (*7*), non-small cell lung cancer (*8*), and gastric cancer (*9*) in Japan. Moreover, in the future, it will be used in more patients as an alternative to PTX. In the current case, nab-PTX was delivered to an aged patient with recurrent angiosarcoma that had disseminated in the pleura. This

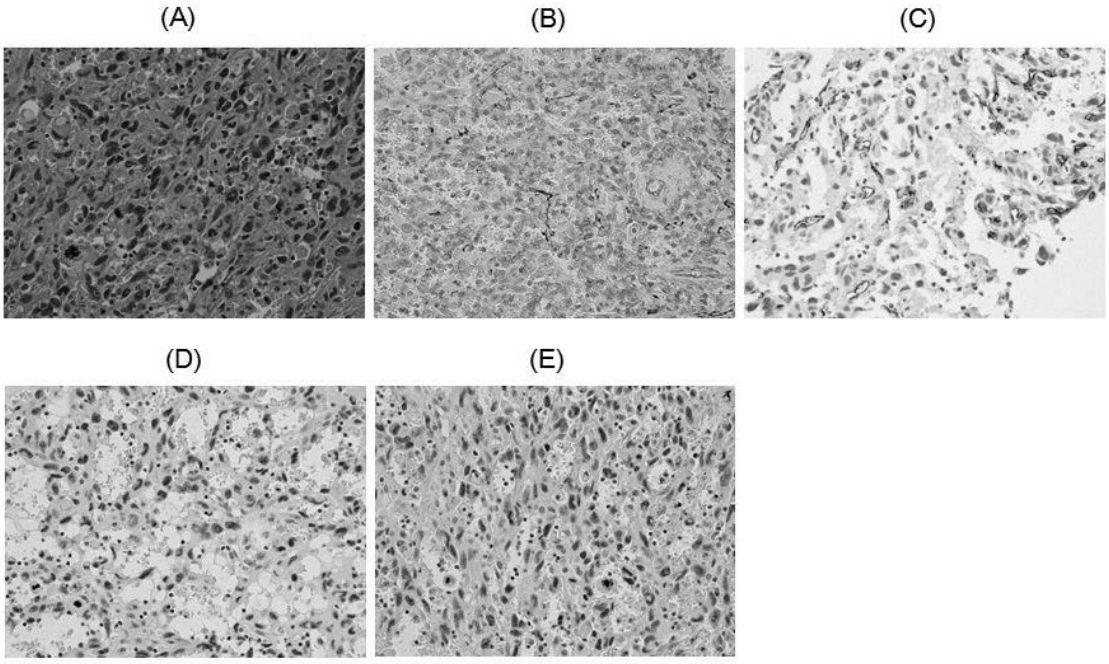


**Figure 2. Pathological analyses. (A)** Resected tumor specimen showed disarrayed growth of hyperchromatic and vasoformative mesenchymal tumor cells with abnormal mitosis (×40). Immunohistochemical analyses revealed that the cells were positive for CD31 **(B)** and CD34 **(C)**, but negative for epithelial markers, S-100 **(D)** and D2-40 **(E)** (×40).

treatment elicited a favorable response and few adverse events, though the tumor acquired resistance eventually. To our knowledge, the current case was the first to show that angiosarcoma significantly responded to nab-PTX. Our results suggested that weekly administration of nab-PTX may be an effective treatment option for recurrent angiosarcoma.

In conclusion, we described a case of angiosarcoma in the pleura, which showed a significant response to nab-PTX.

**Acknowledgements**

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# Significant Relationship Between the Extent of Pleural Plaques and Pulmonary Asbestos Body Concentration in Lung Cancer Patients With Occupational Asbestos Exposure

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**Background** *The aim of this study was to elucidate whether there is a relationship between the extent of pleural plaques and pulmonary asbestos body concentration (PABC).*

**Methods** *The subjects were 207 lung cancer patients with occupational asbestos exposure. We determined the plaque extent by findings on chest images using our own criteria. PABCs were measured in resected or autopsy lung specimens.*

**Results** *There was a significant relationship between plaque extent and PABC. Seventy-five percent of the patients determined to have extensive plaques based on our criteria had a PABC of ≥5,000 asbestos bodies per gram of dry lung tissue, which is one of the certification criteria of lung cancer caused by asbestos for workers' compensation in Japan.*

**Conclusions** *In lung cancer patients, the plaque extent had a significant positive relationship with the PABC. The plaque extent would be useful as a proxy for PABC for lung cancer compensation purposes.* Am. J. Ind. Med. 58:444–455, 2015.


***KEY WORDS: pleural plaques; asbestos bodies; asbestos exposure; chest images; workers'compensation***

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## INTRODUCTION

In Japan, lung cancer patients can receive compensation under workers' compensation if the lung cancer can be attributed to occupational asbestos exposure. If people develop lung cancer attributable to asbestos exposure but are not eligible for workers' compensation, they can receive another benefit under the Act on Asbestos Health Damage Relief [Morinaga et al., 2011]. In these compensation and relief systems, lung cancer is deemed to have been caused by asbestos if the patient has a cumulative asbestos exposure that increases the risk of lung cancer twofold or more. A cumulative exposure of 25 fiber-years is estimated to increase the risk of lung cancer twofold [Consensus report, 1997]. However, asbestos fiber levels had not been thoroughly assessed in working environments for various occupations in Japan. Therefore, it is difficult to estimate cumulative asbestos exposure by "fiber-years".

When an individual is exposed to asbestos, asbestos fibers are inhaled through the respiratory tract and deposited in the lungs. A portion of these asbestos fibers are covered with iron-containing protein and forms asbestos bodies. Asbestos bodies can be observed as ferruginous bodies under a light microscope. Pulmonary asbestos body concentration (PABC; the number of asbestos bodies per gram of dry lung tissue) can be measured in lung tissue from a resected specimen or autopsy specimen. The ease of pulmonary asbestos body formation has been reported to differ by the type of asbestos and the size of asbestos fibers. Asbestos body formation occurs more frequently on amphibole fibers than chrysotile fibers [Pooley et al., 1972; Morgan et al., 1985]. Thus, PABC is potentially a useful indicator of cumulative exposure level of asbestos, particularly of amphibole fibers.

In the Japanese compensation and relief systems, PABC has been used as one of the eligible criteria for lung cancer caused by asbestos. People are deemed to have lung cancer caused by asbestos and are eligible for compensation and relief benefits if they have a PABC of $\geq$5,000 asbestos bodies per gram of dry lung tissue [Kishimoto et al., 2010]. Our research group has been measuring PABC to evaluate the cumulative exposure levels in lung cancer patients. Before measuring PABC, we select the patients who have an occupational history of possible asbestos exposure. However, PABC can be measured only in patients from whom lung tissue is obtained at surgery or autopsy. In addition, PABC measurement requires special technical skills, and consequently such measurements can be performed in few medical facilities. If the cumulative exposure levels could be estimated using medical findings other than in lung tissue specimens, this would contribute to the certification steps in the compensation and relief systems for lung cancer caused by asbestos.

Pleural plaques are changes in the parietal pleura that manifest as localized thickening. Asbestos is thought to be the only cause of pleural plaques among people living in Japan. That is, if people living in Japan have pleural plaques, they must have been exposed to asbestos in the past. A relationship has not been elucidated between the extent (the level of spread and thickening) of pleural plaques and factors related to asbestos exposure. There are some reports that suggest a relationship between the plaque extent and cumulative asbestos exposure [Kishimoto et al., 1989; Bianchi et al., 1999; Paris et al., 2009].

The aim of this study was to elucidate whether or not there is a relationship between the extent of pleural plaques and PABC.

## METHODS

### Subjects

Patients were selected as subjects as follows. The patients were treated at one of seven medical institutions located in the northern, central, and western regions of Japan, with which the authors are affiliated. The subjects were selected from consecutive patients who had undergone surgery or autopsy between 2003 and 2012 at the institutions (the time period differed by institution) and who were diagnosed histologically with primary lung cancer. The steps described below were used to select patients who had occupational asbestos exposure from the sample patients who had satisfied the aforementioned conditions.

(1) An occupational history was elicited from all patients admitted to any of the seven medical institutions. They were requested to answer questions on a self-administered questionnaire, which was the same for all seven institutions and included questions about their occupational history and residential history. (2) The primary physicians selected patients with an occupational history of possible asbestos exposure based on the answers to the questionnaire. An occupation with potential asbestos exposure was defined as one of the occupations on the list of occupations with asbestos exposure [Takahashi et al., 1993]. (3) The primary physician or one of the authors of this study examined the following items by interviewing the patients with potential occupational asbestos exposure: whether or not they handled asbestos in their work or worked near areas where asbestos was handled, duration of asbestos exposure (years), and time since first asbestos exposure (years). (4) One author of this study selected patients who had definite or probable occupational asbestos exposure based on the survey records. The probable cases were patients in whom the presence or absence of asbestos exposure was not clear based on patient statements. However, the patients were assumed to have had asbestos exposure based on the job descriptions. (5) The aforementioned patient selection process was performed regardless of the presence or absence of pleural plaques and regardless of whether or not PABCs were measured.

The patients were narrowed down to 215 after the above process. Among these patients, 207 had chest x-rays (CXR) and computed tomography (CT) images that were taken within 3 months of surgery or autopsy and that could withstand evaluation. These 207 patients were the subjects of this study. In seven of these patients, there was insufficient record of the time since first asbestos exposure and duration of asbestos exposure. Thus, such information was unknown.

## Image Evaluation and Diagnostic Criteria for Pleural Plaques

Evaluations of imaging findings were made by two teams of respiratory specialists. They were chest physicians, including a chest radiologist and a chest surgeon, with expertise in asbestos-related diseases (T.Y., F.S., T.K., K.O., I.U., T.M., and K.M.). Each team consisted of two or three of the specialists. Two teams independently evaluated the imaging findings. If two teams disagreed on their decision, the final decision was made by consensus.

Our diagnostic criteria for pleural plaques based on a frontal view CXR were either of the following conditions. (1) Linear or patchy calcified opacities were observed bilaterally or unilaterally along the diaphragmatic dome, and there was no obliteration of the costophrenic angle. (2) There were bilateral asymmetric opacities derived from localized pleural thickening. These opacities occurred with or without calcification and were located on the inner lateral chest walls in the area of the sixth to tenth ribs. In addition, there was no obliteration of the costophrenic angle. It should be noted that there may be pleural thickening-like findings in the inner lateral chest wall which include opacities from thoracic muscles, subpleural adipose tissue, and ossification of old fracture of the rib. Thus, such opacities need to be differentiated from localized pleural thickening. Also, since obliteration of the costophrenic angle could have been a sequela of pleural diseases such as pleurisy, pyothorax, and diffuse pleural thickening, a diagnosis of the absence or presence of pleural plaque was not made on the side with obliteration of the costophrenic angle. In diagnosis of diffuse pleural thickening on CXR, we used the criteria described in International Labour Office 2000 Guidelines [International Labour Office, Geneva, 2000].

Regarding CT, patients were diagnosed with pleural plaques if the CT findings indicated localized thickening of the parietal pleura and if all of (1), (2), (3), and (4) below were satisfied. (1) The CT attenuation value of the thickened area was the same or greater than that of the thoracic muscles. (2) In CT sections showing pleural thickening, the thickened area had spread like a sheet in a horizontal direction and the borders of the thickening were well defined. (3) The spread of the thickened area was observed in the craniocaudal direction in multiple consecutive sections. (4) Findings of pleural thickening were not due to pleurisy, pyothorax, hemothorax, or thoracotomy, nor arose from intercostal vessels, subpleural adipose tissue, or peripheral lung lesions. The aforementioned determination was made based on CT images using both lung window (window level: −600 to −700 Hounsfield units (HU) and window width: 1,000–2,000 HU) and mediastinal window (window level: 0–50 HU and window width: 300–500 HU) settings.

In this study, the subjects were selected from multiple institutions for a retrospective examination. Therefore, the CT imaging method was not standardized. The images used in this study were confirmed to be suitable for diagnosis and to have satisfied the conditions as follows. Imaging was performed in deep inspiration, and all areas of the diaphragm, rib cage, and lung fields were imaged. Images had 5–7 mm scan collimation and 5–7 mm scan intervals. Contrast-enhanced CT had been performed in some patients. High-resolution CT had not been performed in the majority of the patients.

## Classifications of Plaque Extent Based on Chest Imaging

In this study, we used the aforementioned diagnostic criteria and classified the extent of pleural plaques as described below. CXRs were used to classify patients into two groups: a group with pleural plaques (positive group) and a group without pleural plaques (negative group). As we have reported in our previous study, CT findings were used to semi-quantitatively classify patients by the plaque extent [Yusa et al., 2011].

- Class 0: There were no pleural plaques on CT.
- Class 1: Pleural plaques were present and their spread was as follows. A CT section was selected with the greatest extent of pleural plaques on either the left or right side. The plaques extended to less than one fourth of the inner chest wall.
- Class 2: The same conditions were used as in Class 1, except the plaque extent was one fourth or more of the inner chest wall.

Figure 1A shows the method of dividing the inner chest wall into quarters and Figure 1B, a representative case of Class 2.

Preliminary inspection showed that pleural plaques of the positive group by the CXR-based plaque classification were obviously more extensive in the level of spread and/or thickening than those of the negative group, when the plaques were examined on CT. In this study, we defined "extensive pleural plaques" as follows: pleural plaques of the positive group by the CXR-based plaque classification or Class 2 group by the CT-based plaque classification.

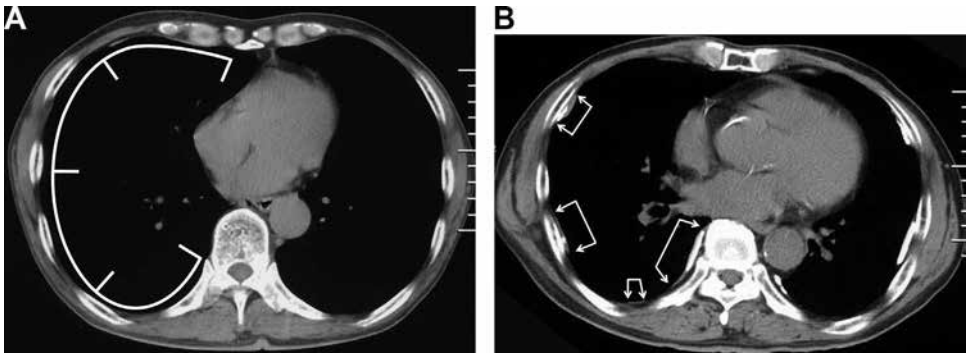


**FIGURE 1.** (**A**) Division into quarters of the inner chest wall on computed tomography (CT) image. A CT section was selected with the most extensive pleural plaque in the left or right side of the chest. The inner chest wall was divided into quarters. The extent of the inner chest wall line on the left or right side was established ventrally from the sternal border and dorsally to the origin of the ribs. If there were multiple plaques (including plaques observed in mediastinal pleura in the same section), the sum was calculated by adding the extent of each plaque. (**B**) Class 2 patient according to the computed tomography (CT)-based pleural plaque classification. The chest CT showed multiple pleural plaques with calcification. All CT sections were examined, and the section with the most extensive pleural plaques was selected. The total area of four pleural plaques (shown in the figure) was more than one fourth of the inner chest wall. Thus, the patient was determined to be Class 2.

## PABC Measurement

PABCs were measured in resected or autopsy lung specimens using the method of Smith and Naylor [1972] that was revised by Kohyama and Suzuki [1991]. Briefly, a peripheral lung specimen of 2–5 g was dried in a drying oven for 2–4 h at 110°C. The dry weight was measured, and then the dried lung tissue was digested in laboratory bleach (Clean 99-K200®: a combined solution of 30% sodium hypochlorite, 4% potassium hydroxide, and an anionic surface active agent) for 3–4 h at 60°C. The reaction solution was centrifuged. The supernatant was removed, and the pellet was washed with distilled water and centrifuged again. Distilled water was added to adjust the volume to 50 ml. A portion of this solution was filtered through a 25-mm cellulose membrane filter (pore size 0.45 μm), and the filter was dried. The filter was mounted on a glass microscope slide and cleared using acetone vapor, and a cover slip was placed over the filter. The slide specimen was magnified 400 times under a phase-contrast microscope, and the ferruginous bodies were counted. In our study, a ferruginous body was defined as a morphologically "typical" body, which had a yellow iron-protein coat and a central core, as described by Churg et al. [1977]. Most ferruginous bodies consist of asbestos fibers [Moulin et al., 1988]. Thus, we deemed all ferruginous bodies to be asbestos bodies. The number of ferruginous bodies per gram of dry lung tissue was calculated and reported as the PABC. The result of the PABC measurement was expressed as the number of asbestos bodies per gram of dry lung tissue (AB/g dry lung). PABCs were measured for all the subjects at our five medical institutions using the aforementioned method. Technicians performed the measurements following the same manual. They were thoroughly trained by the same instructors (N.K. and Y.S.).

## Statistical Analysis

The unpaired *t*-test was used as the significance test between two groups for variables with normal distributions. Multiple comparisons between three groups were performed using Tukey-Kramer's test, as a post hoc test for analysis of variance. A chi-square test was used to test for significance in frequency by gender between two groups. Initial inspection showed that the log-transformed values of PABC could be considered to have a normal distribution, because the values of skewness and kurtosis were approximately zero. Thus the log-transformed values of PABC ($\log_{10}$ PABC) were used for statistical analyses. Multiple logistic regression analysis was performed to examine the factors that predicted the extent of pleural plaques. The significance level was set at 0.05. Statistical calculations were performed using the statistical software JMP ver.11 (SAS Institute, NC).

## Ethics

This study was approved by the ethics committee of Tokyo Women's Medical University (approval number: 2081 approval date: January 30, 2011) with which the principal investigator is affiliated. In addition, approval was obtained by an ethics review of each medical institution from which patients' medical record data and images were obtained. This study was a retrospective study and used anonymized

**TABLE I.** Patient Characteristics (N = 207)

| | N |
|---|---|
| Gender | |
| Men | 204 |
| Women | 3 |
| Age (years) | |
| 40–49 | 1 |
| 50–59 | 12 |
| 60–69 | 70 |
| 70–79 | 88 |
| 80–89 | 34 |
| 90–99 | 2 |
| Site of materials for PABC measurements | |
| Right lung | |
| Upper lobe | 63 |
| Middle lobe | 17 |
| Lower lobe | 47 |
| Left lung | |
| Upper lobe | 43 |
| Lower lobe | 37 |

N, number of patients; PABC, pulmonary asbestos body concentration.

medical record data and images. Therefore, written consent was not obtained from some patients, including autopsy cases.

# RESULTS

## Patient Characteristics

The subjects were 207 patients, consisting of 204 men and 3 women. There were 159 surgical patients and 48 autopsy patients. There were 181 patients with definite asbestos exposure and 26 patients with probable exposure. The mean age at surgery or autopsy was 71.6 years (range: 41–91 years, standard deviation (SD): 7.9 years) (Table I).

In Table II, the number of patients is shown by occupation type. There were diverse occupation types. The majority of the patients had worked on construction sites or in manufacturing industries.

## Agreement Rates Among Observers

The two teams of respiratory specialists had substantial agreement rates on plaque classifications based on CXR and CT. In the CXR-based classification, the agreement rate was 89.6% with a kappa coefficient of 0.72 (95% confidence interval: 0.61–0.87). In the CT-based classification, the agreement rate was 85.8% with a kappa coefficient of 0.69 (95% confidence interval: 0.59–0.79).

## Image Findings and PABC

The patients were classified by the extent of pleural plaques. When patients were grouped based on their CXR findings, there were 167 patients who were negative for pleural plaques (negative group) and 40 patients who were positive (positive group). When they were grouped based on their CT findings, there were 76 patients in the Class 0 group, 61 patients in the Class 1 group, and 70 patients in the Class 2 group. No patient in any group had diffuse pleural thickening.

The PABCs were below the lower limit of measurement and were unmeasurable in 4 of 207 patients. The lower limit of PABC measurement ranged from 33 to 102 AB/g dry lung in these four patients. All four patients were in the negative group in the CXR-based classification and in the Class 0 group in the CT-based classification. In the preliminary examination, a value was substituted that was below the lower limit of measurement for these four patients to check if there would be a change in the statistical significance. The results of a statistical significance test by group did not show any change. Thus in the examination thereafter the lower limit value of measurement was used as the PABC value for each of these four patients.

**TABLE II.** Patients With Asbestos Exposure by Occupation Type

| Occupation type | N |
|---|---|
| Work at construction sites | 67 |
| Shipyard work | 34 |
| Work at iron and steel foundries | 19 |
| Work at oil refineries and chemical plants | 13 |
| Work involving asbestos product manufacturing | 11 |
| Work involving manufacturing and repair of cars and railway cars | 10 |
| Manufacturing and repair work of electronics or industrial machinery | 8 |
| Work involving operation of cars, trucks, or trains | 8 |
| Glass manufacturing work | 7 |
| Work related to insulation and furnace installation | 6 |
| Work in rooms, buildings, and warehouses with sprayed-on asbestos | 6 |
| Work in warehouses housing raw asbestos fiber or asbestos products | 5 |
| Work involving demolition/disassembly of buildings | 4 |
| Work involving brick and cement product manufacturing | 3 |
| Work at power plants or other electrical facilities | 2 |
| Work involving the use of heat-resistant clothing and gloves | 2 |
| Work involving garbage disposal facilities and waste collection | 2 |
| Total | 207 |

N, number of patients.

PABCs were compared by site from which lung tissue was obtained for measurement. The geometric mean of PABC was 3,741 AB/g dry lung and 4,207 AB/g dry lung in the right lung and left lung, respectively. When their $\log_{10}$ PABC values were compared, there was no significant difference (unpaired *t*-test; $P = 0.69$). The geometric mean of PABC was 3,802 AB/g dry lung and 3,990 AB/g dry lung in the upper/middle lobes and lower lobe respectively. There was no significant difference (unpaired t-test; $P = 0.87$). Based on these results, no correction was made to the PABC values for lung tissue site in the examination below.

Table III shows the number of patients by PABC level and using the CXR- and CT-based plaque classification. The patients with PABCs of $\geq$1,000 AB/g dry lung accounted for 71% of the total patients and those with PABCs of $\geq$5,000 AB/g dry lung accounted for 44%.

Figure 2 shows dots which represent the PABC value by patient in each group of the CXR-based plaque classification. The PABC of the positive group was significantly higher than that of the negative group (Table IV). In the negative group, there were 113 patients (68%) with PABC of $<$5,000 AB/g dry lung and 54 patients (32%) with PABC of $\geq$5,000 AB/g dry lung. In the positive group, there were 3 patients (8%) and 37 such patients (93%), respectively.

Figure 3 shows PABC by patient in each group of the CT-based plaque classification. The PABC of the Class 2 group was significantly higher than that of the Class 1 or 0 group, and the PABC of the Class 1 group was significantly higher than that of the Class 0 group (Table V). In the Class 0 group, there were 63 patients (83%) with PABC of $<$5,000 AB/g dry lung and 13 patients (17%) with PABC of $\geq$5,000 AB/g dry lung. The equivalent division was 34 patients (56%) and 27 patients (44%) in the Class 1 group, and 19 patients (27%) and 51 patients (73%) in the Class 2 group.

Eighty-one patients had "extensive pleural plaques" when such plaques were defined as being positive by CXR finding or Class 2 by CT finding. Of these patients, 61 (75%) had PABC of $\geq$5,000 AB/g dry lung. Of 126 patients other than those with "extensive pleural plaques", 30 (24%) had PABC of $\geq$5,000 AB/g dry lung. When patients with "extensive pleural plaques" were compared to patients with PABC of $\geq$5,000 AB/g dry lung, the positive predictive value and negative predictive value of the plaques as an indicator of the PABC of $\geq$5,000 AB/g dry lung were 0.75 and 0.77, respectively.

## Relationships Between Pleural Plaque Extent and Factors Related to Asbestos Exposure

Data on the duration of asbestos exposure and the time since first asbestos exposure were identified in 200 (men: 198, women: 2) of 207 patients. The mean duration of asbestos exposure was 28.0 years (SD: 12.8 years), and the mean time since first asbestos exposure was 47.7 years (SD: 9.8 years).

Table IV shows the following by CXR-based group: gender, age, $\log_{10}$ PABC, duration of asbestos exposure, and time since first asbestos exposure. It was determined that the variables of each group could be considered to have a normal distribution because the values of skewness and kurtosis were approximately zero. The unpaired *t*-test was used to compare these factors between the negative group and positive groups. These two groups showed a statistically significant difference in age, $\log_{10}$ PABC, and time since first asbestos exposure.

In Table V the same factors as in Table IV are shown by the CT-based classification of plaque extent. Multiple comparisons between the Class 0 group, Class 1 group, and Class 2 group were performed using Tukey-Kramer's test, as a post hoc test for analysis of variance. There was a significant difference in age between the Class 0 group and Class 2 group, in $\log_{10}$ PABC among the Class 0 group, Class 1 group, and Class 2 group, and in time since first asbestos exposure between the Class 0 group and Class 2 group and between the Class 1 group and Class 2 group.

Multiple logistic regression analysis was performed by CXR-based pleural plaque classification and by CT-based

**TABLE III.** Number of Patients According to PABC Level

| | | CXR-based plaque classification | | CT-based plaque classification | | |
|---|---|---|---|---|---|---|
| **PABC (AB/g dry lung)** | **Total** | **Negative group** | **Positive group** | **Class 0** | **Class 1** | **Class 2** |
| 0–999 | 59 | 59 | 0 | 40 | 14 | 5 |
| 1,000–4,999 | 57 | 54 | 3 | 23 | 20 | 14 |
| 5,000–49,999 | 65 | 40 | 25 | 11 | 21 | 33 |
| 50,000–499,999 | 23 | 13 | 10 | 2 | 4 | 17 |
| 500,000–999,999 | 3 | 1 | 2 | 0 | 2 | 1 |
| Total | 207 | 167 | 40 | 76 | 61 | 70 |

PABC, pulmonary asbestos body concentration; AB, asbestos bodies; CXR, chest X-ray; CT, computed tomography.

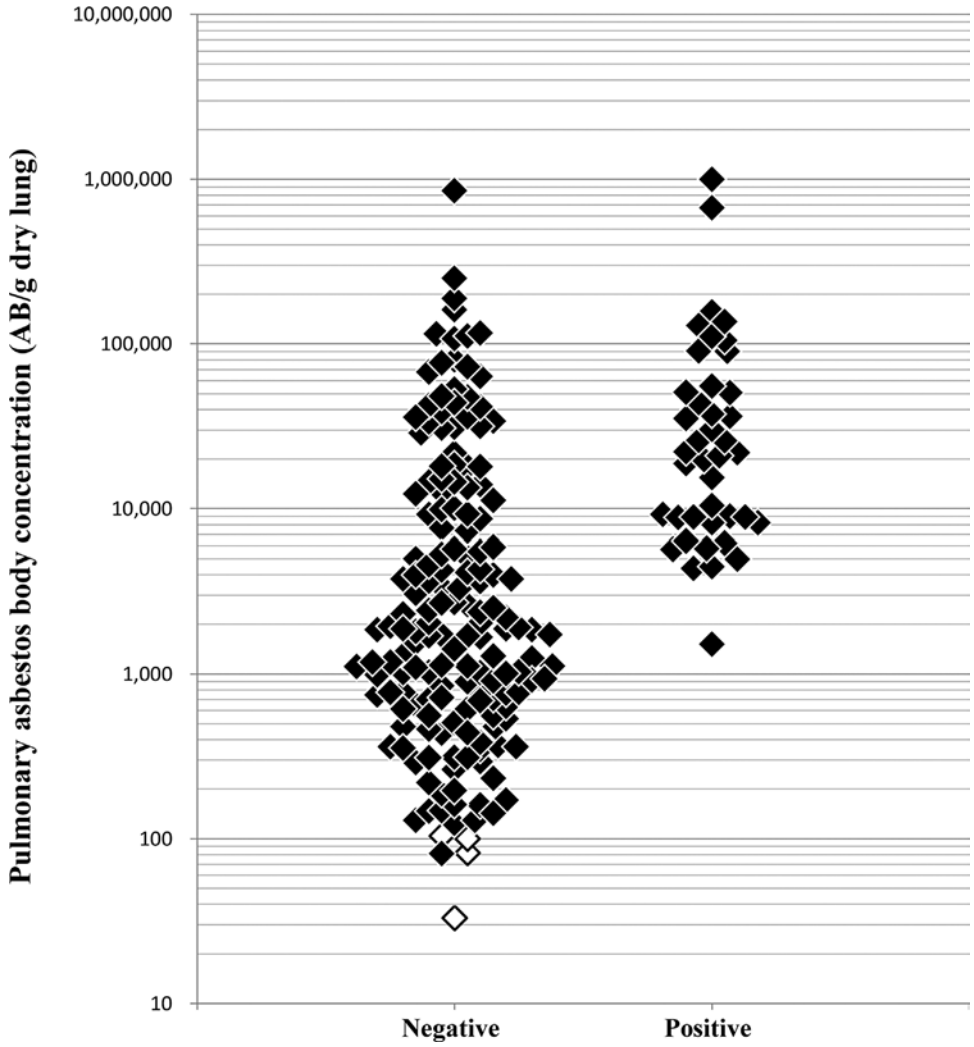


**FIGURE 2.** Pulmonary asbestos body concentration (PABC) by patient in each group of the chest x-ray (CXR)-based plaque classification. The patients were divided into the negative group and positive group based on the CXR-based plaque classification. A dot represents the PABC of each patient. The white dots are patients with PABC values that were below the lower limit of measurement. The geometric mean of PABC was 2,506 asbestos bodies (AB)/g dry lung in the negative group (n = 167) and 25,061 AB/g dry lung in the positive group (n = 40). There was a significant difference between these groups (Table IV).

pleural plaque classification to examine the factors that predict the extent of pleural plaques. Independent variables were age, $\log_{10}$ PABC, duration of asbestos exposure, and time since first asbestos exposure. There were only two women among 200. Thus, gender was excluded from the analysis. For the CXR-based plaque classification, the negative group and positive group were established as dependent variables. For the CT-based plaque classification, the Class 0 group, Class 1 group, and Class 2 group were established as dependent variables. Since there were three groups in the CT-based classification, multinomial logistic regression was used.

Table VI shows the analysis results. The $\log_{10}$ PABC value and time since first asbestos exposure were significant factors that predicted the presence of pleural plaques in the CXR-based classification. Only the $\log_{10}$ PABC value was a significant factor that predicted the extent of pleural plaques in the CT-based classification.

## DISCUSSION

In our study, we set up the CXR- and CT-based diagnostic criteria for pleural plaques. We used our own method to classify the extent of pleural plaques using CT images. Using these criteria, two teams of respiratory specialists with expertise in asbestos-related diseases reached substantial agreement on the presence and absence

**TABLE IV.** Gender, Age, and Factors Related to Asbestos Exposure by the CXR-based Plaque Classification (N = 207)

| | | | CXR-based plaque classification | | | | |
|---|---|---|---|---|---|---|---|
| | Total | | Negative group | | Positive group | | |
| | N | Mean (SD) | N | Mean (SD) | N | Mean (SD) | *P*-value[a] |
| Gender | | | | | | | |
| Men | 204 | | 165 | | 39 | | |
| Women | 3 | | 2 | | 1 | | 0.53 |
| Age (years) | 207 | 71.6 (7.9) | 167 | 71.0 (8.0) | 40 | 74.3 (6.7) | 0.018 |
| $Log_{10}$ PABC | 207 | 3.593[b] (0.906) | 167 | 3.399[c] (0.861) | 40 | 4.399[d] (0.600) | <0.0001 |
| Duration of asbestos exposure (years) | 200 | 28.0 (12.8) | 160 | 27.6 (12.8) | 40 | 29.7 (13.0) | 0.35 |
| Time since first asbestos exposure (years) | 200 | 47.7 (9.8) | 160 | 46.3 (9.8) | 40 | 53.3 (8.0) | <0.0001 |

CXR, chest X-ray; N, number of patients; PABC, pulmonary asbestos body concentration; SD, standard deviation.
[a]Comparison between the negative group and positive group.
[b,c,d]The values are equal to 3,957 asbestos bodies (AB)/g dry lung, 2,506 AB/g dry lung, and 25,061 AB/g dry lung, respectively.
The values were calculated by chi-square test for gender, unpaired t-test for other factors.

of plaques based on CXR and on the classification of plaque extent based on CT. Our CT-based plaque classification method is simpler than other reported classification methods and resulted in substantial agreement among respiratory specialists [Meirelles et al., 2006; Suganuma et al., 2009].

The results of this study showed a significant relationship between the extent of pleural plaques and PABC in lung cancer patients with a history of occupational asbestos exposure. In addition, the lung cancer patients with extensive plaques based on our imaging criteria (positive group in the CXR-based

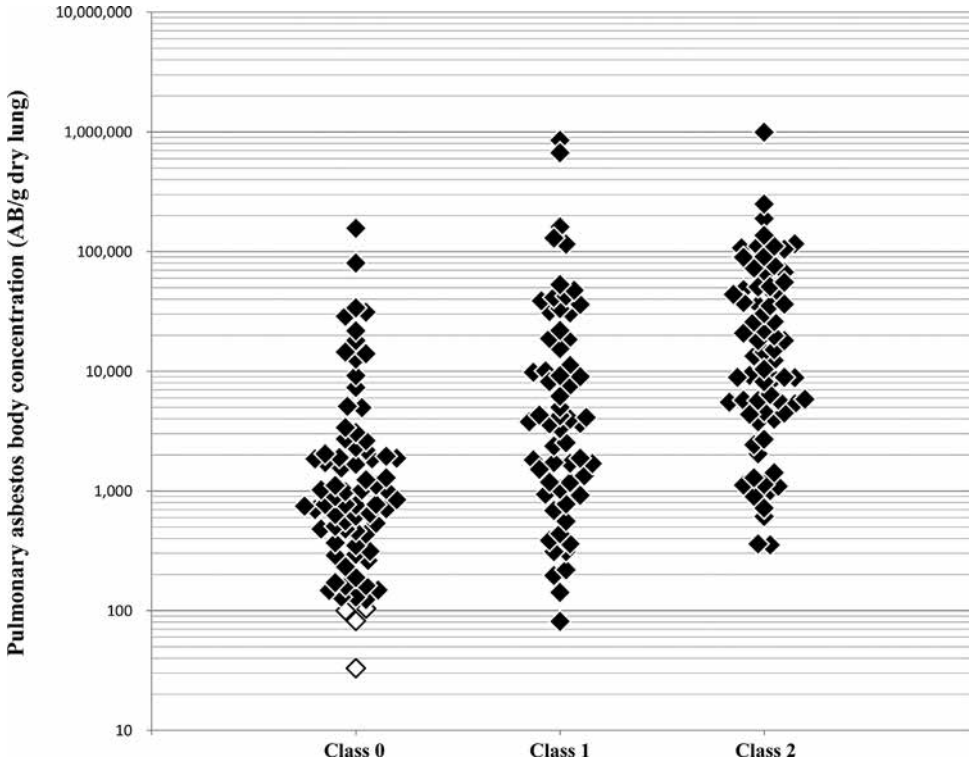


**FIGURE 3.** Pulmonary asbestos body concentration (PABC) by patient in each group of the computed tomography (CT)-based plaque classification. The patients were divided into the Class 0, 1, and 2 groups based on the CT-based plaque classification. A dot represents the PABC of each patient. The white dots are patients with PABC values that were below the lower limit of measurement. The geometric mean of PABC was 1,114 asbestos bodies (AB)/g dry lung in the Class 0 group (n = 70), 4,656 AB/g dry lung in the Class 1 group (n = 60), and 13,152 AB/g dry lung in the Class 2 group (n = 70). There were significant differences among these groups (Table V).

**TABLE V.** Gender, Age, and Factors Related to Asbestos Exposure by the CT-based Plaque Classification (N = 207)

| | CT-based plaque classification | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Class 0 | | Class 1 | | Class 2 | | | | |
| | N | Mean (SD) | N | Mean (SD) | N | Mean (SD) | *P*-value[a] | *P*-value[b] | *P*-value[c] |
| Gender | | | | | | | | | |
| Men | 75 | | 59 | | 70 | | ND | ND | ND |
| Women | 1 | | 2 | | 0 | | | | |
| Age (years) | 76 | 70.2 (7.6) | 61 | 71.3 (7.9) | 70 | 73.5 (7.9) | 0.67 | 0.030 | 0.25 |
| $Log_{10}$ PABC | 76 | 3.047[d] (0.739) | 61 | 3.668[e] (0.886) | 70 | 4.119[f] (0.750) | <0.0001 | <0.0001 | 0.0037 |
| Duration of asbestos exposure (years) | 70 | 26.7 (13.2) | 60 | 28.8 (11.0) | 70 | 28.8 (13.9) | 0.24 | 0.14 | 0.97 |
| Time since first asbestos exposure (years) | 70 | 46.2 (8.6) | 60 | 45.8 (10.2) | 70 | 50.8 (10.0) | 0.47 | 0.0003 | 0.024 |

[a,b,c]Comparisons between Class 0 and Class 1, Class 0 and Class 2, and Class 1 and Class 2, respectively. Multiple comparisons between the three groups were performed using Tukey-Kramer's test, as a post hoc test for analysis of variance.
[d,e,f]The values are equal to 1,114 asbestos bodies (AB)/g dry lung, 4,656 AB/g dry lung, and 13,152 AB/g dry lung, respectively.

classification or Class 2 group in the CT-based classification) were highly likely to have had cumulative asbestos exposure levels corresponding to PABC of ≥5,000 AB/g dry lung, with a positive predictive value of 0.75.

Some lung cancer patients with extensive plaques had low PABCs, albeit a small number of patients. On the other hand, many lung cancer patients without extensive plaques (negative group in the CXR-based classification or Class 0 or 1 group in the CT-based classification) had high PABCs. They indicate that PABC is not the only factor predicting the extent of pleural plaques. PABC is not necessarily correlated with pulmonary asbestos fiber concentration because asbestos body formation occurs more frequently on amphibole fibers than chrysotile fibers as mentioned earlier. The potential factors that affect the extent of pleural plaques are thought to be pulmonary asbestos fiber concentration, type of asbestos and size of asbestos fibers, time since first asbestos exposure, and various host factors.

The subjects of this study were patients with various cumulative asbestos exposure levels. Some patients were inferred to have low cumulative asbestos exposure levels corresponding to PABCs of <1,000 AB/g dry lung. However, individuals exposed to substantial levels of chrysotile could have been included among patients with low PABC, because chrysotile fibers are less likely to form pulmonary asbestos bodies than amphibole fibers [De Vuyst et al., 1998].

This study retrospectively examined only surgical cases and autopsy cases of lung cancer. Thus, a certain level of selection bias was inevitable. In this study, data of lung cancer patients with a history of occupational asbestos

**TABLE VI.** Factors Related to the Extent of Pleural Plaques by the CXR- and CT-based Plaque Classifications (Multiple Logistic Regression Analysis) (N = 200)

| Dependent variable | Independent variable | OR | 95% CI | *P*-value |
|---|---|---|---|---|
| CXR-based plaque classification[a] | Age | 0.94 | 0.86–1.07 | 0.097 |
| | $Log_{10}$ PABC | 4.56 | 2.68–8.42 | <0.0001 |
| | Duration of asbestos exposure | 0.98 | 0.94–1.03 | 0.14 |
| | Time since first asbestos exposure | 1.14 | 1.06–1.23 | 0.0006 |
| CT-based plaque classification[b] | Age | 1.00 | 0.96–1.05 | 0.88 |
| | $Log_{10}$ PABC | 3.02 | 2.15–4.34 | <0.0001 |
| | Duration of asbestos exposure | 0.99 | 0.97–1.02 | 0.51 |
| | Time since first asbestos exposure | 1.03 | 0.99–1.07 | 0.20 |

CXR, chest X-ray; CT, computed tomography; PABC, pulmonary asbestos body concentration; OR, odds ratio; 95% CI, 95% confidence interval.
[a]For the CXR-based plaque classification, the negative group and positive group were established as dependent variables, and each was treated as a nominal variable of 0 and 1, respectively.
[b]For the CT-based plaque classification, the Class 0 group, Class 1 group, and Class 2 group were established as dependent variables, and each was treated as a nominal variable of 0, 1, and 2, respectively.

exposure were gathered from multiple medical institutions in widespread areas in Japan. The percentage of subjects by occupation was roughly consistent with the percentage of lung cancer patients by occupation who had been approved for workers' compensation for lung cancer attributable to occupational asbestos exposure in Japan during periods from April, 2010 to March, 2013 (based on data published by the Japanese Ministry of Health, Labour and Welfare, 2013). These results suggest that our subjects reasonably reflect the overall population of lung cancer patients with a history of occupational asbestos exposure in Japan.

Kishimoto et al. [1989] examined the relationship between the extent of pleural plaques and PABC. They used autopsy patients from one medical institution in Japan. They classified the patients by the findings of pleural plaques on the CXR according to the criteria of Askergren and Szamosi [1978]. They reported that patients with definite pleural plaques on the CXR such as types IV and V in that system had many more asbestos bodies than those with indefinite pleural plaques (type IIIb). Bianchi et al. [1999] examined autopsy cases of lung cancer from one medical institution in Italy. They reported a correlation between the size of pleural plaques and PABC. Paris et al. [2009] conducted a large-scale French study on workers with asbestos exposure. They used high-resolution CT and found a significant correlation between the detection frequency of pleural plaques and the cumulative exposure based on the exposure history. This result suggests that there is a positive relationship between the plaque extent and cumulative asbestos exposure.

As mentioned earlier, when an individual is exposed to asbestos, asbestos fibers are inhaled through the respiratory tract and retained in the lungs. A portion of the asbestos fibers forms asbestos bodies. Karjalainen et al. [1996] examined surgical cases of lung cancer in Finland. They found a significant correlation between pulmonary asbestos fiber concentration and the number of asbestos bodies in the lung tissue sections. In addition, pulmonary asbestos fiber concentration and the number of asbestos bodies in the lung tissue section were significantly higher in patients with definite exposure than in those with unlikely exposure. De Klerk et al. [1996] examined autopsy cases of former crocidolite miners in Australia. They reported positive correlations among cumulative crocidolite exposure (fiber-years), pulmonary crocidolite fiber concentration, and PABC. Thus, PABC has been reported to be an indicator that reflects the level of cumulative exposure to asbestos (mainly amphiboles).

According to the Helsinki criteria, the presence of 5,000–15,000 asbestos bodies per gram of dry lung tissue corresponds to a cumulative asbestos exposure of 25 fiber-years [Consensus report, 1997]. This cumulative exposure level increases the risk of lung cancer twofold. In workers' compensation and relief systems in Japan, the presence of $\geq$5,000 asbestos bodies per gram of dry lung tissue is one of the certification criteria of lung cancer caused by asbestos.

This criterion may not be applicable to some patients with chrysotile exposure. For the certification of workers' compensation, other administratively estimated criteria are applicable to such patients, including (i) asbestosis on CXR; (ii) pleural plaques with more than 10 years' occupational asbestos exposure; and (iii) asbestos bodies or fibers in the lung tissues with more than 10 years' occupational asbestos exposure [Kishimoto et al., 2010]. In addition, lung cancer patients who have PABCs between 1,000 and 5,000 AB/g dry lung have their lung tissue examined for their asbestos fibers by analytical transmission electron microscopy. If two million asbestos fibers $>5$ μm or five million asbestos fibers $>1$ μm are quantified per gram of dry lung tissue, they receive the benefits of compensation or relief.

The results of our study indicate that lung cancer patients determined to have extensive plaques (plaque positive by CXR or Class 2 by CT) are likely to have PABC of $\geq$5,000 AB/g dry lung. Our results suggest that pleural plaques provide evidence of sufficient asbestos exposure for workers' compensation and relief systems in the absence of PABC measurement. This statement shows that the extent of pleural plaques from imaging is a useful medical finding to deduce whether or not a patient meets the certification criterion of lung cancer caused by asbestos for workers' compensation and relief in Japan: PABC of $\geq$5,000 AB/g dry lung.

Diffuse pleural thickening is another pleural change caused by asbestos. Patients with bilateral diffuse pleural thickening are considered to have moderate or heavy asbestos exposures [Gibbs et al., 1991; Consensus report, 1997]. There were no patients with diffuse pleural thickening in the subjects we examined.

Several reports have examined the progression of pleural plaques over time. Hillerdal [1978] examined residents of Uppsala, Sweden. He reported multiple cases with long-term follow-up which showed gradual progression of pleural plaques in CXR. Epler et al. [1982] examined the development of pleural plaques observed on CXR. They reported that pleural plaques were not observed in CXR taken within 10 years since first asbestos exposure. However, plaques were seen in approximately 10% of the individuals (10–19 years) since first exposure, approximately 30% of the individuals (20–29 years) since first exposure, and approximately 60% of the individuals (40–49 years) since first exposure. Thus, the percentage increased over time. Paris et al. [2009] reported a positive correlation between the detection frequency of pleural plaques in high-resolution CT and the time since first asbestos exposure. Larson et al. [2010] examined retrospectively CXR of workers exposed to amphiboles. They reported that all workers had pleural lesions and most of them showed progression of radiographic changes over time. These findings suggest that the extent of pleural plaques progress over time after exposure.

The results of our study show a significant relationship between the presence or absence of pleural plaques on CXR and the time since first asbestos exposure. This result is

consistent with the results of reports described above. It needs more than 10 years since first asbestos exposure for pleural plaques to appear on CXR. Our subjects had a long duration since first asbestos exposure (mean: 47.7 years, SD: 9.8 years). This duration may be long enough for pleural plaques to appear on CXR.

Our results did not, however, show a significant relationship between the extent of pleural plaques in the CT-based classification and the time since first asbestos exposure. In our diagnostic criteria of pleural plaques, the level of calcification occurring in pleural plaques affects the diagnosis of the presence or absence of pleural plaques using CXR. On the other hand, such calcification does not affect the CT-based classification of the plaque extent, because our classification criteria do not include the presence or absence of calcification in pleural plaques. This difference could be a cause of the difference in our results using CXR and CT.

Our study had several limitations. (1) This was a retrospective study and the subject selection may have been biased, because the subjects were limited to those who had undergone surgery or autopsy. (2) The CT imaging methods were not standardized among the institutions. (3) There were considerably more male subjects than female subjects. Thus, gender could not be analyzed as a predictor. (4) Our study did not have information on different asbestos types or different sizes of asbestos fibers. Thus, we could not analyze the difference in the plaque extent nor PABC between patients with exposure to chrysotile and amphiboles.

In conclusion, our study showed the significant relationship between the extent of pleural plaques and PABC in lung cancer patients with occupational asbestos exposure. The plaque extent was determined by findings on chest images using our own criteria. The patients determined to have "extensive pleural plaques" based on our imaging criteria are likely to have had cumulative asbestos exposure levels corresponding to PABC of $\geq$5,000 AB/g dry lung, which is one of the certification criteria of lung cancer caused by asbestos for workers' compensation and relief in Japan.

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*Review Article*

# Functional Alteration of Natural Killer Cells and Cytotoxic T Lymphocytes upon Asbestos Exposure and in Malignant Mesothelioma Patients

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Malignant mesothelioma is caused by exposure to asbestos, which is known to have carcinogenic effects. However, the development of mesothelioma takes a long period and results from a low or intermediate dose of exposure. These findings have motivated us to investigate the immunological effects of asbestos exposure and analyze immune functions of patients with mesothelioma and pleural plaque, a sign of exposure to asbestos. Here, we review our knowledge concerning natural killer (NK) cells and cytotoxic T lymphocytes (CTL). NK cells showed impaired cytotoxicity with altered expression of activating receptors upon exposure to asbestos, while induction of granzyme$^+$ cells in CD8$^+$ lymphocytes was suppressed by asbestos exposure. It is interesting that a decrease in NKp46, a representative activating receptor, is common between NK cells in PBMC culture with asbestos and those of mesothelioma patients. Moreover, it was observed that CD8$^+$ lymphocytes may be stimulated by some kind of "nonself" cells in plaque-positive individuals and in mesothelioma patients, whereas CTL in mesothelioma is impaired by poststimulation maintenance of cytotoxicity. These findings suggest that analysis of immunological parameters might contribute to the evaluation of health conditions of asbestos-exposed individuals and to a greater understanding of the pathology of malignant mesothelioma.

## 1. Introduction

Inhalation of naturally occurring particles and fibers causes not only pulmonary fibrosis following an inflammatory response, but also tumor and autoimmune diseases. To date, we have focused on and examined the effect of asbestos exposure on the functions of various kinds of immune competent cells. These studies confirmed that functional decreases in T helper (Th) cells, natural killer (NK) cells, and cytotoxic T lymphocytes (CTLs) were caused by exposure to asbestos, decreases that were also partly observed in patients with malignant mesothelioma, following examination of cell lines and primary cells cultured with asbestos and analyzing cells prepared from the peripheral blood of patients (Table 1) [1–8]. Recently, we have concentrated on the analysis of CTL function in individuals exposed to asbestos and in patients with malignant mesothelioma and found interesting similarities and differences between the groups [9]. These studies give us the opportunity to think in an integrated manner regarding alteration of tumor immunity, and the role played by NK cells and CTLs upon asbestos exposure and in mesothelioma patients. Therefore, here we first show

Table 1: The major part of our previous studies about immunological effects of asbestos exposure and analysis for immune functions of patients.

| Analyses for | Asbestos in cultures or name of diseases | Results | References |
|---|---|---|---|
| (i) Natural killer (NK) cells | | | |
| Human NK cell line, YT-A1 | Culture with chrysotile | Decreases in natural cytotoxicity, cell surface NKG2D, and 2B4 and phosphorylation of ERK | [5, 6] |
| Peripheral blood $CD56^+$ NK cells | Malignant mesothelioma | Low cytotoxicity, low expression of cell surface NKp46 | [5] |
| Human NK cells in PBMC | Culture with chrysotile | Decrease in cell surface NKp46 | [5] |
| (ii) T helper cells | | | |
| Human T cell line, MT-2 | Culture with chrysotile | Resistance against asbestos-induced apoptosis, increases in secretion of IL-10 and expression of bcl-2 mRNA, decreases in secretion of IFN-$\gamma$, TNF-$\alpha$, IL-6, and CXCL10, and surface expression and mRNA of CXCR3 | [1, 2] |
| | Culture with crocidolite | Resistance against asbestos-induced apoptosis, increases in secretion of IL-10 and ratio of bcl-2/bax mRNAs, and decreases in secretion of IFN-$\gamma$ and TNF-$\alpha$ | [4] |
| Peripheral blood $CD4^+$ T cells | Malignant mesothelioma | Very low expression of cell surface CXCR3, low IFN-$\gamma$ mRNA, and high bcl-2 mRNA | [1, 3] |
| | Pleural plaque | Low expression of cell surface CXCR3 | [3] |
| Isolated human $CD4^+$ T cells | Culture with chrysotile | Decreases in cell surface CXCR3 and intracellular IFN-$\gamma$ | [3] |
| (iii) Cytotoxic T lymphocytes (CTL) | | | |
| Human $CD8^+$ T cells in mixed lymphocyte reaction | Culture with chrysotile | Decreases in allogeneic cytotoxicity and intracellular IFN-$\gamma$ and granzyme B | [7] |
| Peripheral blood $CD8^+$ T cells | Malignant mesothelioma | High percentage of $perforin^+$ cells, stimulation-induced decrease in $perforin^+$ cells | [9] |
| | Pleural plaque | High percentage of $perforin^+$ cells | [9] |

findings concerning NK cells and then investigate CTLs as found in a cell culture exposed to asbestos as well as in individuals exposed to asbestos and patients with malignant mesothelioma. Before discussing these subjects, we first describe the background of our studies, various aspects of asbestos, malignant mesothelioma, and the relationship between asbestos exposure and immune function.

## 2. Asbestos, Malignant Mesothelioma, and Immune Function

Asbestos is a kind of naturally occurring mineral fiber that has valuable physical and chemical characteristics including flexibility, as well as fire and heat resistance, which has resulted in the enormous use of asbestos globally. However, in the latter part of the 20th century many reports established that inhalation of asbestos causes malignant mesothelioma, which marked asbestos as one of the representative carcinogenic materials [10–16]. Malignant mesothelioma begins in mesothelial cells covering the inner surface of pleural, pericardial, and peritoneal cavities, as well as the tunica vaginalis, and pleural mesothelioma is the major condition [17]. Asbestos is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC), and it is thought that almost all cases of malignant mesothelioma are caused by exposure to asbestos. Asbestos causes cellular toxicity and mutagenicity and induces the generation of reactive oxygen species (ROS). In addition, it is known that the amounts of oxidized pyrimidine and alkalized nucleic acid base components correlate with the period of exposure to asbestos and that intratracheal instillation of asbestos induces an increase in the mutation frequency of lung DNA in rats [17–21]. However, the relationship between asbestos and malignant mesothelioma cannot be attributed to a "dose-dependent relationship," which is regarded as a general rule in toxicology. It is thought that malignant mesothelioma is caused by a relatively low or intermediate dose of asbestos exposure, whereas a high dose of exposure causes asbestosis [22]. It is also known that malignant mesothelioma occurs

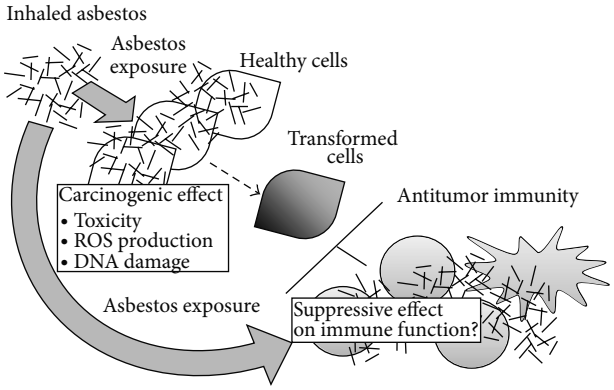



Figure 1: Possible effect of asbestos exposure on antitumor immunity. It is illustrated that there might be a suppressive effect of asbestos exposure on antitumor immunity in the pathology of tumor diseases caused by exposure to asbestos. It is well known that asbestos has a carcinogenic effect, but the development of malignant mesothelioma takes a long time after exposure to asbestos, suggesting the existence of effective antitumor immunity and subsequent impairment caused by asbestos exposure.

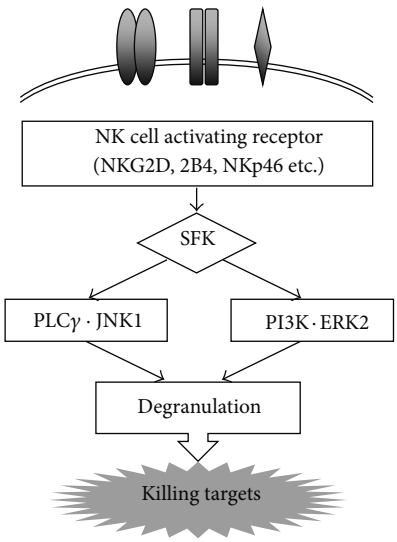



Figure 2: NK cell activating receptors and signal transduction leading to killing targets. NK cells recognize target cells by various kinds of activating and inhibitory receptors. Activation uses common machinery that induces cytotoxicity for targets. The bindings of activating receptors with each ligand transduce through the Src family kinase- (SFK-) dependent phosphoinositide-3 kinase (PI3K) → extracellular signal-regulated kinase 2 (ERK2) pathway and the phospholipase Cγ → c-Jun N-terminal kinase 1 (JNK1) pathway. Finally, degranulation is induced, by which perforin and granzymes in cytotoxic granules are released and work on target cells to induce apoptosis.

even in people exposed environmentally to asbestos. In addition, it takes about 40 years to develop malignant mesothelioma after the initial exposure to asbestos. These findings suggest that mesothelioma may not be caused just by the direct effect of asbestos on mesothelial cells, in which asbestos exposure might exert some kind of alternative effect on the body and allowed us to consider that one possible candidate is its effect on immune function (Figure 1). Inhaled asbestos reaches the pleural cavity through the trachea, bronchus, and pulmonary alveoli, and some asbestos arrive at the regional lymph nodes. Accumulation of asbestos in lymph nodes was observed in people exposed to asbestos nonoccupationally and occupationally [23, 24]. Dodson et al. reported that the total amount of asbestos in the lung was quite low, whereas most cases having asbestos in the lymph nodes showed larger amounts of asbestos in the nodes than in the lung [23, 24]. Thus, immune competent cells may have contact with asbestos not only in nonlymphoid tissue and the area of pulmonary parenchyma and pleural cavity with the inflammatory response, but also in lymphoid tissue of bronchoalveolar, mediastinal, and intercostal nodes, and so on.

## 3. Activating Receptors on NK Cells

Our study focused on and examined the expression of activating receptors on the surface of NK cells, which play a crucial role in recognition for target cells leading to induction of cytotoxicity. Instead of antigen-specific receptors such as the T cell receptor and immunoglobulin for T and B cells, NK cells express various kinds of activating and suppressive receptors [25, 26]. Suppressive and activating receptors on NK cells recognize MHC class I and the ligands derived from infected viruses and tumor cells to contribute to suppression and activation of cytotoxicity, respectively. Some of the killer cell immunoglobulin-like receptors (KIRs) and heterodimers CD94 and NKG2A or NKG2B are suppressive receptors. On the other hand, the homodimer of NKG2D, 2B4, which is a member of the signaling lymphocyte activation molecule (SLAM) family, and NKp46, a member of the natural cytotoxicity receptor (NCR) family, are receptors that play a role in the induction of cytotoxicity. It has been found that activating receptors employ the same pathway of intracellular signal transduction [27]. After binding of ligands, degranulation of cytotoxic granules including perforin and granzymes is induced through the Src family kinase- (SFK-) dependent phosphoinositide-3 kinase (PI3K) → extracellular signal-regulated kinase 2 (ERK2) pathway and the phospholipase Cγ → c-Jun N-terminal kinase 1 (JNK1) pathway. Perforin and granzymes released by degranulation cause target cells to undergo apoptosis (Figure 2).

## 4. Impaired Cytotoxicity and Altered Expression of Activating Receptors in an NK Cell Line Exposed to Asbestos

We began examining the effect of asbestos exposure on cell lines. The human NK cell line of YT-A1 was cultured with continuous exposure to chrysotile B (CB) asbestos at 5 $\mu$g/mL, named the YT-CB5 subline and was then examined periodically concerning cytotoxicity for K567 cells and expression of cell surface receptors, with results being

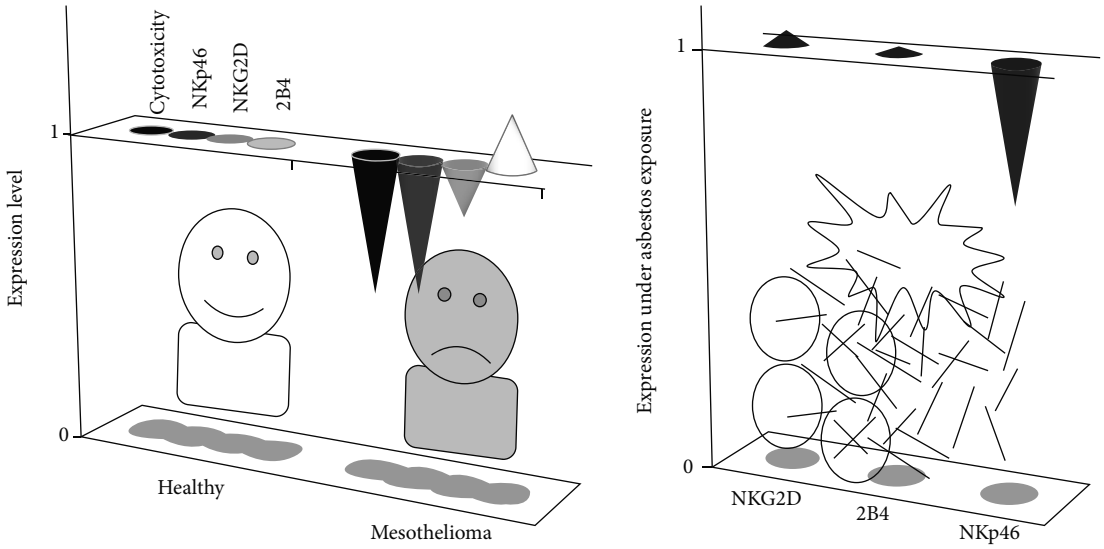



Figure 3: The characteristic decrease of NKp46 on NK cells shared by *in vitro* asbestos exposure and malignant mesothelioma patients. Peripheral blood NK cells in 7 patients with malignant mesothelioma showed decreased cell surface NKp46, but not NKG2D or 2B4, among activating receptors, compared with 10 healthy individuals, and it is interesting that this was also shown by NK cells in PBMCs cultured with asbestos. The relative alterations of expression level are shown.

compared to those of the control subline of YT-Org. Initially, the effect of asbestos exposure on viability of the cell line was checked and 5 $\mu$g/mL of CB was chosen and utilized as a concentration having no effect on cell growth and apoptosis. Although YT-CB5 showed a normal level of cytotoxicity comparable to YT-Org until 1 month after the start of culture, it showed impaired cytotoxicity after around 5 months [5]. In accord with the impaired cytotoxicity, YT-CB5 showed decreases in cell surface expression of NKG2D and 2B4, whereas NKG2A and CD94 showed no changes in expression. Although it is known that cytotoxicity against K562 cells is independent of 2B4, the decrease in 2B4-dependent cytotoxicity in YT-CB5 was confirmed by a reverse antibody-dependent cell-mediated cytotoxicity (ADCC) assay. Moreover, YT-CB5 showed the decrease in phosphorylation of ERK1/2 following incubation with K562 cells, and the SFK inhibitor of pp2 or the PI3K inhibitor of wortmannin caused the decrease in phosphor-ERK1/2 of YT-Org. In addition, YT-CB5 also showed a low level of phosphor-ERK1/2 under stimulation with antibody to NKG2D [6]. Thus, it was found that asbestos exposure causes impairment of cytotoxicity with altered expression of activating receptors in NK cells.

## 5. Decrease in NKp46 on NK Cells in Culture upon Asbestos Exposure and in Patients with Malignant Mesothelioma

After the study of the cell line exposed to asbestos, we examined the function of peripheral blood NK cells in patients with malignant mesothelioma. Peripheral blood mononuclear cells (PBMCs) prepared from peripheral blood were assayed for cytotoxicity against K562 cells and the expression level of activating receptors on the cell surface of NK cells, and results were compared between healthy and mesothelioma individuals. To evaluate the lytic activity of a given cell number, the cytotoxicity per 5000 NK cells was calculated from the percentage of NK cells in PBMCs. Mesothelioma patients showed lower cytotoxicity of NK cells than healthy individuals, and also exhibited alteration in expression of activating receptors in their NK cells, which differed from YT-CB5. The NK cells of mesothelioma patients exhibited a characteristic decrease in expression of NKp46, whereas NKG2D and 2B4 showed normal expression (Figure 3) [5]. PBMCs were then cultured in media supplemented with IL-2 and exposed to CB at 5 $\mu$g/mL for 7 days and assayed for the expression of activating receptors on the cell surface of NK cells. As shown by NK cells of mesothelioma patients, NK cells showed a decrease in NKp46 in the culture upon CB exposure, whereas NKG2D and 2B4 did not differ from the control culture (Figure 3). In addition, glass wool, which represents a man-made mineral fiber and a substitute for asbestos, did not cause such an alteration in expression of activating receptors, unlike CB asbestos. It was therefore interesting to discover that peripheral blood NK cells in mesothelioma patients showed a characteristic decrease in cell surface NKp46 with low cytotoxicity, similar to that of NK cells in the culture with asbestos, suggesting the possibility that impairment of NK cell function might be caused by inhaled asbestos and may be related to the pathology of malignant mesothelioma.

## 6. Relationship between Cytotoxicity, Expression of Activating Receptors, and Signal Transduction in NK Cells

To examine the relationship between low levels of cytotoxicity and activating receptors, peripheral blood NK cells were isolated from the PBMCs of healthy individuals and assayed

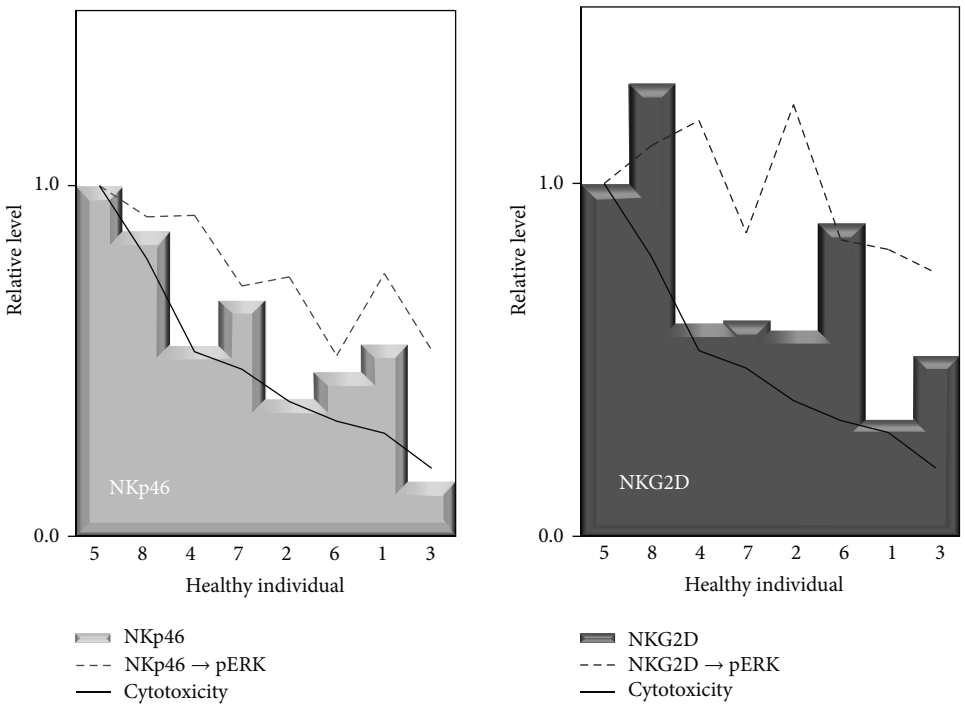



Figure 4: Relationship among cytotoxicity, expression of NKp46 or NK2D, and ERK phosphorylation. When the NK cells of healthy individuals were analyzed, an individual with high cytotoxicity showed high expression of NKp46 and high phosphorylation of ERK induced through NKp46, and there was also a similar relationship among cytotoxicity, NKG2D expression, and ERK phosphorylation. These findings suggest that low expression of NKp46 may be attributed to decreased cytotoxicity of NK cells in patients with malignant mesothelioma. The levels in each individual relative to an individual with the highest cytotoxicity are shown.

regarding cytotoxicity for K562 cells, cell surface expression of activating receptors, and phosphorylation of ERK1/2 after stimulation with antibodies to receptors, and results were compared between individuals. When individuals were put in descending order of cytotoxicity, an individual with high cytotoxicity showed high expression of NKp46 and NKG2D, whereas another with low cytotoxicity showed the opposite trend (Figure 4). In accord with this finding, an individual with high cytotoxicity showed a high level of phosphor-ERK1/2 following stimulation with antibodies to NKp46 or NKG2D, whereas another with low cytotoxicity showed a low level of phosphorylation of ERK1/2 [6]. In contrast, the expression level of 2B4 and the phosphorylation level of EKR1/2 following stimulation with 2B4 did not show such a relationship with cytotoxicity. These findings indicate that expression levels of NKG2D and NKp46 are related to the degree of cytotoxicity induced by stimulation with those receptors through signal transduction downstream of the receptors, suggesting that decreased cytotoxicity of NK cells in mesothelioma patients might be attributed to low expression of NKp46.

## 7. Cytotoxic T Lymphocytes and Inhalation Exposure to Asbestos

In antitumor immunity, $CD8^+$ T lymphocytes play a more crucial role in cytotoxicity against target cells in an antigen-specific manner, together with the natural cytotoxicity of NK cells [28]. CTLs also utilize the same tools to injure targets such as NK cells, in which perforin and granzymes are released from CTLs into an intercellular space and induce apoptosis of target cells [29]. However, $CD8^+$ T lymphocytes have to be selected clonally and proliferate and differentiate into functional CTLs in order to exert matured cytotoxicity for targets [30]. The differentiation of functional CTLs is a very complex event, in which various kinds of immune cells contribute to CTL differentiation. At first, dendritic cells transfer antigen to lymphoid organs as regional lymph nodes, where they or node-resident dendritic cells are ready to present antigen for T cells as antigen presenting cells (APC) [31]. Naïve $CD8^+$ T lymphocytes then migrate into the nodes and communicate with APC, and those having specificity for the antigen presented are chosen [32]. Moreover, $CD4^+$ T lymphocytes capable of recognizing the same antigen also have to migrate into the nodes and communicate with APC to lead to differentiation of functional CTLs [33]. Thus, the lymph node is a place where immune cells necessary for CTL differentiation meet and communicate with each other, while inhaled asbestos also migrates into regional nodes and accumulates there as mentioned above. These findings indicated that inhaled asbestos might affect differentiation of functional CTLs and motivated us to examine this possibility.

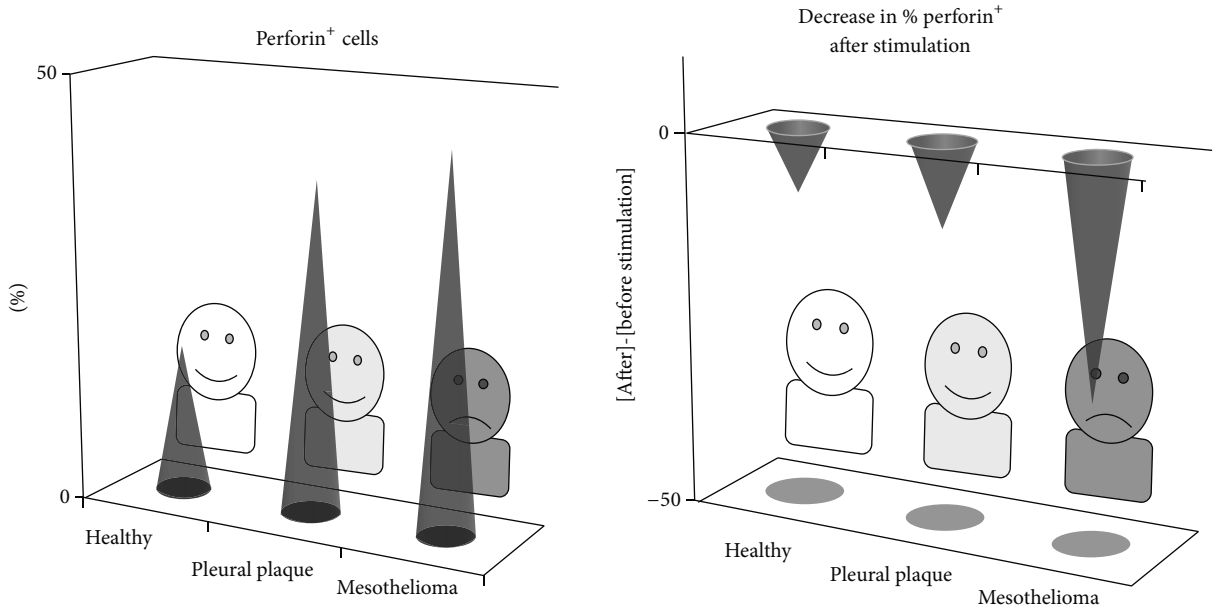



Figure 5: CTL function in patients with malignant mesothelioma and individuals positive for pleural plaque. Both 16 plaque-positive individuals and 14 mesothelioma patients showed a high percentage of perforin$^+$ cells in CD8$^+$ lymphocytes, compared with 16 healthy volunteers, whereas a decrease after stimulation was observed in mesothelioma. These findings indicate that CD8$^+$ lymphocytes are stimulated by some kind of "nonself" cells in both plaque-positive individuals and mesothelioma patients, and that poststimulation maintenance of cytotoxicity is impaired in mesothelioma.

## 8. Effect of Asbestos on Induction of Cytotoxic T Lymphocytes by Mixed Lymphocyte Reaction

We thus attempted to investigate the effect of asbestos on acquired immunity in the antitumor response using the mixed lymphocyte reaction (MLR), an experimental method to induce cell-mediated acquired immunity using an allogeneic set of PBMCs or lymphoid cells, and an easy tool to mimic *in vitro* induction of CTL function from naïve CD8$^+$ T cells. PBMCs were cultured with a stimulator of allogeneic PBMCs upon exposure to CB asbestos, and examined for the characteristics and allogeneic cytotoxicity of CD8$^+$ T cells [7]. CB exposure suppressed the increase in cell number of CD8$^+$ lymphocytes, which when sorted from the culture showed a decrease in cytotoxicity against allogeneic targets compared with those from the control culture. CD8$^+$ lymphocytes from the CB-exposed culture also showed low percentages of intracellular granzyme B and IFN-$\gamma$ and cell surface CD25 and CD45RO, and a high percentage of CD45RA. In addition, suppressed cell proliferation of CD8$^+$ lymphocytes upon exposure to CB was also confirmed by the CFSE labeling method. In contrast, those lymphocytes did not differ in apoptosis from those of the control group. Moreover, the productions of TNF-$\alpha$ and IFN-$\gamma$ in the supernatant from the CB-exposed culture were low, whereas IL-2 did not differ in production. These findings therefore indicate that the induction of CTL function in MLR was suppressed by asbestos, and it was found that asbestos exposure has the potential to exert a suppressive effect on CTL induction following antigen stimulation.

## 9. Functional Properties of CD8$^+$ Lymphocytes in Patients with Pleural Plaque and Malignant Mesothelioma

Malignant mesothelioma is attributed to asbestos exposure, which can be determined by examination for pleural plaque using image analyses involving X-ray and CT-scan methods. Pleural plaque is an objective sign of previous asbestos inhalation, and is known to be whitish, sharply circumscribed, fibrous, hyaline, sometimes calcified, forms patches involving parietal pleura, and is regarded as harmless [34]. Our recent analysis of peripheral blood CD8$^+$ lymphocytes in individuals positive for pleural plaque and patients with malignant mesothelioma revealed the similarities and differences between these groups. Individuals in the pleural plaque and malignant mesothelioma groups showed higher percentages of perforin$^+$ cells and CD45RA$^-$ cells in fresh CD8$^+$ lymphocytes than healthy individuals. However, patients in the mesothelioma group showed a decrease in perforin$^+$ cells following stimulation with PMA and ionomycin, whereas most of the healthy and plaque-positive individuals retained those cells after stimulation (Figure 5) [9]. The decrease in cells positive for intracellular perforin following stimulation might have been attributed to enhanced degranulation of cytotoxic granules, indicating increased cytotoxicity in mesothelioma, since degranulation is a process that releases perforin and granzymes, which act as factors to injure target cells. However, we confirmed that the CD8$^+$ lymphocytes did not show an increase of cell surface CD107a, a representative marker of degranulation, following stimulation. Thus, it was clarified that patients with malignant mesothelioma have

characteristics of impairment in stimulation-induced cytotoxicity of peripheral blood $CD8^+$ lymphocytes. Additionally, it is also important that they showed a similar alteration of function, namely, an increase in $perforin^+$ cells, compared to $CD8^+$ lymphocytes in plaque-positive individuals, which suggests that such a characteristic might be related with inhalation exposure to asbestos.

## 10. Significance of Our Study Results

As described above, our studies demonstrated that asbestos exposure has the potential to cause suppressed function of NK cells and CTLs. Malignant mesothelioma is caused by exposure to asbestos, but its development is limited by the parts that have been exposed to asbestos, suggesting the existence of effective antitumor immunity against transformed cells at an initial phase in the body of individuals exposed to asbestos. In addition, it is well known that asbestos-exposed individuals take a very long time to develop malignant mesothelioma after exposure, suggesting that antitumor immunity fought transformed cells until the individual began to suffer from malignant mesothelioma. These findings highlight the importance of the monitoring and intervention of immune function in asbestos-exposed people.

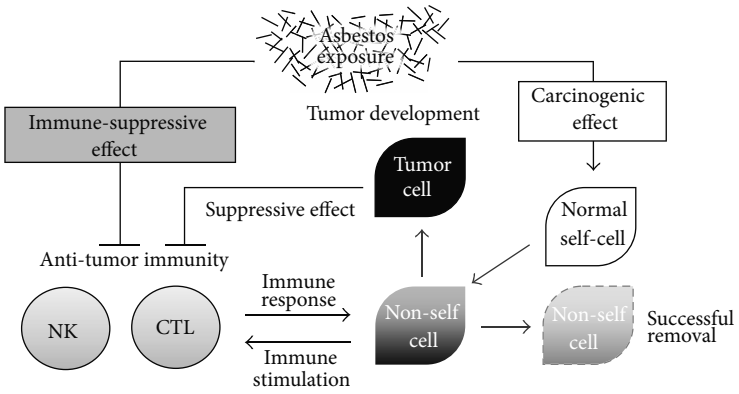
In fact, our study identified one appealing candidate for antitumor immunity in relation to asbestos exposure and malignant mesothelioma, namely, NKp46. NK cells in PBMCs showed decreased cell surface expression of NKp46 following exposure to asbestos, which was also shown by patients with malignant mesothelioma. Although our study using the cell line showed alteration in expression of activating receptors in a different manner, in which NKG2D and 2B4 decreased, these findings indicated that the decrease in activating receptors is attributed to low cytotoxicity through a decrease in signal transduction downstream of those receptors, and allowed us to understand that expression of activating receptors should be examined for primary cell cultures and specimens of malignant mesothelioma. It is interesting that the expression of activating receptors on both NK cells of asbestos-exposed PBMCs and patients with malignant mesothelioma is altered in a characteristic manner and is similar between these groups, in which there is a decrease of NKp46 but not NKG2D or 2B4, which suggests a relationship between the decrease in NKp46, asbestos exposure, and malignant mesothelioma. NK cells play a primary role in cytotoxicity against nonself targets in innate immunity before lymphocytes specific for those targets are clonally selected, proliferate, and acquire fully matured cytotoxicity. NKp46 might therefore be a useful tool for the evaluation of health conditions in asbestos-exposed individuals.

In addition, asbestos exposure suppressed development of CTL function during MLR, in which $CD8^+$ lymphocytes showed decreases in cytotoxicity and the percentage of intracellular granzyme B. These observations allowed us to speculate that $CD8^+$ lymphocytes in patients with malignant mesothelioma might show a decrease in granzymes or perforin similar to that shown by the MLR culture. However, there were no decreases in perforin or granzyme, but rather an increase in perforin in fresh $CD8^+$ lymphocytes from individuals with malignant mesothelioma, as well as those with pleural plaque, when compared with healthy individuals. These findings appear to be paradoxical, but careful discussion leads to clarification. As mentioned above, CTL function differs in induction and maturation from NK cell function. The former is induced by antigen stimulation, whereas the latter is ready to injure targets without stimulation. It is important to note in the results obtained from the analysis of blood specimens that $CD8^+$ lymphocytes showed an increase of perforin in plaque-positive individuals, which had been exposed to asbestos but did not develop any tumors. This means that they have some kind of "non-self" cells prior to tumors, probably caused by exposure to asbestos, which stimulate immune responses in the body. That explanation helped us to realize that both fresh $CD8^+$ lymphocytes of pleural plaque and malignant mesothelioma show such a similar character. On the other hand, healthy individuals have no stimulation with "non-self" cells, including no exposure to asbestos. Therefore, it is difficult to compare healthy and plaque-positive individuals as control and asbestos-exposed cultures in MLR, respectively, which could not have been anticipated before the study was performed. However, it is noteworthy that $CD8^+$ lymphocytes in plaque-positive individuals show an increase in perforin, suggesting that they are specifically fighting against "nonself."

Furthermore, it is interesting that the $CD8^+$ lymphocytes in patients with malignant mesothelioma showed characteristic impairment, in which the percentage of $perforin^+$ cells decreased after stimulation, even though it was as high as that of plaque-positive individuals before stimulation. These findings suggest that such impairment in CTL function might be related to the pathology or development of malignant mesothelioma. We can consider the two scenarios for impaired CTL function in malignant mesothelioma. The first may be caused just by the immune-suppressive effect of tumor cells after the onset of malignant mesothelioma. The second may be caused by the immune-suppressive effect of asbestos exposure before malignant mesothelioma, as suggested by results obtained from our experiment using the MLR culture. Although the study concerning $CD8^+$ cells did not find any impairment of function in individuals with pleural plaque, we have reported that $CD4^+$ T lymphocytes in individuals with pleural plaque showed a decrease in cell surface expression of CXCR3, a chemokine receptor dominantly expressed on Th1 cells [3], which supports the second aforementioned scenario. However, further studies are needed to conclude this matter. In either case, it is clear that CTL function is impaired in patients with malignant mesothelioma, which may be related to the pathology of this disease. Our study results and discussion are summarized in Figure 6.

## 11. Conclusion

Our overall findings highlight the following points. (1) Exposure to asbestos has the potential to suppress the function of NK cells and CTLs. (2) It is possible that analysis of immunological parameters, such as NKp46 expression, might




| | Asbestos-exposed NK cell line or PBMC culture | Malignant mesothelioma | Pleural plaque |
|---|---|---|---|
| NK | • Cytotoxicity: ↓<br>• NKG2D and 2B4: ↓ (cell line)<br>• NKp46: ↓ (PBMC) | • Cytotoxicity: ↓<br>• NKp46: ↓ | |
| CTL | • Cytotoxicity induction: ↓<br>• Perf and GranB: ↓<br>• IFN-$\gamma$ production: ↓ | • Perf in fresh: ↑<br>• Perf after stimulation: ↓ | • Perf in fresh: ↑<br>• Perf after stimulation: no change |

Figure 6: Summary of our study results and discussions. It is illustrated that asbestos exposure may exert not only a carcinogenic effect, but also an immune-suppressive effect, and that there might be an interaction between antitumor immunity and "nonself" cells that results either in the successful removal of these cells or the development of tumor. Our study results are summarized below. Abbreviations: Perf, perforin; GranB, granzyme B.

contribute to the evaluation of health conditions in asbestos-exposed individuals. (3) $CD8^+$ lymphocytes in individuals with pleural plaque may be stimulated by some kind of "non-self" cells. (4) CTL function is impaired in patients with malignant mesothelioma in comparison to plaque-positive individuals. Following these studies, we have continued to examine the effect of asbestos exposure on immune function and analyze specimens from asbestos-exposed individuals and mesothelioma patients. We hope that our studies will contribute to a greater understanding of asbestos exposure-related health disorders, including malignant mesothelioma, in order to improve the cure rate of those diseases.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

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# A risk prediction model for colorectal cancer using genome-wide association study-identified polymorphisms and established risk factors among Japanese: results from two independent case–control studies

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**Most genome-wide association studies of colorectal cancer (CRC) carried out to date have been in populations with European ancestry, and the extent to which the identified variants contribute as predictors of CRC among Japanese populations has not been clarified. We analyzed 23 genetic variants identified in previous genome-wide association studies in a derivation case–control study with 558 cases and 1116 age-matched and sex-matched controls. Six single nucleotide polymorphisms were selected for synthesis of the genetic risk score. A dose-dependent association was observed between CRC risk and genetic risk score, which is the aggregate number of alleles in six selected variants: 8q24 – rs6983267, 15q13 – rs4779584 and rs1696961, 14q22 – rs444435, 16q22 – rs9929218, and 3q26.2 – rs1093599. The *c* statistic for a model that included the genetic risk score and conventional risk factors was 0.7167, versus 0.7009 with the conventional risk factors only ($P = 0.0013$). This model was evaluated in a replication study with 547 cases and 547 age-matched and sex-matched controls, and corresponding *c* statistics were 0.6356 and 0.6391 with no statistical significance. When two studies were combined, the corresponding *c* statics were 0.6132 and 0.6198 ($P = 0.0126$). We developed a risk model that incorporates a genetic risk score and established risk factors, but this model was not satisfactory in the replication study. The results in the combined study still encourage further attempts to using a similar approach among individual countries.** ***European Journal of Cancer Prevention*** **00:000–000** 

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## Introduction

The incidence rate of colorectal cancer (CRC), the most common cancer worldwide, increased rapidly in Japan until the mid-1990s (Katanoda *et al.*, 2013). This rapid increase in CRC incidence can be related to changes in the prevalence of environmental risk factors (Tajima *et al.*, 1985) coupled with genetic background, as suggested by studies of immigrants from Japan to the USA (Dunn, 1975; Shimizu *et al.*, 1987; Marchand, 1999).

Genome-wide association studies (GWASs) have identified genetic susceptibility loci that are associated with CRC (Matsuo *et al.*, 2009; Thompson *et al.*, 2009; Elliott *et al.*, 2010; Houlston *et al.*, 2010; Lascorz *et al.*, 2010; Slattery *et al.*, 2010; von Holst *et al.*, 2010; Cui *et al.*, 2011; He *et al.*, 2011; Tomlinson *et al.*, 2011; Xing *et al.*, 2011). Compared with high-penetrance germline mutations, such as *APC* in familial adenomatous polyposis or mismatch repair genes in hereditary nonpolyposis colorectal cancer, these genetic susceptibility loci are low-penetrance polymorphisms with only weak associations with CRC risk (Matsuo *et al.*, 2009; Thompson *et al.*, 2009; Elliott *et al.*, 2010; Houlston *et al.*, 2010; Lascorz *et al.*, 2010; Slattery *et al.*, 2010; von Holst *et al.*, 2010; Cui *et al.*, 2011; He *et al.*, 2011; Tomlinson *et al.*, 2011; Xing *et al.*, 2011). Although each low-penetrance variant confers only a small increase in risk, their combination may contribute toward an increased risk, and it is accordingly possible that a combination of single variants might be useful in identifying individuals at a relatively higher risk among general populations for targeted cancer prevention.

Most GWASs to date have been carried out in individuals of European ancestry. Given the possibility of considerable differences in genetic architecture, however, such as in allele frequencies or the extent of linkage

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disequilibrium (LD) across populations (Frazer *et al.*, 2009), evaluation in specific populations of non-European ancestry appears to be reasonable. Here, we carried out a case–control study to evaluate associations between 23 GWAS-identified single nucleotide polymorphisms (SNPs) as potential risk variants and the risk of CRC in a Japanese population. Furthermore, we developed a genetic risk predictor for CRC with validated SNPs and evaluated the extent to which the predictor could estimate the probability of CRC risk in this population.

## Methods

### Study population

#### *Derivation population*

The case participants were 558 patients with no previous history of cancer who were histologically diagnosed with CRC between January 2001 and November 2005 at Aichi Cancer Center Hospital in Japan. All participants were recruited after obtaining written informed consent within the framework of the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC) (Inoue *et al.*, 1997; Tajima *et al.*, 2000; Hamajima *et al.*, 2001), and all participants provided blood samples. Among the 558 participants, 289 (51.8%) had colon cancer, 267 (48.0%) had rectal cancer, and two (0.4%) had both.

The control participants were 1116 first-visit outpatients during the same period who were confirmed to have no cancer and no history of neoplasm. Noncancer status was confirmed by medical examinations, including radiographic examinations, with participants suspected of having CRC first examined by physical or endoscopic inspection, and subsequently radiographically when indicated. Controls were selected randomly and were individually matched by age (±5 years) and sex (male, female) with a case–control ratio of 1 : 2. A total of 1674 participants (558 cases and 1116 controls) were included in the study. The study was approved by the institutional ethics committee at Aichi Cancer Center.

#### *Replication population*

The external replication population was also selected from among HERPACC study participants between December 2005 and March 2013. In the same way as the derivation population, 547 CRC patients [301 (55.0%) colon cancer and 246 (45.0%) rectal cancer] were newly detected. Noncancer controls were selected randomly and were individually matched by age (±2 years) and sex (male, female) with a case–control ratio of 1 : 1. The replication study included a total of 1094 participants (547 cases and 547 controls).

### Examination of genetic polymorphisms

DNA of each participant was extracted from the buffy coat fraction using a DNA Blood Mini Kit (Qiagen). For this study, we selected 23 loci reported in GWASs published up to September 2012 to have an association with CRC cancer risk (Table 1) (Matsuo *et al.*, 2009; Thompson *et al.*, 2009; Elliott *et al.*, 2010; Houlston *et al.*, 2010; Lascorz *et al.*, 2010; Slattery *et al.*, 2010; von Holst *et al.*, 2010; Cui *et al.*, 2011; He *et al.*, 2011; Tomlinson *et al.*, 2011; Xing *et al.*, 2011). Genotyping of each locus was based on SNPType Assays by Fluidigm (South San Francisco, California, USA) with the EP1 system using 192.24 dynamic arrays (Wang *et al.*, 2009; Chan *et al.*, 2011) or TaqMan Assays with the 7500 Applied Biosystems Fast System (Life Technologies, Carlsbad, California, USA). The quality of genotyping at our center is routinely assessed statistically using the Hardy–Weinberg test and by retyping of a random sampling of 5% of participants.

### Information on exposures and family history of colorectal cancer

Information on conventional risk factors for CRC was collected from first-visit outpatients aged 20–79 years using a self-administered questionnaire. Each participant was asked at the time of the first visit to our hospital about their exposure status before the development of the current symptoms that led to their visit to our hospital.

Information on smoking status was obtained in the three categories of nonsmoker, former smoker, and current smoker, with former smokers defined as those who had quit at least 1 year before study enrollment. Cumulative exposure to smoking was categorized into five groups by pack-years (PY), the product of the number of packs of cigarettes smoked per day, and the number of years of smoking, namely, as never, $PY < 15$, $PY < 30$, $PY < 45$, and $PY \geq 45$.

Daily alcohol consumption of various common beverages (Japanese sake, beer, shochu, whiskey, and wine) was determined in terms of the average number of drinks per day. This was then converted into a Japanese sake (rice wine) equivalent measure of 180 ml; termed as 'go', this is a standard measure in Japan and contains 23 g of ethanol. Drinking status was then classified into the five categories of never drinker, less than 5 g ethanol/day, less than 23 g ethanol/day, less than 46 g ethanol/day, and more than or equal to 46 g ethanol/day.

Consumption of folate was determined using a food frequency questionnaire, which included 43 single food items in eight frequency categories. The food frequency questionnaire was validated using a 3-day weighed dietary record as standard, which showed that reproducibility and validity were satisfactory (Tokudome *et al.*, 2005; Imaeda *et al.*, 2007). Correlation coefficients for folate were 0.36 for men and 0.38 for women. Consumption of supplemental folate was not considered in total consumption because the questionnaire for multivitamins

**Table 1 Information on candidate loci in this study**

| dbSNP rs numbers | Chr. | Chr. position | Gene | Risk allele | Allele major/minor in HapMap JPT | MAF in HapMap JPT | References |
|---|---|---|---|---|---|---|---|
| rs6983267 | 8q24 | 128 413 305 | Unknown | G | T/G | 0.341 | Elliott *et al.* (2010), Hamajima *et al.* (2001), He *et al.* (2011), Houlston *et al.* (2010) |
| rs4779584 | 15q13 | 32 994 756 | Unknown | T | T/C | 0.156 | Hamajima *et al.* (2001), Houlston *et al.* (2010), Lascorz *et al.* (2010) |
| rs3802842 | 11q23 | 111 171 709 | *C11orf93* | C | A/C | 0.300 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs10795668 | 10q14 | 8 701 219 | Unknown | G | G/A | 0.405 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs4939827 | 18q21 | 46 453 463 | *SMAD7* | T | C/T | 0.205 | Frazer *et al.* (2009), Hamajima *et al.* (2001) He *et al.* (2011), Houlston *et al.* (2010), Jia *et al.* (2013) |
| rs12953717 | 18q21 | 46 453 929 | *SMAD7* | T | C/T | 0.178 | Frazer *et al.* (2009), Jia *et al.* (2013) |
| rs16892766 | 8q23 | 117 630 683 | Unknown | C | A (monomorphic) | 0 | Hamajima *et al.* (2001), He *et al.* (2011) |
| rs719725 | 9g24 | 6 365 683 | Unknown | A | A/C | 0.318 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs4444235 | 14q22 | 54 410 919 | Unknown | C | C/T | 0.422 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs9929218 | 16q22 | 68 820 946 | *CDH1* | G | G/A | 0.133 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs10411210 | 19q13 | 33 532 300 | *RHPN2* | T | C/T | 0.182 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs961253 | 20p12 | 6 404 281 | Unknown | A | C/A | 0.200 | Hamajima *et al.* (2001), Houlston *et al.* (2010) |
| rs4464148 | 18q21 | 46 459 032 | *SMAD7* | C | T/C | 0.034 | Frazer *et al.* (2009), Jia *et al.* (2013) |
| rs6691170 | 1q41 | 222 045 446 | Unknown | G | G (monomorphic) | 0 | Katanoda *et al.* (2013) |
| rs6687758 | 1q41 | 222 164 948 | Unknown | A | A/G | 0.330 | Katanoda *et al.* (2013) |
| rs10936599 | 3q26.2 | 169 492 101 | *MYNN* | T | T/C | 0.284 | Katanoda *et al.* (2013) |
| rs11169552 | 12q13.13 | 51 155 663 | Unknown | C | C/T | 0.239 | Katanoda *et al.* (2013) |
| rs7136702 | 12q13.13 | 50 880 216 | Unknown | C | T/C | 0.420 | Katanoda *et al.* (2013) |
| rs4925386 | 20q13.33 | 60 921 044 | *LAMA5* | T | C/T | 0.216 | Katanoda *et al.* (2013) |
| rs1957636 | 14p11.2 | 54 560 018 | Near *BMP4* | A | A/G | 0.378 | Lascorz *et al.* (2010) |
| rs4813802 | 20p12 | 6 699 595 | Near *BMP2* | G | T/G | 0.170 | Lascorz *et al.* (2010) |
| rs16969681 | 15q13 | 32 993 111 | Unknown | T | C/T | 0.489 | Lascorz *et al.* (2010) |
| rs11632715 | 15q13 | 33 004 247 | Unknown | A | A/G | 0.222 | Lascorz *et al.* (2010) |

Chr., chromosome; MAF, minor allele frequency.

was not quantitative. Participants were divided into three groups on the basis of the distribution of folate consumption among controls (tertiles).

BMI was calculated as the self-reported weight (kg) divided by the square of self-reported height (m). A family history of CRC in a first-degree relative was based on self-reporting, as described elsewhere (Suzuki *et al.*, 2007). The questionnaire also covered the regularity of physical exercise: participants were asked to report the frequency and intensity of recreational exercise, with average daily exercise hours of any intensity calculated and categorized into the three levels of none, and less than 0.5 h/day and more than or equal to 0.5 h/day.

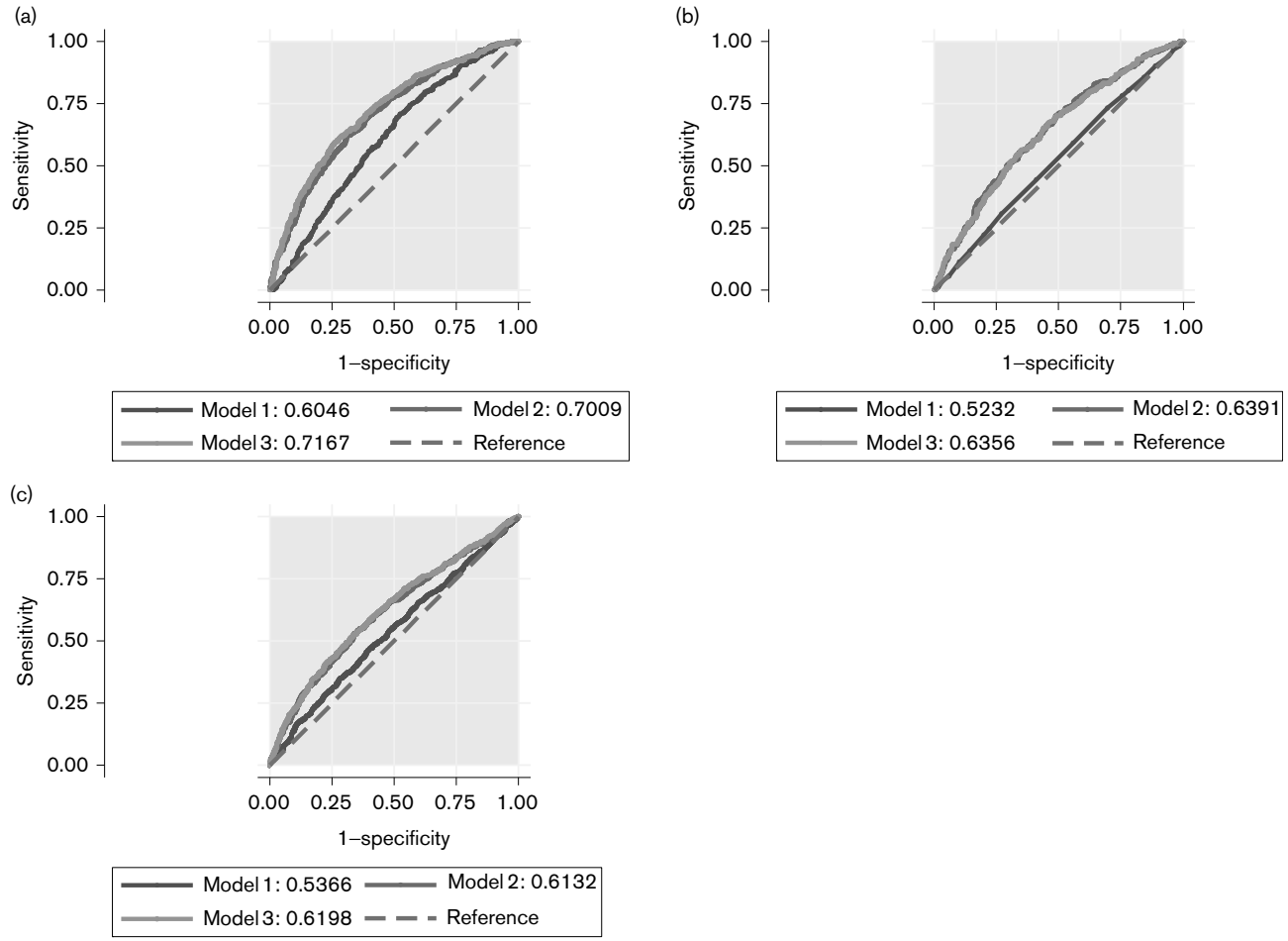
### Data analyses

Differences in categorized demographic variables between cases and controls were evaluated using the $\chi^2$-test. Participants with unknown data were excluded. Mean ages were compared using the Mann–Whitney test. Linear trend, odds ratios (ORs), and 95% confidence intervals for assessment of the main effect of each SNP were calculated using conditional logistic regression models (per-allele model) adjusted for established risk factors, namely age, smoking (never, PY < 15, PY < 30, PY < 45, and PY ≥ 45, daily ethanol consumption (0, < 5, < 23, < 46, and ≥ 46 g/day), dietary folate intake (tertiles), current BMI (< 18.5, 18.5–21.9, 22–24.9, ≥ 25 kg/m$^2$), regular exercise (no, < 0.5, or ≥ 0.5 h/day), family history of CRC (yes, no), and referral pattern to our hospital (patient discretion, family or friend recommendation, referral from another clinic, secondary screening after primary screening, or other). Missing values for covariates were treated as dummy variables in the models.

On the basis of the results for individual candidate variants among the derivation population, we created a polygenetic risk score to measure the cumulative effect of multiple genetic risk variants using those SNPs with a statistically marginally significant association with CRC risk ($P < 0.1$) in per-allele logistic regression models. If several SNPs located on the same gene or chromosome were selected and were in strong LD in terms of $R^2$ (≥0.8), we selected the variant showing the lowest $P$-value. On the basis of the total number of risk-conferring variant alleles (one allele for heterozygotes and two alleles for homozygotes) of the selected SNPs, we divided the participants into three risk score groups (1, 2, and 3) that produced the biggest area under the receiver operating characteristic (AUC) curve on the basis of the logistic regression model adjusted for established risk factors. We evaluated the association between risk score and CRC risk in the logistic regression for categorical variables, adjusted for established risk factors as described above.

For risk model construction and validation, we used the derivation and replication population and combined total population separately. Among each population, we evaluated prediction models for the risk of CRC that combined the genetic risk score with established risk factors (model 3 in Fig. 1) by comparing models with (model 1 in Fig. 1) and without the genetic risk score (model 2 in

**Fig. 1**




AQ6 ■■■■.

Fig. 1) using the AUC curve, which is also known as a concordance (*c*) statistic, to assess model discrimination. Hosmer and Lemeshow (2000) have suggested that a *c* statistic or an AUC value between 0.7 and 0.8 is acceptable for model discrimination, whereas a value greater than 0.8 is excellent. The AUC/*c* statistics were compared using the method described by DeLong *et al.* (1988) and Demler *et al.* (2012). All analyses were carried out using Stata SE (version 13.1; STATA Corp., College Station, Texas, USA).

## Results

Demographic characteristics and selected lifestyle habits of participants among the derivation and replication population are shown in Table 2. The groups were appropriately matched for age and sex. The proportion of heavier smokers and heavier drinkers was higher among cases than the controls. Cases were exposed to a higher alcohol dose than controls. In contrast, no significant difference was observed in the distribution of current BMI. Those with a family history of CRC were significantly more prevalent among cases. A similar distribution was observed on stratification by population, except for smoking, physical activity, or folate consumption.

Table 3 presents the association between six individual candidate variants (based on Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJCP/A32*) and CRC risk among the derivation and replication population. In the derivation population, six loci, namely, 8q24 – rs6983267, 15q13 – rs4779584 and rs1696961, 14q22 – rs444435, 16q22 – rs9929218, and 3q26.2 – rs1093599, showed an association with a *P*-value less than 0.1, the threshold value for selection in this study. Rs4779584 and rs1696961 are located in the same region, 15q13, and the coefficient of LD ($R^2$) was 0.17. On the basis of these, we used these six loci for the construction of a polygenic risk score for validation in the replication population. Among the replication population, only rs4779584 and rs1696961 showed a significant

**Table 2 Characteristics of study participants in the derivation and replication population**

| | Derivation [*n* (%)] | | | Replication [*n* (%)] | | |
|---|---|---|---|---|---|---|
| | CRC case | Control | *P*-value | CRC case | Control | *P*-value |
| All (male + female) | 558 | 1116 | 1.000 | 547 | 547 | 1.000 |
| Male | 350 (62.7) | 700 (62.7) | | 358 (65.5) | 358 (65.5) | |
| Female | 208 (37.3) | 416 (37.3) | | 189 (34.6) | 189 (34.6) | |
| Age [mean (SD)] | 60 (10.3) | 60 (10.1) | 0.815 | 61 (10.0) | 61 (10.0) | 0.996 |
| < 40 | 24 (4.3) | 54 (4.8) | | 18 (3.3) | 20 (3.7) | |
| 40–49 | 60 (10.8) | 116 (10.4) | | 63 (11.5) | 60 (11.0) | |
| 50–59 | 192 (34.4) | 378 (33.9) | | 145 (26.5) | 146 (26.7) | |
| 60–69 | 189 (33.9) | 402 (36.0) | | 215 (39.3) | 216 (39.5) | |
| AQ7 > 70 | 93 (16.7) | 166 (14.9) | | 106 (19.4) | 105 (19.2) | |
| Pack-years of smoking | | | 0.030 | | | 0.764 |
| 0 | 231 (41.4) | 536 (48.0) | | 228 (41.7) | 246 (45.0) | |
| < 15 | 60 (10.8) | 130 (11.7) | | 59 (10.8) | 62 (11.3) | |
| < 30 | 73 (13.1) | 144 (12.9) | | 77 (14.1) | 76 (13.9) | |
| < 45 | 84 (15.1) | 138 (12.4) | | 81 (14.8) | 73 (13.4) | |
| > 45 | 106 (19.0) | 161 (14.4) | | 96 (17.6) | 85 (15.5) | |
| Unknown | 4 (0.7) | 38 (0.6) | | 6 (1.1) | 5 (0.9) | |
| Daily ethanol consumption (g/day) | | | 0.025 | | | 0.036 |
| 0 | 225 (40.3) | 475 (42.6) | | 186 (34.0) | 216 (39.5) | |
| < 5 | 69 (12.4) | 145 (13.0) | | 70 (12.8) | 83 (15.2) | |
| < 23 | 98 (17.6) | 231 (20.7) | | 110 (20.1) | 107 (19.6) | |
| < 46 | 73 (13.1) | 127 (11.4) | | 66 (12.1) | 62 (11.3) | |
| > 46 | 85 (15.2) | 114 (10.2) | | 114 (20.8) | 78 (14.3) | |
| Unknown | 8 (1.4) | 63 (1.5) | | 1 (0.2) | 1 (0.2) | |
| Current BMI in categories (kg/m$^2$) | | | 0.453 | | | 0.450 |
| < 22.5 | 238 (42.7) | 473 (42.4) | | 255 (46.6) | 257 (47.0) | |
| < 25 | 185 (33.2) | 358 (32.1) | | 173 (31.6) | 170 (31.1) | |
| < 27.5 | 83 (14.9) | 197 (17.7) | | 76 (13.9) | 86 (15.7) | |
| > 27.5 | 47 (8.4) | 80 (7.2) | | 42 (7.7) | 30 (5.5) | |
| Unknown | 5 (0.9) | 8 (0.7) | | 1 (0.2) | 4 (0.7) | |
| Daily physical activity in any intensity (h/day) | | | 0.263 | | | 0.012 |
| None | 143 (25.6) | 263 (23.6) | | 131 (24.0) | 101 (18.5) | |
| < 0.5 | 240 (43.0) | 459 (41.1) | | 266 (48.6) | 256 (46.8) | |
| > 0.5 | 175 (31.4) | 394 (35.3) | | 150 (27.4) | 190 (34.7) | |
| Family history of CRC in first-degree relative | | | 0.027 | | | 0.021 |
| None | 494 (88.5) | 1025 (91.9) | | 461 (84.3) | 487 (89.0) | |
| Yes | 64 (11.5) | 91 (8.2) | | 86 (15.7) | 60 (11.0) | |
| Dietary folate intake (maximum–minimum) (μg) | | | 0.436 | | | 0.009 |
| Lowest tertile (152.2–270.1/172.9–336.3) | 197 (35.3) | 367 (32.9) | | 206 (37.8) | 182 (33.3) | |
| Medium tertile (270.3–351.1/336.8–430.9) | 167 (29.9) | 367 (32.9) | | 202 (37.1) | 181 (33.1) | |
| Highest tertile (351.1–975.1/431.2–937.0) | 184 (33.0) | 367 (32.9) | | 135 (24.8) | 181 (33.1) | |
| Unknown | 10 (1.8) | 15 (1.3) | | 2 (0.4) | 3 (0.6) | |
| Reason for visit | | | < 0.001 | | | < 0.001 |
| Patient discretion | 93 (16.7) | 333 (29.8) | | 65 (11.9) | 91 (16.6) | |
| Family recommendation | 116 (20.8) | 175 (15.7) | | 90 (16.5) | 45 (8.2) | |
| Referral from other clinic | 239 (42.8) | 280 (25.1) | | 237 (43.3) | 177 (32.4) | |
| Secondary screening after primary screening | 102 (18.3) | 301 (27.0) | | 104 (19.0) | 168 (30.7) | |
| Others | 3 (0.5) | 5 (0.5) | | 5 (0.9) | 3 (0.6) | |
| Unknown | 5 (0.9) | 22 (2.0) | | 46 (8.4) | 63 (11.5) | |

CRC, colorectal cancer.

association. Supplementary Table 2 (Supplemental digital content 1, *http://links.lww.com/EJCP/A32*) shows the distribution of the aggregate number of alleles in the six selected loci and their ORs in the derivation and replication population. The larger the number of risk alleles, the higher the estimated ORs in both age-adjusted and multivariate models (both $P$ for trend $< 0.001$) in the derivation population. However, a similar significant association was not observed among the replication population ($P = 0.217$).

To avoid insufficient numbers of participants because of extensive categorization and the resulting instability of risk estimates, we categorized participants into three risk score groups: group 1 (1–4 risk alleles), group 2 (5–7 risk alleles), and group 3 (≥8 risk alleles). As shown in Table 4, among the derivation population, ORs for group 2 and group 3 compared with group 1 were 1.40 (1.07–1.83) and 2.23 (1.50–3.30), with this trend being significant ($P$ for trend $< 0.001$). Although a significant trend was not observed among the replication population ($P$ for trend $= 0.669$), a significant association remained among the combined total population ($P$ for trend $< 0.001$).

Finally, we compared the risk prediction capacities of models of (i) a genetic risk score-only model, (ii) an established risk factors model, and (iii) a combination of (i) and (ii) according to the three study populations. As shown in Fig. 1a, AUC for the respective models in the

**Table 3 Associations between six selected loci and colorectal cancer risk in the derivation and replication population**

| | Derivation | | | | | | Replication | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Per-allele model | | | | Per-allele model | | |
| dbSNP rs numbers | Cases/controls (*n*) | RAF in controls | HWE *P* in controls | Odds ratio[a] | 95% CI | *P*-value | Cases/controls (*n*) | Odds ratio[a] | 95% CI | *P*-value |
| rs6983267[b] | | 0.353 | 0.974 | 1.16 | 1.00–1.35 | 0.048[b] | | 1.02 | 0.86–1.21 | 0.830 |
| TT | 213/468 | | | | | | 223/222 | | | |
| TG | 257/509 | | | | | | 239/246 | | | |
| GG | 88/139 | | | | | | 85/79 | | | |
| rs4779584[b] | | 0.821 | 0.426 | 1.20 | 0.98–1.46 | 0.076[b] | | 1.26 | 1.00–1.58 | 0.046[b] |
| CC | 9/37 | | | | | | 16/16 | | | |
| TC | 147/311 | | | | | | 133/168 | | | |
| TT | 402/768 | | | | | | 398/363 | | | |
| rs4444235[b] | | 0.582 | 0.218 | 1.20 | 1.04–1.40 | 0.015[b] | | 1.00 | 0.84–1.18 | 0.965 |
| TT | 82/185 | | | | | | 81/85 | | | |
| CT | 254/563 | | | | | | 269/260 | | | |
| CC | 222/368 | | | | | | 197/202 | | | |
| rs9929218[b] | | 0.177 | 0.992 | 1.17 | 0.97–1.41 | 0.092[b] | | 0.84 | 0.67–1.05 | 0.126 |
| GG | 352/756 | | | | | | 386/359 | | | |
| GA | 188/325 | | | | | | 145/172 | | | |
| AA | 18/35 | | | | | | 16/16 | | | |
| rs10936599[b] | | 0.344 | 0.244 | 1.14 | 0.98–1.33 | 0.090[b] | | 1.05 | 0.88–1.25 | 0.618 |
| TT | 221/472 | | | | | | 216/224 | | | |
| TC | 258/521 | | | | | | 263/258 | | | |
| CC | 79/123 | | | | | | 68/65 | | | |
| rs16969681[b] | | 0.468 | 0.795 | 1.24 | 1.07–1.44 | 0.004[b] | | 1.19 | 1.00–1.40 | 0.046[b] |
| CC | 113/314 | | | | | | 140/118 | | | |
| CT | 310/560 | | | | | | 271/268 | | | |
| TT | 135/242 | | | | | | 68/65 | | | |

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; RAF, risk allele frequency.
[a]Odds ratios were estimated from conditional logistic regression models considering age–sex matching in the model.
[b]Six loci were selected on the basis of a $P < 0.1$ in the per-allele model.

**Table 4 Association between colorectal cancer risk and risk scores according by allele number group**

| | Derivation | | Replication | | Total | |
|---|---|---|---|---|---|---|
| Risk score group (number of risk alleles) | Cases/controls (*n*) | Multivariate OR (95% CI)[a] | Cases/controls (*n*) | Multivariate OR (95% CI)[a] | Cases/controls (*n*) | Multivariate OR (95% CI)[a] |
| Group 1 (0–4) | 110/309 | 1.00 (reference) | 131/153 | 1.00 (reference) | 241/462 | 1.00 (reference) |
| Group 2 (5–7) | 358/686 | 1.40 (1.07–1.83) | 338/316 | 1.14 (0.84–1.54) | 696/1002 | 1.29 (1.06–1.56) |
| Group 3 (8–12) | 90/121 | 2.23 (1.50–3.30) | 78/78 | 1.06 (0.69–1.63) | 168/199 | 1.63 (1.23–2.15) |
| Trend *P* | | < 0.001 | | 0.669 | | < 0.001 |

CI, confidence interval; OR, odds ratio.
[a]Multivariate models adjusted for age, referral pattern, current BMI, smoking, alcohol consumption, regular exercise, family history of colorectal cancer in a first-degree relative, and dietary folate intake.

derivation population was 0.6046, 0.7009, and 0.7167, with these differences in prediction with each pairwise comparison being significant ($P < 0.001$). Although the combined total population also showed significant differences with each pairwise comparison ($P < 0.001$), the replication population did not show a significant difference between models 2 and 3 ($P = 0.5607$) (Fig. 1b and c).

## Discussion

In this study, we observed that six of 23 GWAS-identified CRC risk variants were associated suggestively with CRC risk in a Japanese population. These six variants contributed toward CRC risk among this population with a PAF of 27.2, indicating that they had a considerable population impact. Further, we used these six selected variants to develop a genetic predictor of CRC risk. The combination of this genetic risk score with established risk factors for CRC yielded a risk model with adequate discrimination power of more than 70% of AUC. These results indicate that this genetic risk model can be used to stratify patients by their degree of risk of CRC, and can thus be used for targeted cancer prevention.

We aimed to replicate GWAS-identified loci in a Japanese population. Our results were mostly but not completely consistent with the findings of a very recent report from the Asian Colorectal Cancer Consortium, of which we are members (Jia *et al.*, 2013). The loci showing inconsistency were rs3802842 in 11q23 [minor allele frequency (MAF): 0.41 in Asian Colorectal Cancer Consortium and 0.32 in this study], rs10795668 in 10q14 (MAF: 0.38 and 0.40), rs4939827 in *SMAD7* (MAF: 0.26

and 0.21), rs10411210 in 19q13 (MAF: 0.19 and 0.14), rs961253 in 20p12 (MAF: 0.09 and 0.11), and rs6687758 in 1q41 (MAF: 0.22 and 0.29). Some of these were differences in MAF, indicating potential heterogeneity by ethnicity within Asian populations, and might be a reason for this inconsistency. Further replication using a similar approach among individual countries is warranted.

Although each of the variants selected for the risk score is relatively common among populations (risk allele frequency in controls range from 0.172 to 0.468; Table 3) and the effect size of each individual variant is small, we speculated that the combination of such alleles together may be useful in developing a prediction model. Although genetic risk factors cannot be changed, an understanding of the genetic risk background may enable the modification of established risk factors in high-risk groups (primary prevention) or secondary prevention. For instance, it might be useful to calibrate age at initial screening and the frequency of screening according to the individual's genetic risk of CRC. The application of these genetic risk factors to individualized prevention warrants further investigation.

Our study had several methodological strengths. First, it was carried out in a single region in central Japan within the framework of the HERPACC study, with a considerable number of participants and high response rates (95%) to the completion of questionnaires and provision of blood. Second, potential known confounding factors such as age, sex, and lifestyle factors were adjusted for by matching and statistical adjustment. Third, given that our allele frequencies were comparable with those reported previously in public databases, such as HapMap JPT (*http://www.ncbi.nlm.nih.gov/snp*), bias in the distribution of selected polymorphisms was negligible. Fourth, as the genotypes of selected variants do not change throughout life, we can assume that the impact of these polymorphisms is subject to Mendelian randomization.

The present study is based on hospital-based case–control studies, and is thus subject to several methodological limitations. The values for self-reported lifestyle factors might have been inaccurate. Any such misclassification would be assumed to be nondifferential, however, and likely to underestimate the association. In addition, the sample size of our study was limited, particularly in the stratified risk group. Our findings therefore require validation in larger studies.

In conclusion, we evaluated 23 genetic variants for their association with the risk of CRC in a Japanese population and constructed a genetic risk score using a combination of six selected variants. Combination of this score with established risk factors provided a model that distinguishes individuals at high risk for CRC and may be useful in individualized prevention.

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### Conflicts of interest

There are no conflicts of interest.

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# Genetic variants of *SLC17A1* are associated with cholesterol homeostasis and hyperhomocysteinaemia in Japanese men

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**Hyperuricaemia is an undisputed and highly predictive biomarker for cardiovascular risk. *SLC17A1*, expressed in the liver and kidneys, harbours potent candidate single nucleotide polymorphisms that decrease uric acid levels. Therefore, we examined *SLC17A1* polymorphisms (rs1165196, rs1179086, and rs3757131), which might suppress cardiovascular risk factors and that are involved in liver functioning, via a large-scale pooled analysis of the Japanese general population in a cross-sectional study. Using data from the Japan Multi-Institutional Collaborative Cohort Study, we identified 1842 participants of both sexes, 35–69-years-old, having the requisite data, and analysed their SLC17A1 genotypes. In men, logistic regression analyses revealed that minor alleles in SLC17A1 polymorphisms (rs1165196 and rs3757131) were associated with a low-/high-density lipoprotein cholesterol ratio >2.0 (rs1165196: odds ratio [OR], 0.703; 95% confidence interval [CI], 0.536–0.922; rs3757131: OR, 0.658; 95% CI, 0.500–0.866), and with homocysteine levels of >10.0 nmol/mL (rs1165196: OR, 0.544; 95% CI, 0.374–0.792; rs3757131: OR, 0.509; 95% CI, 0.347–0.746). Therefore, these polymorphisms had dominant negative effects on cholesterol homeostasis and hyperhomocysteinaemia, in men, independent of alcohol consumption, physical activity, or daily energy and nutrition intake. Thus, genetic variants of SLC17A1 are potential biomarkers for altered cholesterol homeostasis and hyperhomocysteinaemia in Japanese men.**

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Cardiovascular diseases, including coronary heart disease, stroke, and peripheral arterial disease, are the most prevalent conditions and leading causes of mortality, worldwide. In addition, age, sex, overweight, hypertension, smoking, and diabetes are widely accepted as major risk factors for the development of cardiovascular disease[1]. Furthermore, recent studies have reported that hyperuricaemia[2–4] and elevated levels of homocysteine[5,6] are also associated with cardiovascular disease, and a significant positive correlation has been observed between the serum concentrations of uric acid and homocysteine[7].

In addition to the above-mentioned factors, altered lipid metabolism, such as the disruption of cholesterol homeostasis via altered liver function, is another major risk factor for cardiovascular disease[8]. Cholesterol is a critical lipid that is a component of biological cell membranes and is an important precursor of steroid hormones and bile acids. Unfortunately, the disruption of cholesterol homeostasis significantly increases the risk of premature cardiovascular disease[9]. To monitor for this disruption, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) are among the most commonly used clinical biomarkers[10], with high LDL-C and low HDL-C levels indicating major risks for the development of coronary heart disease[11]. In addition, prospective studies investigating different racial and ethnic populations have confirmed that HDL-C is a strong, consistent, and independent predictive factor for the incidence of cardiovascular disease[10,12]. Furthermore, the Framingham study demonstrated that a combination of high LDL-C and low HDL-C levels is a strong predictor of the relative risk of cardiovascular events[13]. Moreover, the LDL-C/HDL-C ratio is a precise marker of cholesterol homeostasis, and can help predict cardiovascular events[14].

Previous genome-wide association studies have examined the genetic factors that control serum uric acid concentrations, and have identified 14 candidate causal single nucleotide polymorphisms (SNPs) and two pathways involving the *PKD2*, *SLC17A1*, *SLC17A3*, *SLC17A4*, and *SLC2A9* genes[15]. One of these genes (*SLC17A1*) encodes the solute carrier family 17 (organic anion transporter), member 1, which is also known as sodium phosphate transport protein 1. This protein is expressed in the sinusoidal membrane of hepatocytes and in the proximal tubules in the kidneys[16]; in the proximal tubules, it acts as a renal transporter of uric acid and mediates the co-transport of sodium and inorganic phosphate[17]. Additionally, *SLC17A1* SNPs are associated with kidney function indicators, such as suppressed serum uric levels and gout, in Japanese men[18]. Because hyperuricaemia is an undisputed and highly effective biomarker for predicting cardiovascular risk, *SLC17A1* SNPs might also suppress cardiovascular risk factors. However, whether *SLC17A1* SNPs, associated with liver function, are also associated with cardiovascular risk factors remains unknown. Cholesterol and homocysteine are both metabolised in the liver; therefore, we analysed three *SLC17A1* SNPs (rs1165196, rs1179086, and rs3757131) to evaluate their association with cholesterol homeostasis and homocysteine levels, and their roles as predictors of cardiovascular events. This analysis was conducted as a cross-sectional study using a large-scale pooled analysis of the Japanese general population.

## Results

**Participant characteristics.** Table 1 shows the participants' characteristics, including their anthropometric measures, blood chemistry data, questionnaire responses, total energy and macronutrient intake, and distribution of the three polymorphisms according to sex. The mean age of the included men was 55.6 years, compared to 54.8 years for the women. Significant differences were not observed in comparisons of the metabolic equivalents (METs) and polymorphism allele frequency data between the men and women. Furthermore, the allele frequencies for the polymorphisms were similar to those in other Japanese populations[18], and were in agreement with the Hardy-Weinberg equilibrium (rs1165196 for men: $\chi^2 = 0.571$, $p = 0.319$; rs1165196 for women: $\chi^2 = 1.241$, $p = 0.265$; rs1179086 for men: $\chi^2 = 0.023$, $p = 0.877$; rs1179086 for women: $\chi^2 = 1.350$, $p = 0.245$; rs3757131 for men: $\chi^2 = 0.284$, $p = 0.593$; rs3757131 for women: $\chi^2 = 1.053$, $p = 0.304$).

**Associations between polymorphisms and participant characteristics, according to sex.** The distributions of the three polymorphism genotypes are listed in Tables 2–4. For each genotype, the major homozygotes had significantly higher levels of uric acid, compared to the heterozygotes and minor homozygotes, in both sexes. Similarly, the major homozygous alleles in rs1165196 and rs3757131 exhibited high homocysteine and low folic acid levels in men. We also compared the major homozygotes with the heterozygotes and minor homozygotes. In men, rs1165196 and rs3757131 exhibited similar statistical significance. When compared to the major homozygous alleles, the combined minor genotype (TC + CC in rs1165196 and CT + TT in rs3757131) was associated with significantly higher folic acid levels, and significantly lower uric acid and homocysteine levels. In contrast, women did not demonstrate any significant variables associated with uric acid and homocysteine levels in each genotype.

**Associations of polymorphism genotypes with LDL-C/HDL-C ratios and homocysteine levels.** Table 5 shows the proportion of participants with LDL-C/HDL-C ratios $\leq 2.0$ and those $> 2.0$, according to the polymorphism genotypes. For the logistic regression analysis, the major homozygous genotypes were used as the reference group and the heterozygous and minor homozygous genotypes were used as the exposed group in the dominant model. When we combined the TC and CC genotypes for rs1165196 as the low-risk genotype comparison group (assuming a dominant effect for the variant C allele), the

| | Men (n = 995) | | Women (n = 847) | | |
|---|---|---|---|---|---|
| | Mean ± SD (%) | CV (%) | Mean ± SD (%) | CV (%) | p-value |
| Age (years) | 55.6 ± 8.8 | 15.8 | 54.8 ± 8.5 | 15.5 | 0.052 |
| BMI (kg/m$^2$) | 23.8 ± 3.1 | 13.0 | 22.9 ± 3.3 | 14.4 | <0.001 |
| Triglycerides (mg/dL) | 120.7 ± 61.0 | 50.5 | 92.2 ± 49.3 | 53.4 | <0.001 |
| Total cholesterol (mg/dL) | 204.3 ± 31.4 | 15.3 | 216.9 ± 35.4 | 16.3 | <0.001 |
| LDL-C (mg/dL) | 120.6 ± 29.7 | 24.6 | 129.5 ± 33.7 | 26.0 | <0.001 |
| HDL-C (mg/dL) | 59.5 ± 15.7 | 26.3 | 68.9 ± 15.2 | 22.0 | <0.001 |
| LDL-C/HDL-C | 2.17 ± 0.78 | 35.9 | 2.00 ± 0.77 | 38.5 | <0.001 |
| Uric acid (mg/dL) | 6.03 ± 1.17 | 19.4 | 4.43 ± 0.99 | 22.3 | <0.001 |
| Folic acid (ng/mL)* | 8.61 ± 2.25 | 26.1 | 9.75 ± 3.26 | 33.4 | <0.001 |
| Homocysteine (nmol/mL)** | 9.37 ± 3.48 | 37.1 | 7.36 ± 2.51 | 34.1 | <0.001 |
| Energy intake (kcal/day) | 1,930 ± 360 | 18.6 | 1,547 ± 251 | 16.2 | <0.001 |
| Protein intake (g/day) | 55.8 ± 11.2 | 20.0 | 51.3 ± 10.5 | 20.4 | <0.001 |
| Fat intake (g/day) | 41.8 ± 10.5 | 25.1 | 44.6 ± 11.7 | 26.2 | <0.001 |
| Carbohydrate intake (g/day) | 278.6 ± 70.3 | 25.2 | 216.4 ± 45.1 | 20.8 | <0.001 |
| METs (h/day) | 14.7 ± 14.0 | 95.2 | 15.0 ± 13.7 | 91.3 | 0.693 |
| Alcohol drinking | | | | | |
| 0 g/d | 393 (39.4) | | 593 (70.0) | | |
| 0.1–22.9 g/d | 414 (41.6) | | 238 (28.0) | | <0.001 |
| 23.0–45.9 g/d | 122 (12.2) | | 15 (1.77) | | |
| ≥46.0 g/d | 66 (6.63) | | 1 (0.11) | | |
| Smoking | | | | | |
| Current | 272 (27.3) | | 45 (5.31) | | <0.001 |
| Former | 433 (43.5) | | 25 (2.95) | | |
| Never | 290 (29.1) | | 777 (91.7) | | |
| rs1165196 | | | | | |
| TT | 694 (69.7) | | 612 (72.2) | | 0.496 |
| TC | 271 (27.2) | | 211 (24.9) | | |
| CC | 30 (3.01) | | 24 (2.83) | | |
| rs1179086 | | | | | |
| AA | 542 (54.4) | | 467 (55.1) | | 0.634 |
| AT | 386 (38.7) | | 315 (37.1) | | |
| TT | 67 (6.73) | | 65 (7.67) | | |
| rs3757131 | | | | | |
| CC | 703 (70.6) | | 610 (72.0) | | 0.795 |
| CT | 264 (26.5) | | 213 (25.1) | | |
| TT | 28 (2.81) | | 24 (2.83) | | |

**Table 1. Clinical characteristics and genotype frequencies of the participants, according to sex.** Data are means ± standard deviation. CV, coefficient of variance BMI, body mass index; LDL-C, low-density lipoprotein cholesterol. HDL-C, high-density lipoprotein cholesterol; METs, metabolic equivalents. Sex-related differences were analysed using the *t*-test, Chi-square tests were used for smoking and alcohol habits and genotypes. *Men = 751, Women = 620, **Men = 719, Women = 606.

combined genotype (TC + CC) in men was associated with a significantly lower proportion of participants with an LDL-C/HDL-C ratio of >2.0 (OR, 0.703; 95% CI, 0.536–0.922; OR adjusted for age, body mass index [BMI], research area, alcohol consumption and smoking habits, 0.642; 95% CI, 0.483–0.853), relative to the TT genotype. The combined genotype (CT + TT) in rs3757131 was also associated with a significantly lower proportion of participants with an LDL-C/HDL-C ratio >2.0 (OR, 0.658; 95% CI, 0.500–0.866; OR adjusted for age, BMI, research area, alcohol consumption and smoking habits, 0.607; 95% CI, 0.455–0.808), relative to the major homozygous alleles. When we compared the rs1165196 and rs3757131 alleles in men using an LDL-C/HDL-C ratio of >2.0, rs1165196 had a significantly lower

| Sex | Men | | | | | | Women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype (N) | TT (694) | TC (271) | CC (30) | p* | TC + CC | p** (vs. TT) | TT (612) | TC (211) | CC (24) | p* | TC + CC | p** (vs. TT) |
| Age (years) | 55.5 ± 8.8 | 55.5 ± 8.9 | 58.2 ± 7.9 | 0.271 | 55.7 ± 8.8 | 0.730 | 54.7 ± 8.7 | 54.8 ± 8.1 | 58.2 ± 7.0 | 0.131 | 55.1 ± 8.1 | 0.491 |
| BMI (kg/m$^2$) | 23.7 ± 3.0 | 24.1 ± 3.1 | 23.7 ± 2.9 | 0.145 | 24.1 ± 3.1 | 0.062 | 22.9 ± 3.3 | 22.7 ± 3.2 | 23.5 ± 4.1 | 0.483 | 22.8 ± 3.3 | 0.570 |
| Triglycerides (mg/dL) | 120.6 ± 59.4 | 121.8 ± 64.9 | 112.8 ± 64.2 | 0.741 | 120.9 ± 64.8 | 0.946 | 93.4 ± 50.0 | 88.3 ± 45.8 | 94.0 ± 59.5 | 0.433 | 88.9 ± 47.3 | 0.238 |
| Total cholesterol (mg/dL) | 204.2 ± 31.4 | 204.0 ± 31.5 | 211.0 ± 32.1 | 0.503 | 204.7 ± 31.6 | 0.818 | 217.7 ± 35.1 | 214.0 ± 36.9 | 222.0 ± 27.1 | 0.324 | 214.8 ± 36.0 | 0.287 |
| LDL-C (mg/dL) | 121.1 ± 29.9 | 119.3 ± 29.2 | 121.5 ± 30.0 | 0.690 | 119.5 ± 29.2 | 0.441 | 129.8 ± 33.1 | 128.1 ± 36.0 | 133.8 ± 27.1 | 0.668 | 128.7 ± 35.2 | 0.665 |
| HDL-C (mg/dL) | 58.9 ± 15.5 | 60.3 ± 16.0 | 66.8 ± 14.3 | 0.017 | 60.9 ± 16.0 | 0.062 | 69.1 ± 15.4 | 68.1 ± 15.0 | 69.4 ± 11.4 | 0.705 | 68.3 ± 14.6 | 0.454 |
| LDL-C/HDL-C | 2.20 ± 0.79 | 2.12 ± 0.75 | 1.92 ± 0.71 | 0.069 | 2.10 ± 0.75 | 0.053 | 2.00 ± 0.77 | 2.00 ± 0.81 | 1.99 ± 0.58 | 0.999 | 2.00 ± 0.79 | 0.977 |
| Uric acid (mg/dL) | 6.10 ± 1.14 | 5.87 ± 1.21 | 5.94 ± 1.40 | 0.020 | 5.88 ± 1.23 | 0.005 | 4.49 ± 1.00 | 4.27 ± 0.92 | 4.28 ± 1.22 | 0.016 | 4.27 ± 0.95 | 0.004 |
| Folic acid (ng/mL)* | 8.42 ± 2.13 | 8.98 ± 2.51 | 9.69 ± 1.80 | 0.001 | 9.04 ± 2.46 | 0.001 | 9.71 ± 3.08 | 9.84 ± 3.74 | 9.84 ± 2.97 | 0.912 | 9.84 ± 3.66 | 0.667 |
| Homocysteine (nmol/mL)** | 9.66 ± 3.85 | 8.70 ± 2.34 | 8.51 ± 1.53 | 0.003 | 8.69 ± 2.29 | <0.001 | 7.36 ± 2.72 | 7.38 ± 1.92 | 7.20 ± 1.92 | 0.957 | 7.36 ± 1.91 | 0.982 |
| Energy intake (kcal/day) | 1,929 ± 356 | 1,933 ± 376 | 1,919 ± 284 | 0.976 | 1,931 ± 367 | 0.925 | 1,546 ± 248 | 1,552 ± 257 | 1,542 ± 267 | 0.957 | 1,551 ± 258 | 0.813 |
| Protein intake (g/day) | 56.0 ± 11.4 | 55.2 ± 11.0 | 55.1 ± 9.52 | 0.587 | 55.2 ± 10.9 | 0.303 | 51.4 ± 10.6 | 51.4 ± 10.3 | 50.2 ± 12.3 | 0.866 | 51.3 ± 10.5 | 0.893 |
| Fat intake (g/day) | 41.9 ± 10.8 | 41.4 ± 9.89 | 43.1 ± 8.78 | 0.654 | 41.6 ± 9.78 | 0.676 | 44.5 ± 11.7 | 45.0 ± 11.7 | 45.1 ± 11.8 | 0.830 | 45.0 ± 11.7 | 0.542 |
| Carbohydrate intake (g/day) | 279.0 ± 71.1 | 278.8 ± 69.8 | 267.8 ± 56.8 | 0.695 | 277.7 ± 68.6 | 0.797 | 215.9 ± 43.9 | 218.6 ± 47.4 | 211.1 ± 56.6 | 0.630 | 217.9 ± 48.3 | 0.565 |
| METs (h/day) | 14.9 ± 14.1 | 14.4 ± 14.5 | 13.7 ± 8.7 | 0.847 | 14.4 ± 14.0 | 0.606 | 15.0 ± 13.5 | 15.2 ± 14.3 | 13.2 ± 11.4 | 0.790 | 15.0 ± 14.1 | 0.976 |

**Table 2. The distribution of the participants, according to sex, for rs1165196.** Data are presented as means ± standard deviation; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; METs, metabolic equivalents. Genotype differences were analysed using one-way analysis of variance*, or the *t*-test**. *Men (TT = 524, TC = 208, CC = 19) Women (TT= 439, TC = 161, CC = 20). **Men (TT = 502, TC = 201, CC = 16) Women (TT = 431, TC = 156, CC = 19).

frequency of the C allele, whereas rs3757131 had a significantly lower frequency of the T allele (T vs. C alleles in rs1165196: OR, 0.726; 95% CI, 0.573–0.920; C vs. T alleles in rs3757131: OR, 0.689; 95% CI, 0.542–0.876). Furthermore, we found that physical activity and daily energy or nutritional intake did not affect the LDL-C/HDL-C ratio in rs1165196 or rs3757131. In contrast, the proportions of women with an LDL-C/HDL-C ratio of >2.0 were not significantly different when the various polymorphism genotypes were compared.

Similar analyses were performed for homocysteine levels (Table 6). In men, when we compared the alleles for rs1165196 and rs3757131 using homocysteine levels of >10.0 nmol/mL, rs1165196 had a significantly lower frequency of the C allele, whereas rs3757131 had a significantly lower frequency of the T allele (T vs. C alleles in rs1165196: OR, 0.589; 95% CI, 0.420–0.826; C vs. T alleles in rs3757131: OR, 0.564; 95% CI, 0.399–0.797). Moreover, we confirmed that the tendency attenuated the relationship between hyperhomocysteinaemia and the *SLC17A1* polymorphisms, after adjusting for folic acid levels. In contrast, the proportion of women with homocysteine levels >10.0 nmol/mL were not significantly different when we compared the various polymorphism genotypes.

## Discussion

High levels of uric acid are historically associated with gout, although recent studies have revealed that they might also be associated with cardiovascular disease and the incidence of coronary heart disease, hypertension, stroke, metabolic syndrome, and other disorders. Therefore, patients presenting with high uric acid levels should be screened and treated for comorbid cardiovascular risk factors[19]. Furthermore, a previous genome-wide association study of uric acid transporters revealed that several SNPs were significant genetic determinants of uric acid levels[20]. In that study, the *SLC17A1* polymorphisms exhibited an association with low serum levels of uric acid. The polymorphisms of interest for *SLC17A1* are rs1165196, rs1179086, and rs3757131; these polymorphisms are known to be associated with low levels of uric acid in Japanese men[18]. However, because these three SNPs exhibit strong linkage disequilibrium[18], determining which SNP is responsible for the functional changes in SLC17A1 is difficult. In agreement with these previous studies, our findings revealed significant associations between the *SLC17A1* polymorphisms and uric acid levels in Japanese men. Furthermore, in the present study, we also compared the associations between various *SLC17A1* genotypes and cardiovascular risk factors via altered cholesterol homeostasis and hyperhomocysteinaemia, after combining the heterozygous and minor homozygous alleles due to the small number of minor homozygotes. Our results indicated that, among male participants, the presence of minor alleles in rs1165196 and rs3757131 were associated with significantly lower homocysteine levels, compared to the major alleles. In addition, the odds of having a high LDL-C/

| Sex | Men | | | | | | Women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype (N) | AA (542) | AT (386) | TT (67) | p* | AT + TT | p** (vs. AA) | AA (467) | AT (315) | TT (65) | p* | AT + TT | p** (vs. AA) |
| Age (years) | 55.3 ± 8.9 | 55.7 ± 8.8 | 56.5 ± 8.3 | 0.537 | 55.9 ± 8.7 | 0.371 | 55.1 ± 8.5 | 54.5 ± 8.4 | 54.0 ± 8.9 | 0.501 | 54.4 ± 8.5 | 0.289 |
| BMI (kg/m²) | 23.6 ± 2.9 | 24.0 ± 3.2 | 23.7 ± 2.9 | 0.161 | 24.0 ± 3.2 | 0.086 | 22.9 ± 3.3 | 22.8 ± 3.3 | 23.1 ± 3.4 | 0.844 | 22.9 ± 3.3 | 0.850 |
| Triglycerides (mg/dL) | 118.3 ± 58.4 | 125.2 ± 64.7 | 114.6 ± 59.6 | 0.168 | 123.6 ± 64.0 | 0.175 | 94.7 ± 52.4 | 87.5 ± 41.7 | 96.6 ± 58.6 | 0.099 | 89.0 ± 45.1 | 0.096 |
| Total cholesterol (mg/dL) | 204.0 ± 30.9 | 203.9 ± 31.8 | 209.1 ± 33.4 | 0.438 | 204.7 ± 32.1 | 0.741 | 218.7 ± 35.5 | 214.8 ± 35.7 | 214.2 ± 32.9 | 0.258 | 214.7 ± 35.2 | 0.101 |
| LDL-C (mg/dL) | 121.0 ± 29.2 | 119.8 ± 30.2 | 122.1 ± 31.2 | 0.769 | 120.2 ± 30.3 | 0.662 | 130.2 ± 33.4 | 129.2 ± 34.5 | 125.7 ± 32.2 | 0.599 | 128.6 ± 34.1 | 0.501 |
| HDL-C (mg/dL) | 59.3 ± 15.7 | 59.0 ± 15.8 | 64.0 ± 14.3 | 0.049 | 59.7 ± 15.7 | 0.662 | 69.5 ± 15.8 | 68.0 ± 14.5 | 69.0 ± 14.3 | 0.403 | 68.2 ± 14.4 | 0.212 |
| LDL-C/ HDL-C | 2.19 ± 0.79 | 2.17 ± 0.78 | 2.00 ± 0.70 | 0.198 | 2.15 ± 0.77 | 0.417 | 1.99 ± 0.78 | 2.01 ± 0.78 | 1.93 ± 0.72 | 0.721 | 2.00 ± 0.77 | 0.964 |
| Uric acid (mg/dL) | 6.13 ± 1.18 | 5.94 ± 1.14 | 5.83 ± 1.26 | 0.016 | 5.92 ± 1.16 | 0.005 | 4.53 ± 0.98 | 4.31 ± 0.97 | 4.23 ± 1.04 | 0.002 | 4.30 ± 0.98 | 0.001 |
| Folic acid (ng/mL) | 8.44 ± 2.16 | 8.79 ± 2.39 | 9.05 ± 1.95 | 0.054 | 8.82 ± 2.34 | 0.021 | 9.78 ± 3.24 | 9.76 ± 3.37 | 9.53 ± 2.78 | 0.888 | 9.72 ± 3.28 | 0.838 |
| Homocysteine (nmol/mL)** | 9.53 ± 3.92 | 9.23 ± 2.95 | 8.63 ± 1.79 | 0.217 | 9.16 ± 2.83 | 0.156 | 7.19 ± 2.16 | 7.50 ± 2.98 | 7.86 ± 2.29 | 0.142 | 7.56 ± 2.88 | 0.076 |
| Energy intake (kcal/day) | 1,938 ± 350 | 1,921 ± 381 | 1,908 ± 308 | 0.687 | 1,919 ± 371 | 0.413 | 1,553 ± 253 | 1,543 ± 251 | 1,530 ± 241 | 0.740 | 1,541 ± 249 | 0.494 |
| Protein intake (g/day) | 56.1 ± 11.2 | 55.5 ± 11.4 | 54.4 ± 10.1 | 0.407 | 55.3 ± 11.2 | 0.272 | 51.4 ± 10.2 | 51.5 ± 10.9 | 50.4 ± 11.3 | 0.748 | 51.3 ± 11.0 | 0.884 |
| Fat intake (g/day) | 41.8 ± 10.8 | 41.6 ± 9.94 | 42.3 ± 11.1 | 0.881 | 41.7 ± 10.1 | 0.891 | 44.3 ± 11.1 | 45.0 ± 12.1 | 45.9 ± 13.9 | 0.490 | 45.1 ± 12.4 | 0.290 |
| Carbohydrate intake (g/day) | 282.4 ± 69.7 | 274.8 ± 72.9 | 269.6 ± 57.6 | 0.152 | 274.1 ± 70.9 | 0.063 | 216.8 ± 44.8 | 216.7 ± 45.1 | 212.2 ± 48.6 | 0.728 | 216.0 ± 45.7 | 0.777 |
| METs (h/day) | 14.8 ± 13.7 | 14.5 ± 14.9 | 15.3 ± 12.3 | 0.899 | 14.6 ± 14.5 | 0.844 | 14.6 ± 13.2 | 15.3 ± 14.4 | 16.0 ± 13.9 | 0.596 | 15.5 ± 14.3 | 0.342 |

**Table 3. The distribution of participants, according to sex, for rs1179086.** Data are presented as means ± standard deviation; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; METs, metabolic equivalents. Genotype differences were analysed using one-way analysis of variance*, or the *t*-test**. *Men (TT = 412, TC = 294, CC = 45) Women (TT = 335, TC = 237, CC = 48). **Men (TT = 399, TC = 281, CC = 39) Women (TT = 330, TC = 231, CC = 65).

HDL-C ratio or hyperhomocysteinaemia were significantly lower for patients with the minor alleles (vs. the major homozygous alleles), both before and after adjusting for age, BMI, research area, daily energy and nutritional intake, and alcohol consumption and smoking habits.

In healthy humans, uric acid is excreted in the urine, although kidney disease can impair this excretion route, leading to hyperuricaemia. In addition, hyperuricaemia can be caused by the increased generation of uric acid. Furthermore, exposure to lead or diets involving excessive intake of alcohol, purine nucleotides, protein, and carbohydrates can also contribute to high levels of uric acid[3]. However, in the present study, we found that alcohol consumption, physical activity, and daily energy or nutritional intake were not different for each SNP. Another potential mechanism leading to high uric acid levels is mutation(s) in the genes coding for the glucokinase regulatory protein; PDZ domain-containing proteins; and transporters of organic anions, glucose, ATP-binding cassettes, or monocarboxylic acid[15,20,21]; these transport proteins are key regulators of uric acid levels. Interestingly, *SLC17A1* is the gene involved in the renal transport of uric acid, and has been associated with low uric acid levels[18].

HDL-C is an undisputed and highly predictive biomarker of cardiovascular risk. In this context, HDL-C is central to reverse cholesterol transport, which is the cardioprotective mechanism by which cholesterol, synthesized in the peripheral tissues, is transported to the liver for degradation or recycling. Various factors can result in elevated HDL-C levels, including smoking, alcohol consumption, exercise, and statin therapy. However, epidemiological studies have demonstrated that uric acid levels are inversely correlated with HDL-C levels[22], and that there is a strong relationship between LDL-C levels and the incidence of atherosclerotic cardiovascular disease[10,23]. Another recent study has demonstrated that the LDL-C/HDL-C ratio is a precise marker of cholesterol homeostasis, and can be used to predict the risk of cardiovascular events[24]. Nicholls *et al.* showed that an LDL-C/HDL-C ratio >2.0 was associated with plaque progression (despite statin usage), whereas a ratio <1.5 was significantly associated with plaque regression[25]. Many studies have indicated that cholesterol homeostasis is a major mechanism for

| Sex | Men | | | | | | Women | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype (N) | CC (703) | CT (264) | TT (28) | p* | CT+ TT | p** (vs. CC) | CC (610) | CT (213) | TT (24) | p* | CT+ TT | p** (vs. CC) |
| Age (years) | 55.5 ± 8.8 | 55.4 ± 8.9 | 58.0 ± 7.9 | 0.342 | 55.7 ± 8.8 | 0.808 | 54.7 ± 8.7 | 54.9 ± 8.0 | 57.5 ± 7.7 | 0.293 | 55.1 ± 8.0 | 0.496 |
| BMI (kg/m$^2$) | 23.7 ± 3.0 | 24.1 ± 3.2 | 23.6 ± 2.6 | 0.155 | 24.0 ± 3.1 | 0.073 | 22.9 ± 3.3 | 22.7 ± 3.2 | 23.3 ± 4.0 | 0.630 | 22.8 ± 3.3 | 0.570 |
| Triglycerides (mg/dL) | 120.6 ± 59.2 | 122.0 ± 65.6 | 111.9 ± 64.0 | 0.708 | 121.0 ± 65.4 | 0.932 | 93.1 ± 49.6 | 89.3 ± 47.3 | 94.1 ± 59.4 | 0.628 | 89.8 ± 48.5 | 0.394 |
| Total cholesterol (mg/dL) | 203.9 ± 31.4 | 204.6 ± 31.4 | 212.2 ± 32.8 | 0.391 | 205.3 ± 31.6 | 0.536 | 217.8 ± 35.1 | 213.8 ± 37.0 | 220.7 ± 27.7 | 0.310 | 214.5 ± 36.2 | 0.219 |
| LDL-C (mg/dL) | 121.0 ± 29.8 | 119.3 ± 29.3 | 122.7 ± 30.6 | 0.673 | 119.6 ± 29.4 | 0.501 | 129.9 ± 33.2 | 128.0 ± 35.9 | 131.1 ± 28.6 | 0.749 | 128.3 ± 35.2 | 0.530 |
| HDL-C (mg/dL) | 58.7 ± 15.4 | 60.8 ± 16.3 | 67.0 ± 14.2 | 0.007 | 61.4 ± 16.2 | 0.014 | 69.2 ± 15.4 | 67.8 ± 14.8 | 70.7 ± 12.7 | 0.449 | 68.1 ± 14.6 | 0.362 |
| LDL-C/ HDL-C | 2.20 ± 0.79 | 2.10 ± 0.76 | 1.94 ± 0.73 | 0.058 | 2.09 ± 0.76 | 0.032 | 2.00 ± 0.77 | 2.00 ± 0.80 | 1.93 ± 0.62 | 0.919 | 1.99 ± 0.79 | 0.962 |
| Uric acid (mg/dL) | 6.10 ± 1.15 | 5.86 ± 1.21 | 5.95 ± 1.42 | 0.015 | 5.87 ± 1.23 | 0.004 | 4.48 ± 1.00 | 4.29 ± 0.92 | 4.19 ± 1.19 | 0.029 | 4.28 ± 0.95 | 0.009 |
| Folic acid (ng/mL) | 8.43 ± 2.12 | 9.00 ± 2.52 | 9.72 ± 1.85 | 0.001 | 9.05 ± 2.48 | <0.001 | 9.70 ± 3.08 | 9.88 ± 3.74 | 9.71 ± 2.86 | 0.830 | 9.86 ± 3.65 | 0.572 |
| Homocysteine (nmol/mL)** | 9.67 ± 3.84 | 8.63 ± 2.29 | 8.60 ± 1.60 | 0.001 | 8.63 ± 2.25 | <0.001 | 7.38 ± 2.73 | 7.34 ± 1.92 | 7.18 ± 1.93 | 0.940 | 7.32 ± 1.91 | 0.803 |
| Energy intake (kcal/day) | 1,928 ± 355 | 1,934 ± 379 | 1,923 ± 294 | 0.973 | 1,933 ± 372 | 0.854 | 1,546 ± 247 | 1,550 ± 260 | 1,553 ± 267 | 0.979 | 1,550 ± 260 | 0.843 |
| Protein intake (g/day) | 56.0 ± 11.4 | 55.2 ± 11.0 | 55.0 ± 9.86 | 0.586 | 55.2 ± 10.9 | 0.303 | 51.3 ± 10.5 | 51.4 ± 10.4 | 50.5 ± 12.3 | 0.918 | 51.3 ± 10.6 | 0.957 |
| Fat intake (g/day) | 41.8 ± 10.8 | 41.6 ± 9.81 | 42.6 ± 8.92 | 0.891 | 41.7 ± 9.71 | 0.918 | 44.5 ± 11.7 | 45.0 ± 11.7 | 46.0 ± 11.6 | 0.720 | 45.1 ± 11.7 | 0.483 |
| Carbohydrate intake (g/day) | 279.2 ± 70.6 | 278.2 ± 70.8 | 268.1 ± 58.8 | 0.711 | 277.2 ± 69.7 | 0.683 | 216.1 ± 43.7 | 217.9 ± 47.9 | 212.3 ± 56.8 | 0.794 | 217.3 ± 48.8 | 0.718 |
| METs (h/day) | 14.9 ± 14.2 | 14.2 ± 14.1 | 13.1 ± 8.82 | 0.649 | 14.1 ± 13.6 | 0.396 | 14.9 ± 13.5 | 15.4 ± 14.4 | 12.8 ± 11.5 | 0.669 | 15.1 ± 14.1 | 0.840 |

**Table 4. The distribution of the participants, according to sex, for rs3757131.** Data are presented as means ± standard deviation; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; METs, metabolic equivalents. Genotype differences were analysed using one-way analysis of variance*, or the *t*-test**. *Men (TT = 530, TC = 204, CC = 17) Women (TT = 435, TC = 165, CC = 20). **Men (TT = 507, TC = 198, CC = 14) Women (TT = 427, TC = 160, CC = 19).

suppressing cardiovascular disease. In the present study, we used the LDL-C/HDL-C ratio to evaluate cholesterol homeostasis because it is a better predictor of future cardiovascular disease than are single lipid parameters (e.g., triglyceride, LDL-C, or HDL-C levels)[14]. However, we did not find a correlation between uric acid levels and cholesterol homeostasis for minor alleles in rs1165196 and rs3757131 (data not shown). These results suggest that the minor alleles in rs1165196 and rs3757131 had independent effects on uric acid levels and cholesterol homeostasis.

Various cardiovascular disease risk factors are reported to be related to homocysteine levels, including total cholesterol, other lipids, smoking, and the presence or absence of hypertension and diabetes mellitus. However, adjusting for these risk factors only weakly attenuates the strong relationship between homocysteine levels and mortality due to cardiovascular causes[5]. In addition, homocysteine levels are higher in patients with gout than in healthy controls, and uric acid levels are known to be correlated with homocysteine levels[22]. In contrast, hyperhomocysteinaemia is not correlated with uric acid levels in patients with gout, although there is an inverse association between homocysteine levels and renal function[26]. These reports indicate that hyperhomocysteinaemia, which may be related to uric acid levels and cardiovascular mortality, is clearly a cardiovascular risk factor, although the precise relationship remains unknown.

Interestingly, an association between hyperlipidaemia and hyperhomocysteinaemia has been suggested. In an animal model, hyperhomocysteinaemia inhibited reverse cholesterol transport by reducing circulating HDL levels, via inhibition of apoA-I protein synthesis, and enhanced HDL-C clearance[27]. Therefore, the liver may be a major organ involved in regulating homocysteine and cholesterol homeostasis[28]. These studies suggest that the interactions between homocysteine and HDL metabolism may be clinically important. Based on our findings, the rs1165196 and rs3757131 polymorphisms may be suitable biomarkers for cardiovascular disease risk factors, although their direct effect(s) on the metabolic pathway remains unknown.

| SNP/genotype | Men | | | | | | | | Women | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LDL-C/HDL-C ratio (N) | | OR | 95% CI | OR† | 95% CI† | OR‡ | 95% CI‡ | LDL-C/HDL-C ratio (N) | | OR | 95% CI | OR† | 95% CI† | OR‡ | 95% CI‡ |
| | ≤2.0 | >2.0 | | | | | | | ≤2.0 | >2.0 | | | | | | |
| rs1165196 | | | | | | | | | | | | | | | | |
| TT | 292 | 402 | Reference | | Reference | | Reference | | 336 | 276 | Reference | | Reference | | Reference | |
| TC | 136 | 135 | 0.721 | 0.544–0.956 | 0.653 | 0.486–0.878 | 0.649 | 0.481–0.877 | 124 | 87 | 0.854 | 0.622–1.173 | 0.844 | 0.594–1.198 | 0.846 | 0.595–1.204 |
| CC | 17 | 13 | 0.555 | 0.266–1.162 | 0.549 | 0.253–1.191 | 0.572 | 0.261–1.253 | 12 | 12 | 1.217 | 0.538–2.753 | 0.920 | 0.376–2.251 | 0.978 | 0.399–2.401 |
| TC+CC | 153 | 148 | 0.703 | 0.536–0.922 | 0.642 | 0.483–0.853 | 0.642 | 0.481–0.857 | 136 | 99 | 0.886 | 0.654–1.201 | 0.845 | 0.604–1.183 | 0.853 | 0.609–1.195 |
| T | 720 | 939 | Reference | | Reference | | Reference | | 796 | 639 | Reference | | Reference | | Reference | |
| C | 170 | 161 | 0.726 | 0.573–0.920 | 0.679 | 0.531–0.869 | 0.684 | 0.533–0.878 | 148 | 111 | 0.934 | 0.715–1.220 | 0.869 | 0.647–1.167 | 0.877 | 0.653–1.179 |
| rs1179086 | | | | | | | | | | | | | | | | |
| AA | 234 | 308 | Reference | | Reference | | Reference | | 256 | 211 | Reference | | Reference | | Reference | |
| AT | 175 | 211 | 0.916 | 0.704–1.191 | 0.866 | 0.657–1.143 | 0.884 | 0.666–1.172 | 177 | 138 | 0.946 | 0.709–1.261 | 0.898 | 0.652–1.237 | 0.894 | 0.647–1.234 |
| TT | 36 | 31 | 0.654 | 0.393–1.089 | 0.671 | 0.391–1.152 | 0.689 | 0.399–1.190 | 39 | 26 | 0.809 | 0.477–1.372 | 0.677 | 0.375–1.222 | 0.668 | 0.369–1.209 |
| AT+TT | 211 | 242 | 0.871 | 0.678–1.120 | 0.828 | 0.636–1.078 | 0.686 | 0.646–1.106 | 216 | 164 | 0.921 | 0.701–1.210 | 0.861 | 0.635–1.167 | 0.860 | 0.633–1.169 |
| A | 643 | 827 | Reference | | Reference | | Reference | | 689 | 560 | Reference | | Reference | | Reference | |
| T | 247 | 273 | 0.859 | 0.703–1.050 | 0.835 | 0.677–1.029 | 0.849 | 0.686–1.050 | 255 | 190 | 0.917 | 0.737–1.141 | 0.855 | 0.671–1.090 | 0.857 | 0.672–1.093 |
| rs3757131 | | | | | | | | | | | | | | | | |
| CC | 293 | 410 | Reference | | Reference | | Reference | | 334 | 276 | Reference | | Reference | | Reference | |
| CT | 136 | 128 | 0.673 | 0.506–0.894 | 0.614 | 0.456–0.828 | 0.614 | 0.453–0.832 | 125 | 88 | 0.852 | 0.621–1.168 | 0.842 | 0.594–1.194 | 0.845 | 0.595–1.201 |
| TT | 16 | 12 | 0.536 | 0.250–1.150 | 0.537 | 0.242–1.189 | 0.563 | 0.251–1.265 | 13 | 11 | 1.024 | 0.452–2.322 | 0.782 | 0.320–1.909 | 0.823 | 0.335–2.021 |
| CT+TT | 152 | 140 | 0.658 | 0.500–0.866 | 0.607 | 0.455–0.808 | 0.609 | 0.455–0.816 | 138 | 99 | 0.868 | 0.641–1.176 | 0.831 | 0.594–1.162 | 0.839 | 0.599–1.175 |
| C | 722 | 948 | Reference | | Reference | | Reference | | 793 | 640 | Reference | | Reference | | Reference | |
| T | 168 | 152 | 0.689 | 0.542–0.876 | 0.649 | 0.506–0.833 | 0.656 | 0.509–0.846 | 151 | 110 | 0.903 | 0.691–1.179 | 0.844 | 0.629–1.133 | 0.853 | 0.635–1.146 |

**Table 5. Associations between the SLC17A1 gene variants and the LDL-C/HDL-C ratio.** SNP, single nucleotide polymorphism; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; OR, odds ratio; CI, confidence interval. †Adjusted for age, body mass index, research area, alcohol consumption, smoking habits. ‡Adjusted for age, body mass index, research area, METs, daily energy and nutritional intake (protein, fat, carbohydrate), and alcohol and smoking habits.

Plasma homocysteine concentrations are typically considered to be inversely related to folic acid levels (a cofactor or substrate for enzymes involved in homocysteine metabolism). Similarly, a previous study reported that homocysteine levels in elderly Japanese individuals are inversely related to folic acid levels[29]. Furthermore, another study reported that folic acid supplementation lowered homocysteine levels, although the change in lipid metabolism was not significant at the end of the treatment period[28,30]. Nevertheless, the preventative effects of folic acid supplementation on cardiovascular disease cannot be excluded[31]. In the present study, high homocysteine and low folic acid levels were associated with the major homozygous alleles in rs1165196 and rs3757131. However, adjusting for folic acid weakly attenuated the relationship between hyperhomocysteinaemia and the *SLC17A1* polymorphisms. Nevertheless, the odds of hyperhomocysteinaemia were significantly different for rs1165196 and rs3757131, even after adjusting for folic acid levels. Therefore, *SLC17A1* polymorphisms appear to independently affect low homocysteine levels.

To the best of our knowledge, the present study was the first to explore the association between rs1165196 and rs3757131 genotypes and cardiovascular risk factors, altered cholesterol homeostasis, and homocysteine levels in Japanese men. However, in women, statistically significant differences in cholesterol homeostasis and homocysteine levels were not identified. Similarly, pronounced sex-related

| SNP/ genotype | Men | | | | | | | | Women | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Homocysteine (N) | | OR | 95% CI | OR† | 95% CI† | OR‡ | 95% CI‡ | Homocysteine (N) | | OR | 95% CI | OR† | 95% CI† | OR‡ | 95% CI‡ |
| | ≤10.0 | >10.0 | | | | | | | ≤10.0 | >10.0 | | | | | | |
| rs1165196 | | | | | | | | | | | | | | | | |
| TT | 336 | 166 | Reference | | Reference | | Reference | | 387 | 44 | Reference | | Reference | | Reference | |
| TC | 158 | 43 | 0.551 | 0.375–0.810 | 0.505 | 0.340–0.751 | 0.560 | 0.371–0.844 | 141 | 15 | 0.936 | 0.505–1.734 | 0.923 | 0.492–1.731 | 0.942 | 0.498–1.781 |
| CC | 13 | 3 | 0.467 | 0.131–1.662 | 0.447 | 0.122–1.634 | 0.542 | 0.143–2.047 | 17 | 2 | 1.035 | 0.231–4.628 | 0.792 | 0.169–3.704 | 0.796 | 0.166–3.814 |
| TC+CC | 171 | 46 | 0.544 | 0.374–0.792 | 0.499 | 0.339–0.734 | 0.560 | 0.375–0.835 | 158 | 17 | 0.946 | 0.525–1.706 | 0.910 | 0.498–1.663 | 0.927 | 0.504–1.707 |
| T | 830 | 375 | Reference | | Reference | | Reference | | 915 | 103 | Reference | | Reference | | Reference | |
| C | 184 | 49 | 0.589 | 0.420–0.826 | 0.550 | 0.389–0.777 | 0.609 | 0.426–0.870 | 175 | 19 | 0.964 | 0.576–1.615 | 0.910 | 0.537–1.540 | 0.923 | 0.542–1.572 |
| rs1179086 | | | | | | | | | | | | | | | | |
| AA | 273 | 126 | Reference | | Reference | | Reference | | 300 | 30 | Reference | | Reference | | Reference | |
| AT | 202 | 79 | 0.847 | 0.606–1.185 | 0.788 | 0.557–1.113 | 0.854 | 0.595–1.227 | 208 | 23 | 1.106 | 0.625–1.958 | 1.028 | 0.571–1.851 | 1.041 | 0.574–1.888 |
| TT | 32 | 7 | 0.474 | 0.204–1.103 | 0.431 | 0.181–1.022 | 0.468 | 0.192–1.137 | 37 | 8 | 2.162 | 0.923–5.066 | 1.868 | 0.763–4.574 | 1.883 | 0.751–4.723 |
| AT+TT | 234 | 86 | 0.796 | 0.575–1.102 | 0.733 | 0.524–1.027 | 0.796 | 0.560–1.131 | 245 | 31 | 1.265 | 0.745–2.149 | 1.164 | 0.675–2.007 | 1.175 | 0.677–2.041 |
| A | 748 | 331 | Reference | | Reference | | Reference | | 808 | 83 | Reference | | Reference | | Reference | |
| T | 266 | 93 | 0.790 | 0.604–1.034 | 0.743 | 0.564–0.980 | 0.788 | 0.591–1.050 | 282 | 39 | 1.346 | 0.899–2.016 | 1.258 | 0.831–1.904 | 1.264 | 0.830–1.925 |
| rs3757131 | | | | | | | | | | | | | | | | |
| CC | 338 | 169 | Reference | | Reference | | Reference | | 383 | 44 | Reference | | Reference | | Reference | |
| CT | 158 | 40 | 0.506 | 0.342–0.750 | 0.466 | 0.311–0.698 | 0.514 | 0.338–0.781 | 145 | 15 | 0.900 | 0.486–1.668 | 0.902 | 0.481–1.691 | 0.926 | 0.490–1.750 |
| TT | 11 | 3 | 0.545 | 0.150–1.981 | 0.536 | 0.144–1.997 | 0.665 | 0.171–2.584 | 17 | 2 | 1.024 | 0.229–4.580 | 0.763 | 0.163–3.569 | 0.763 | 0.160–3.467 |
| CT+TT | 169 | 43 | 0.509 | 0.347–0.746 | 0.468 | 0.316–0.694 | 0.523 | 0.348–0.786 | 162 | 17 | 0.913 | 0.507–1.646 | 0.890 | 0.487–1.625 | 0.910 | 0.495–1.674 |
| C | 834 | 378 | Reference | | Reference | | Reference | | 911 | 103 | Reference | | Reference | | Reference | |
| T | 180 | 46 | 0.564 | 0.399–0.797 | 0.529 | 0.371–0.754 | 0.585 | 0.406–0.843 | 179 | 19 | 0.939 | 0.561–1.571 | 0.893 | 0.528–1.510 | 0.907 | 0.532–1.544 |

**Table 6. Associations between the SLC17A1 gene variants and hyperhomocysteinaemia.** SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval. †Adjusted for age, body mass index, research area, alcohol consumption, smoking habits. ‡Adjusted for age, body mass index, research area, folic acid, alcohol consumption, smoking habits.

differences in the regulation of uric acid levels have been reported in both humans and animals[32]. In the Framingham Heart Study, the association between uric acid levels and mortality risk in men was not significant in the univariate analysis, and the association in women was not significant after adjusting for diuretic use, blood pressure, and total cholesterol levels[33]. Therefore, the specific physiological characteristics of women may be related to the sex-related differences in SNP genotypes that were observed to be associated with uric acid levels, although the underlying mechanism has yet to be elucidated. Furthermore, alcohol consumption, physical activity, and daily energy and nutrition intake did not affect the associations in either men or women. Therefore, identification of the pathogenic genetic factor(s) for cardiovascular disease remains critically important.

The limitations of our study include its cross-sectional design and the relatively small number of participants. Although a study with a low statistical power has a reduced likelihood of detecting a true effect, case-control studies with small sample sizes are still widely used, and can be used to assess previously identified candidate regions and more precisely determine target selections. In addition, we only assessed Japanese participants in the present study, and further studies in other ethnic groups are needed to validate our findings. Therefore, large, prospective trials involving patients from multiple ethnic groups are needed to better assess the effects of *SLC17A1* polymorphisms on cholesterol homeostasis and hyperhomocysteinaemia.

## Conclusion

In conclusion, our results indicate that the rs1165196 and rs3757131 polymorphisms, expressed in the liver, confer dominant negative effects on LDL-C/HDL-C ratios and hyperhomocysteinaemia in Japanese men. Although the exact biological mechanism for this association remains unknown, our findings provide credible evidence that the rs1165196 and rs3757131 polymorphisms may be associated with a decreased risk of cardiovascular disease. This risk reduction may be due to their important role in maintaining cholesterol homeostasis and preventing hyperhomocysteinaemia through altered liver function. However, further studies are needed to interpret the effects of *SLC17A1* polymorphisms on cardiovascular disease.

## Methods

**Study participants.** In the present study, we evaluated participant data collected during the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study. That cohort study evaluated the general Japanese population in 10 research areas, using genetic and clinical data to detect and confirm gene-environment interactions related to lifestyle-associated diseases[34]. The study participants were 35–69 years old, and were enrolled after responding to study announcements in their specific research areas, attending health check-up examinations that were commissioned by their local governments, visiting local health check-up centres, or visiting a cancer hospital. A total of 4490 participants were selected, with approximately 500–600 participants from each research area, except for two areas in which fewer participants were recruited[35].

Among these 4490 participants, 2035 were excluded because they had consumed a meal $<6$ h before having their blood drawn, 228 because they were receiving cholesterol-lowering medication, 24 due to high triglyceride levels ($\geq$400 mg/dL), 338 due to insufficient laboratory data, and 23 individuals were excluded due to the absence of other data. After these exclusions, 1842 individuals (995 men and 847 women) were eligible for analyses. Folic acid (751 men and 620 women) and homocysteine (719 men and 606 women) levels limited the number of participants.

All participants provided written informed consent for the J-MICC Study, and the main study protocol of the J-MICC Study was approved by Ethics Committee at Nagoya University School of Medicine (Approval number 253 and 939). Since actual studies are slightly different from the main J-MICC Study protocol, all procedures involved in this study were performed in accordance with the institutional ethical committees (Chiba Cancer Center, Nagoya University, Nagoya City University, Aichi Cancer Center, Shiga University of Medical Science, Kyoto Prefectural University of Medicine, Tokushima University, Kyushu University, Saga University, and Kagoshima University).

**Blood biochemistry, lifestyle, and nutritional data.** In the present study, we evaluated lifestyle and medical information obtained through self-administered questionnaires (alcohol consumption status [0, 0.1–22.9, 23.0–45.9, or $\geq$46.0 g ethanol/day], smoking habits, current medications, and exercise). In addition, blood chemistry data (serum levels of triglycerides, total cholesterol, HDL-C, uric acid, LDL-C [calculated using the Friedewald formula[36], and plasma levels of homocysteine and folic acid) and anthropometric data were obtained from health check-ups performed in the research areas. Each blood sample was centrifuged and the plasma was separated and stored at −80 °C until analysis. Serum samples were measured by laboratories in each research area. Plasma folic acid concentrations were measured using a chemiluminescent enzyme immunoassay, and plasma homocysteine concentrations were measured using high-performance liquid chromatography by a contract laboratory (SRL, Tokyo, Japan).

For the dietary assessment, a validated food-frequency questionnaire was used to evaluate the intake frequency of 47 foods and beverages over the preceding year, and the total daily energy (kcal), protein (g), fat (g), and carbohydrate (g) intakes were calculated[37–40]. Physical activity was assessed as METs for daily and leisure activities, as previously reported[41]. In brief, METs-hours per day (METs·h/day) of daily activity were estimated for heavy physical work and walking. For leisure activities, METs·h/day were estimated by multiplying the reported daily time spent in each activity by the relevant MET intensity.

**Genotyping *SLC17A1* polymorphisms.** Our previous study described the details of the genetic analysis[35]. Briefly, genomic DNA was extracted from the buffy coat fraction of the participant's blood sample, using a BioRobot M48 Workstation (QIAGEN Group, Tokyo, Japan). The *SLC17A1* polymorphisms (rs1165196, rs1179086, and rs3757131) were genotyped using a multiplex polymerase chain reaction-based Invader assay (Third Wave Technologies, Madison, WI, USA) at the RIKEN Laboratory for Genotyping Development, Center for Genomic Medicine (Yokohama, Japan)[42]. The rs1165196 polymorphism is located at exon 7, where a conversion from thymine to cytosine at nucleotide 806 results in the substitution of threonine for isoleucine. The rs1179086 and rs3757131 polymorphisms are located at intron 12[18].

**Statistical analyses.** Inter-group comparisons were performed using Student's unpaired *t*-tests for continuous variables and chi-square tests for categorical variables (alcohol consumption in current drinkers, smokers, and allele independence [Hardy-Weinberg equilibrium]). Differences in quantitative

data, between the genotypes, were evaluated using one-way analysis of variance. All data were expressed as means ± standard deviation, or as indicated, and the coefficients of variation (CVs) for each parameter were calculated. ORs, 95% CIs, and p-values were calculated using logistic regression analyses; the LDL-C/HDL-C ratio (>2.0) was defined as the dependent variable, and participant age, sex, BMI (kg/$m^2$), research area, METs, daily energy and nutritional intake (protein, fat, and carbohydrate), and alcohol consumption and smoking habits, were included as independent variables. In the general Japanese population, a plasma homocysteine concentration of >10.0 nmol/mL is defined as hyperhomocysteinaemia[29], and this cut-off value was used for our analyses. We performed logistic regression analyses with the homocysteine level defined as the dependent variable, and patient age, sex, BMI, research area, folic acid level, and alcohol consumption and smoking habits were included as independent variables. All statistical tests were two-sided, and differences with a p-value of <0.05 were considered statistically significant. SPSS software (version 18.0, SPSS, Japan, Inc.) was used for all statistical analyses.

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## Author Contributions

T.K. analysed the data and wrote the main manuscript text. N.H., H.T., K.W., K.T., K.A., H.M. and S.S. designed the study. I.O. provided critical comments on the manuscript. R.O., K.S., A.H., N.T., C.N., D.M., E.O. and N.K. contributed to data collection, and M.K. performed the genotyping. All authors contributed to and have approved the final manuscript.

## Additional Information

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# Association between brain-muscle-ARNT-like protein-2 (*BMAL2*) gene polymorphism and type 2 diabetes mellitus in obese Japanese individuals: A cross-sectional analysis of the Japan Multi-institutional Collaborative Cohort Study

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Gene–environment interaction

ABSTRACT

*Aims:* Brain-muscle-Arnt-like protein-1 (*BMAL1*) and *BMAL2* genes are essential components of the circadian clock, and are considered to be involved in glucose homeostasis. We examined whether single nucleotide polymorphisms (SNPs) of *BMAL1* and *BMAL2* were associated with the prevalence of type 2 diabetes (T2DM) in the general Japanese population.
*Methods:* We studied 2467 subjects (1232 men and 1235 women, 35–69 years old), including 105 men and 57 women with T2DM, from the participants of the Japan Multi-institutional Collaborative Cohort Study. The association between SNPs in the *BMAL1* (rs11022775 and rs2290035) and *BMAL2* (rs7958822) genes and T2DM were analyzed by multiple logistic regression after adjustment for potential confounders. Analysis was also performed after stratification by body mass index ($\geq$25 kg/m$^2$ and $<$25 kg/m$^2$) to investigate an interaction between genotypes and obesity.
*Results:* The A/G and A/A genotypes of *BMAL2* rs7958822 showed significantly higher adjusted odds ratios (OR) for T2DM than the G/G genotype among obese men (OR = 2.2, 95% confidence intervals [CI] 1.1, 4.6, *P* for interaction = 0.0495) and obese women (OR = 2.7, 95% CI 1.1, 6.7, *P* for interaction = 0.199). There were no significant associations between *BMAL1* rs11022775 or rs2290035 genotypes and T2DM.
*Conclusions:* To the best of our knowledge, this is the first study to show the significant association between *BMAL2* rs7958822 genotype and T2DM among obese subjects.


## 1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has been increasing worldwide [1]. It is well recognized that several genetic, as well as lifestyle factors are associated with T2DM [2,3]. Although various single nucleotide polymorphisms (SNPs) have significant associations with T2DM [4], the mechanisms have not been fully elucidated. Interestingly, individuals may exhibit different susceptibility to T2DM, due to genetic variation, when their lifestyle is conducive to a high risk of T2DM.

Recently, the relationship between the circadian clock and the development of various diseases has attracted attention, and it has been suggested that SNPs in the clock gene are candidate variants associated with T2DM or glucose intolerance. Brain-muscle-Arnt-like protein 1 (*BMAL1*, *ARNTL*) gene is located in the region of human chromosome 11p15, and is involved in the regulation of circadian rhythm [5]. An AC haplotype of *BMAL1* rs7950226–rs11022775 showed a significant association with T2DM in 1304 individuals from 424 British families [6]. In a Greek population study, a positive relationship between *BMAL1* rs11022775 (T > C) C-allele and gestational diabetes mellitus was observed [7]. A recent report also indicated a significant association between the combination of *NOC* (CCR4 carbon catabolite repression 4-like gene, *CCRN4L*) rs9684900 and *BMAL1* rs2290035 with fasting blood glucose levels among 1510 non-diabetic Chinese subjects [8]. However, there have been no reports regarding the interaction between *BMAL1* rs11022775 and rs2290035, and obesity in T2DM.

*BMAL2* (*ARNTL2*) is another clock gene that lies in the 12p12.2-p11.2 region [9]. Several SNPs of this gene have been linked to psychiatric disorders [10,11], but not to metabolic syndrome or its components in Europe [12]. However, it is unknown whether *BMAL2* rs7958822 is linked to the risk of T2DM in Asian populations.

Therefore, the goal of the present study was to investigate whether intronic SNPs *BMAL1* rs11022775 and rs2290035, and *BMAL2* rs7958822, are associated with T2DM, as well as to determine whether there are gene–environment interactions between *BMAL1*- and *BMAL2*-genotypes and obesity in T2DM.

## 2. Study subjects

Our data were collected from the baseline survey (2005–2008) of the Japan Multi-institutional Collaborative Cohort Study (J-MICC Study), a large genome cohort study that was designed to detect and confirm gene–environment interactions in lifestyle-related diseases. The J-MICC Study involved ten research institutes and universities [13,14]. A total of 4490 subjects (2109 men, 2381 women) aged 35–69 years were selected from the participants in the baseline survey. We excluded subjects who had no information on the *BMAL* genotypes (17 men, 12 women) or glycated hemoglobin (HbA1c) (761 men, 994 women). Furthermore, we excluded participants who had no information on body mass index (BMI) (1 subject), first-degree family history of T2DM (fT2DM) (155 subjects), smoking and drinking habits (1 subject), and leisure time exercise activity (48 subjects). We also excluded participants whose total energy intake was less than 1000 kcal/day or more than 4000 kcal/day (34 subjects). Eventually, the number of participants statistically analyzed was 2467 (1232 men, 1235 women).

Written informed consent was obtained from each participant, and the study protocol was approved by the ethics committees of Nagoya University School of Medicine (the affiliation of the former principal investigator, Nobuyuki Hamajima), Aichi Cancer Center Research Institute (the

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affiliation of the present principal investigator, Hideo Tanaka), and other participating institutions.

## 3. Materials and methods

### 3.1. Samples and diagnostic criteria

HbA1c was measured in laboratories of each study area, and the results of these measurements were collected. To convert HbA1c values from the units of the Japan Diabetes Society (JDS) to the units of the National Glycohemoglobin Standardization Program (NGSP), we used the following officially approved equation: NGSP (%) = 1.02 × JDS (%) + 0.25 [15]. The diagnostic criterion for T2DM was HbA1c ≥ 6.5% in NGSP (47 mmol/mol in IFCC units) and/or being on medication for high blood glucose, taking into account the criteria published by the Japan Diabetes Society [16]. Height (cm) and weight (kg) were measured routinely at health check-ups or by the respective research teams. BMI was calculated using the following formula: weight (kg)/height ($m^2$).

Obesity was defined as a BMI greater than 25 ($kg/m^2$) according to the criteria of the Japan Diabetes Society [16]. We calculated weight change since 20 years old (±kg) by subtracting the self-reported weight at 20 years old from the present body weight.

### 3.2. Questionnaire and measurements

The self-administered questionnaire used in the J-MICC Study included questions about fT2DM, smoking and alcohol drinking habits, leisure time exercise activities, dietary habits, and current medication. A validated, short food-frequency questionnaire (FFQ), which inquired about the frequency of consumption of 47 foods and beverages, was used for evaluating the average total energy intake over the past year [17–19]. Leisure-time physical activity was calculated as: the frequency of exercise per week × the average duration (h/day) × the intensity (metabolic equivalent: MET). The frequency of exercise was categorized as follows: almost none, one to three times/month, one to two times/week, three to four times/week, and five times/week or more. The categories for the average duration of exercise (hours per activity) were as follows: less than 30 min, 30 min to less than 1 h, 1 to less than 2 h, 2 to less than 3 h, 3 to less than 4 h, and 4 h or more. In the calculation of physical activity, 3.4 MET was assigned to light exercise, 7.0 MET to moderate exercise, and 10.0 MET to heavy exercise. Total physical activity was expressed as MET-h/week.

### 3.3. Genotyping of polymorphisms

The details of genotyping performed for the J-MICC Study have been described elsewhere [14]. Briefly, SNPs including the *BMAL1* rs11022775, rs2290035, and *BMAL2* rs7958822 were genotyped using a multiplex polymerase chain reaction (PCR)-based Invader assay (Third Wave Technologies, Madison, WI, USA) [20] at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN. Genotype distributions were tested for Hardy–Weinberg equilibrium [21]. The level of statistical significance was set at $\alpha = 0.05/6$ using Bonferroni's method. In addition, linkage disequilibrium analysis of *BMAL1* rs11022775, rs2290035, and *BMAL2* rs7958822 was performed using the Haploview software [22].

### 3.4. Statistical analyses

To analyze sex differences in background characteristics and genotypes, we used the chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. We estimated the adjusted odds ratio (OR) and 95% confidence intervals (CI) for T2DM using multiple logistic regression models. Possible confounding factors for the study of T2DM were extracted [2], such as age (years), BMI ($kg/m^2$, quartiles), fT2DM (positive, negative, unknown), smoking status (current, past, never), drinking status (current, past or never), exercise activities (MET-h/week, quartiles), total energy intake (kcal/day, quartiles), and study areas (east (Kanto-Tokai), middle (Kinki-Shikoku), and west (Kyushu) Japan). Genotype-T2DM association was analyzed using the dominant model (major allele homozygotes versus heterozygotes + minor allele homozygotes) of each SNP as follows: *BMAL1* rs11022775 (major allele > minor allele; C > T), rs2290035 (T > A), and *BMAL2* rs7958822 (G > A). We also performed fully adjusted multiple logistic regression analyses in the non-obese and obese groups, separately. In this stratified analysis, we classified participants according to the quartiles of exercise activity and total energy intake in the obese and non-obese groups. The significance of the product term for genotype and obesity was examined using the likelihood ratio test. In the analysis of interaction, we classified data on exercise activities and total energy intake into four groups according to quartiles of each sex. We also performed analyses of covariance to determine the relationship between *BMAL2* rs7958822 genotype and weight change since 20 years old (±kg) and BMI ($kg/m^2$), adjusting for age (continuous), smoking (current, past, never), alcohol drinking (current, past, or never), exercise activities (quartiles), total energy intake (quartiles), and study areas (east, middle, west). Calculations and statistical tests were performed using the SAS version 8.2 software (SAS Institute Inc., Cary, NC, USA). All statistical tests were 2-sided and *P*-values <0.05 were considered statistically significant.

## 4. Results

### 4.1. Characteristics of participants and genotype frequencies of BMAL1 and BMAL2

Table 1 shows the characteristics of the participants according to sex. The median age was 59 years for men and 58 years for women. BMI ($kg/m^2$), the proportion of obesity, and weight change since 20 years old (±kg) were significantly higher in men than women. There was a significantly higher proportion of T2DM in men than in women. There were also significant sex differences in the proportion of subjects with first-degree family history of T2DM, smoking and drinking habits, total energy intake, and study areas.

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**Table 1 – Characteristics of the current participants based on the sex.**

| Characteristics | Men | | | Women | | | |
|---|---|---|---|---|---|---|---|
| | *n* | (%) | Median (Q1, Q3) | *n* | (%) | Median (Q1, Q3) | *P*-value[a,b] |
| Age (years) | 1232 | | 59 (52, 64) | 1235 | | 58 (52, 63) | 0.017 |
| Body mass index; BMI (kg/m$^2$) | 1232 | | 23.6 (21.7, 25.4) | 1235 | | 22.4 (20.6, 24.4) | <0.001 |
| Obesity (BMI ≥ 25 kg/m$^2$) | 360 | 29.2 | | 254 | 20.6 | | <0.001 |
| Non-obesity (BMI < 25 kg/m$^2$) | 872 | 70.8 | | 981 | 79.4 | | |
| Weight change from 20 years old (±kg) | 1198 | | 7.0 (2.7, 12.0) | 1209 | | 4.0 (−0.6, 8.4) | <0.001 |
| Unknown | 34 | | – | 26 | | – | |
| Type 2 diabetes | | | | | | | |
| Yes | 105 | 8.5 | | 57 | 4.6 | | <0.001 |
| No | 1127 | 91.5 | | 1178 | 95.4 | | |
| First-degree family history of T2DM | | | | | | | |
| Positive | 202 | 16.4 | | 217 | 17.6 | | <0.001 |
| Negative | 804 | 65.3 | | 869 | 70.4 | | |
| Unknown | 226 | 18.3 | | 149 | 12.1 | | |
| Smoking | | | | | | | |
| Current | 347 | 28.2 | | 75 | 6.1 | | <0.001 |
| Past | 556 | 45.1 | | 53 | 4.3 | | |
| Never | 329 | 26.7 | | 1107 | 89.6 | | |
| Alcohol drinking | | | | | | | |
| Current | 948 | 76.9 | | 431 | 34.9 | | <0.001 |
| Past or never | 284 | 23.1 | | 804 | 65.1 | | |
| Exercise activities (MET-h/week) | 1232 | | 7.7 (1.3, 22.0) | 1235 | | 7.7 (1.3, 19.1) | 0.090 |
| Total energy intake (kcal/day)[a] | 1232 | | 1893 (1723, 2114) | 1235 | | 1569 (1435, 1695) | <0.001 |
| Study areas | | | | | | | |
| East (Kanto-Tokai) | 667 | 54.1 | | 400 | 32.4 | | <0.001 |
| Middle (Kinki-Shikoku) | 161 | 13.1 | | 314 | 25.4 | | |
| West (Kyushu) | 404 | 32.8 | | 521 | 42.2 | | |

Q1, the 25th percentile, Q3, the 75th percentile.
[a] Sex difference was analyzed by Wilcoxon rank-sum test (continuous variables) and chi-square test (categorical variables).

In Table 2, the allele and genotype frequencies for *BMAL1* and *BMAL2* are summarized. The frequencies of all minor alleles were higher than 0.01. Genotype frequencies of all SNPs were in Hardy–Weinberg equilibrium for both sexes (*P* for HWE > Bonferroni corrected $\alpha = 0.05/6$), and there were no significant sex difference in genotype frequencies of any of the SNPs evaluated. The allele frequencies in our study population were consistent with the reference data (C = 0.950 and T = 0.050 for *BMAL1* rs11022775, T = 0.693 and A = 0.307 for rs2290035, and G = 0.789 and A = 0.211 for *BMAL2* rs7958822) [23,24]. It was confirmed that *BMAL1* rs11022775, rs2290035, and *BMAL2* rs7958822 were not in linkage disequilibrium, as

**Table 2 – Allele and genotype frequencies of the genetic polymorphisms in this study.**

| Gene SNP | Chr. | Position (kb) | Region | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Allele frequency[a] | Genotype frequency *n* (%) | | *P* for HWE[b] | Allele frequency[a] | Genotype frequency *n* (%) | *P* for HWE[b] | *P*-value[c] |
| *BMAL1* (*ARNTL*) | | | | | | | | | | | |
| rs11022775 | 11 | 13352217 | Intron | C > T | C/C | 1075 (87.3) | 0.557 | | 1069 (86.6) | 0.096 | 0.588 |
| | | | | C = 0.935 | C/T | 153 (12.4) | | C = 0.932 | 164 (13.3) | | |
| | | | | T = 0.065 | T/T | 4 (0.3) | | T = 0.068 | 2 (0.2) | | |
| rs2290035 | 11 | 13386224 | Intron | T > A | T/T | 668 (54.2) | 0.799 | | 708 (57.3) | 0.739 | 0.166 |
| | | | | T = 0.735 | A/T | 476 (38.6) | | T = 0.758 | 457 (37.0) | | |
| | | | | A = 0.265 | A/A | 88 (7.1) | | A = 0.242 | 70 (5.7) | | |
| *BMAL2* (*ARNTL2*) | | | | | | | | | | | |
| rs7958822 | 12 | 27348173 | Intron | G > A | G/G | 796 (64.6) | 0.709 | | 810 (65.6) | 0.096 | 0.657 |
| | | | | G = 0.803 | A/G | 386 (31.3) | | G = 0.805 | 369 (21.8) | | |
| | | | | A = 0.197 | A/A | 50 (4.1) | | A = 0.195 | 56 (4.5) | | |

SNP, single nucleotide polymorphism.
Chr., location of chromosome.
[a] Indicates major allele > minor allele and an allele frequency in each SNP.
[b] *P*-value for Hardy–Weinberg equilibrium (HWE) (Bonferroni corrected *P* for HWE > 0.05/6).
[c] The frequency of genotype was analyzed by chi-square test for sex differences.

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determined using the Haploview software (pairwise linkage disequilibrium coefficients D'ranged from 0.01 to 0.52).

### 4.2. Association between BMAL1 and BMAL2 genotypes and T2DM

Table 3 shows the non-adjusted (Model 1) and fully adjusted OR (Model 2) for the association between *BMAL1* and *BMAL2* genotypes and T2DM. *BMAL1* rs11022775 and rs2290035, and *BMAL2* rs7958822 showed no significantly increased or decreased OR for T2DM in either sex.

Table 4 shows the fully adjusted OR for T2DM after stratification by obesity. *BMAL1* rs11022775 and rs2290035 showed no significant relationships with T2DM regardless of the presence of obesity in either sex. However, *BMAL2* rs7958822 A/G and A/A genotypes showed significantly higher OR for T2DM than the G/G genotype only among obese men (OR = 2.2, 95% CI = 1.1, 4.6) and women (OR = 2.7, 95% CI = 1.1, 6.7). A significant interaction was observed between *BMAL2* rs7958822 genotypes and obesity for T2DM in men (*P* for interaction = 0.0495) but not in women (*P* for interaction = 0.199).

As shown in Table 5, *BMAL2* rs7958822 G/G and A/G + A/A genotypes were not significantly associated with high adjusted means of weight change since 20 years old (±kg) or BMI (kg/m$^2$) in men and women.

## 5. Discussion

Many physiological functions and behaviors of living organisms involve circadian variation controlled by the circadian clock. In mammals, the transcription factors circadian locomotor output cycles kaput (CLOCK) and BMAL1, together with their target genes *PER* and *CRY* constitute the core feedback loop of the circadian oscillator [25]. BMAL2 is another known transcription factor that also plays an essential role in the core feedback loop of the circadian clock [26]. It has been reported that dysregulation of the circadian clock is closely related to abnormal glucose metabolism. *Clock* and *Bmal1* mutant mice showed impaired glucose tolerance and insulin secretion [27]. In addition, a significant association between the *BMAL1* rs7950226 and rs11022775 haplotypes and T2DM was observed in humans [6]. It was also reported that constitutive expression of *Bmal2* gene restored circadian rhythmicity and insulin action in clock-disrupted *Bmal1*-knockout mice [28].

In the present study population, *BMAL2* rs7958822 polymorphism was not associated with obesity or weight gain since the age of 20 years. However, the prevalence of T2DM was increased when both A alleles of *BMAL2* rs7958822 and obesity were present. Therefore, the association between the rs7958822 variant and T2DM may not be related to a higher frequency of obesity. Rather, obesity may act as an effect modifier in the process of T2DM development when the A allele of *BMAL2* rs7958822 is present. The probability of developing T2DM might become higher when the genetic risk conferred by the A allele of *BMAL2* rs7958822 is combined with obesity-induced insulin resistance. However, the singular effect of this SNP may not be strong in itself according to the result of the interaction with obesity (*P* for interaction: 0.0495 in men, 0.199 in women). In our supplemental analysis, serum levels of lipids (triglycerides, total cholesterol, and HDL-cholesterol) showed no associations with *BMAL2* rs7958822 polymorphism (data not shown). It is currently unclear whether intronic SNP rs7958822 alters *BMAL2* gene function, or whether it is merely in linkage disequilibrium with another functional SNP. It has been reported that the GT haplotype of *BMAL2* rs7958822–rs4964057 is associated with an increased risk of alcohol dependence or abuse in a population-based

**Table 3 – Crude and fully adjusted odds ratio for type 2 diabetes (T2DM) in minor allele carriers compared with homozygous genotypes of major allele.**

| | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|
| | Case/total | %[a] | Model 1 OR (95% CI) | Model 2 OR (95% CI) | Case/total | %[a] | Model 1 OR (95% CI) | Model 2 OR (95% CI) |
| *BMAL1* (*ARNTL*) | | | | | | | | |
| rs11022775 | | | | | | | | |
| C/C | 88/1075 | 8.2 | 1.0 | 1.0 | 47/1069 | 4.4 | 1.0 | 1.0 |
| C/T + T/T | 17/157 | 10.8 | 1.4 (0.76, 2.3) | 1.3 (0.74, 2.3) | 10/166 | 6.0 | 1.4 (0.65, 2.7) | 1.6 (0.72, 3.2) |
| rs2290035 | | | | | | | | |
| T/T | 61/668 | 9.1 | 1.0 | 1.0 | 32/708 | 4.5 | 1.0 | 1.0 |
| A/T + A/A | 44/564 | 7.8 | 0.84 (0.56, 1.3) | 0.87 (0.57, 1.3) | 25/527 | 4.7 | 1.1 (0.61, 1.8) | 0.93 (0.53, 1.6) |
| *BMAL2* (*ARNTL2*) | | | | | | | | |
| rs7958822 | | | | | | | | |
| G/G | 64/796 | 8.0 | 1.0 | 1.0 | 31/810 | 3.8 | 1.0 | 1.0 |
| A/G + A/A | 41/436 | 9.4 | 1.2 (0.78, 1.8) | 1.1 (0.73, 1.7) | 26/425 | 6.1 | 1.6 (0.95, 2.8) | 1.7 (0.94, 2.9) |

95% CI, 95% confidence intervals.
Model 1, multiple logistic regression was used to show crude odds ratio (OR) for T2DM comparing minor allele carrier with homozygous genotypes of major allele as a reference.
Model 2, adjusted items: age (continuous), BMI (quartiles), first-degree family history of T2DM (positive, negative, unknown), smoking (current, past, never), alcohol drinking (current, past or never), exercise activities (quartiles), total energy intake (quartiles), and study areas (east, middle, west).
[a] A proportion of the case among each group.

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**Table 4 – Fully adjusted odds ratio (OR) for type 2 diabetes (T2DM) stratified by obesity in minor allele carriers compared with homozygous genotypes of major allele.**

| | Men | | | | Women | | | |
|---|---|---|---|---|---|---|---|---|
| | Non-obesity | | Obesity | | Non-obesity | | Obesity | |
| | Case/total (%)[a] | OR (95% CI) | Case/ total (%)[a] | OR (95% CI) | Case/ total (%)[a] | OR (95% CI) | Case/ total (%)[a] | OR (95% CI) |
| *BMAL1* (*ARNTL*) rs11022775 | | | | | | | | |
| C/C | 56/758 (7.4) | 1.0 | 32/317 (10.1) | 1.0 | 24/845 (2.8) | 1.0 | 23/224 (10.3) | 1.0 |
| C/T + T/T | 9/114 (7.9) | 1.1 (0.47, 2.2) | 8/43 (18.6) | 2.3 (0.87, 5.8) | 5/136 (3.7) | 1.6 (0.50, 4.1) | 5/30 (16.7) | 2.0 (0.56, 6.2) |
| | *P* for interaction[b] = 0.261 | | | | *P* for interaction[b] = 0.973 | | | |
| *BMAL1* (*ARNTL*) rs2290035 | | | | | | | | |
| T/T | 38/464 (8.2) | 1.0 | 23/204 (11.3) | 1.0 | 19/576 (3.3) | 1.0 | 13/132 (9.8) | 1.0 |
| A/T + A/A | 27/408 (6.6) | 0.87 (0.51, 1.5) | 17/156 (10.9) | 0.83 (0.40 1.7) | 10/405 (2.5) | 0.71 (0.31, 1.6) | 15/122 (12.3) | 1.4 (0.59, 3.2) |
| | *P* for interaction[b] = 0.862 | | | | *P* for interaction[b] = 0.246 | | | |
| *BMAL2* (*ARNTL2*) rs7958822 | | | | | | | | |
| G/G | 43/556 (7.7) | 1.0 | 21/240 (8.8) | 1.0 | 17/636 (2.7) | 1.0 | 14/174 (8.0) | 1.0 |
| A/G + A/A | 22/316 (7.0) | 0.81 (0.46, 1.4) | 19/120 (15.8) | 2.2 (1.1, 4.6) | 12/345 (3.5) | 1.2 (0.55, 2.7) | 14/80 (17.5) | 2.7 (1.1, 6.7) |
| | *P* for interaction[b] = 0.0495 | | | | *P* for interaction[b] = 0.199 | | | |

95% CI, 95% confidence intervals.
The fully adjusted odds ratio (OR) of T2DM stratified by obesity was used to compare minor allele carrier with homozygous genotypes of major allele as a reference.
Adjusted items: age (continuous), first-degree family history of T2DM (positive, negative, unknown), smoking (current, past, never), alcohol drinking (current, past or never), exercise activities (quartiles), total energy intake (quartiles), study areas (east, middle, west)
[a] A proportion of the case among each group.
[b] The interaction between genotype and obesity was analyzed by likelihood ratio test of fully adjusted logistic regression model.

study in Finland [10]. To date, however, there have been no studies that showed a significant association between *BMAL2* rs7958822 genotype and T2DM. Furthermore, we could not find in the literature, any SNPs that are in linkage disequilibrium with rs7958822 and are associated with T2DM or fasting blood glucose levels.

In the present study, *BMAL1* rs2290035 showed no significant association with T2DM. This result is essentially in line with those of earlier studies. It was reported that the combination of *BMAL1* rs2290035 and *NOC* rs9684900 variants were associated with fasting glucose levels in the Taiwanese population, despite the absence of the independent effects of each SNP [8]. There was also no association between *BMAL1* rs2290035 polymorphism and fasting blood glucose in Finland [12]. We could not find any other reports that showed a significant association between *BMAL1* rs2290035 and T2DM or fasting blood glucose.

In one study performed in the United Kingdom, the minor allele T of *BMAL1* rs11022775 was associated with a higher OR of T2DM than allele C in South Asian populations, but the opposite association was observed in white European cohorts [29]. In 346 pregnant Greek women, the frequency of the C allele of *BMAL1* rs11022775 was significantly higher in patients with gestational diabetes mellitus than in the control group

**Table 5 – Analysis of covariance of the relationship between *BMAL2* rs7958822 genotype and weight change from 20 years of age and body mass index.**

| | Men | | | | | Women | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Model 1 | | | Model 2 | | Model 1 | | | Model 2 | |
| | *n* | Mean (SE) | *P*-value | Adjusted mean (SE) | *P*-value | *n* | Mean (SE) | *P*-value | Adjusted mean (SE) | *P*-value |
| Weight change (±kg) | 1198 | | | | | 1209 | | | | |
| *BMAL2* (*ARNTL2*) rs7958822 | | | | | | | | | | |
| G/G | 775 | 7.5 (0.3) | 0.252 | 7.8 (0.7) | 0.310 | 798 | 4.1 (0.3) | 0.614 | 5.5 (1.0) | 0.830 |
| A/G + A/A | 423 | 7.0 (0.4) | | 7.3 (0.7) | | 411 | 4.3 (0.4) | | 5.6 (1.0) | |
| Body mass index (kg/m$^2$) | 1232 | | | | | 1235 | | | | |
| G/G | 796 | 23.5 (1.0) | 0.985 | 24.4 (1.0) | 0.829 | 810 | 22.5 (1.0) | 0.583 | 22.7 (1.0) | 0.372 |
| A/G + A/A | 436 | 23.5 (1.0) | | 24.3 (1.0) | | 425 | 22.4 (1.0) | | 22.5 (1.0) | |

SE, standard error.
Model 1, non-adjusted model.
Model 2, adjusted for age (continuous), smoking (current, past, never), alcohol drinking (current, past or never), exercise activities (quartiles), total energy intake (quartiles), and study areas (east, middle, west).

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[7]. In the present study, there was no statistically significant association between *BMAL1* rs11022775 and T2DM, although the point estimate of the adjusted OR was 1.3 for men and 1.6 for women. A possible reason for the lack of significant association might be that the frequency of minor T allele (6.7%) was lower than that observed in a previous study (16.0%) [29], and was too low to provide sufficient statistical power.

One strength of the present study is that we could obtain information on family history of T2DM and many life-style factors, and adjusted for the effects of these factors in multivariable modeling. However, the study also has limitations. First, we cannot fully infer the cause–effect relationship between *BMAL2* rs7958822 genotype and T2DM due to the nature of the cross-sectional study, although genetic factors precede the development of T2DM. Second, information on treatment for high blood glucose, family history of T2DM, smoking and drinking habits, total energy intake, and physical activity during leisure time was obtained from a self-administered questionnaire. Random misclassification of the history of treatment for T2DM may have attenuated the association between *BMAL1* and *BMAL2* genotypes and T2DM. Furthermore, random misclassification or measurement errors of lifestyle and other factors included in regression models may have led to residual confounding, even after statistical adjustments. Third, only small number of SNPs in *BMAL1* and *BMAL2* genes were examined, and SNPs of other clock genes, such as *CLOCK*, *PER*, and *CRY* were not analyzed [25]. Fourth, the present results may not be applicable to ethnic groups outside of the Japanese population.

In summary, the results of the present study showed that *BMAL2* rs7958822 A/G and A/A genotypes were associated with a higher prevalence of T2DM than the G/G genotype among obese individuals in Japan. Studies involving greater number of SNPs, such as a genome-wide association study, may be useful to elucidate in more detail the association between clock gene variants and T2DM, and its effect modifiers.

## Conflict of interest

The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

# Clinical Characteristics Associated with Long-term Survival in Metastatic Gastric Cancer after Systemic Chemotherapy

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## Abstract

**Background: Systemic chemotherapy for patients with metastatic gastric cancer (MGC) is generally palliative, although some patients experience long-term survival after treatment. Thus, we identified clinical characteristics that are associated with long-term survival of patients with MGC after palliative chemotherapy. Materials and Methods: We retrospectively reviewed 514 MGC patients who received systemic chemotherapy at our institution from 2001 to 2008. To identify clinical predictors of survival beyond 2 years, multivariate logistic regression analyses were performed, and 5-year survival rates were estimated among MGC patients following chemotherapy. Results: Among 514 patients, 96 (19%) and 16 (3%) survived beyond 2 and 5 years, respectively, and performance status of 0 or 1 (odds ratio [OR]=3.39; p=0.01), previous gastrectomy (OR=1.86; p=0.01), single metastatic site (OR=1.80; p=0.03), and normal alkaline phosphatase levels (OR=2.81; p<0.01) were identified as independent predictors of long-term survival. Of the 16 5-year survivors, six were alive at the end of the study and showed no evidence of disease despite cessation of chemotherapy. Conclusions: The present data demonstrate distinct clinical characteristics that are associated with long-term survival of MGC patients, and indicated that palliative chemotherapy can be curative in highly selected patients.**

**Keywords:** Advanced gastric cancer - chemotherapy - predictive factor - survival

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## Introduction

Although the incidence of gastric cancer has recently declined, it remains the second leading global cause of death (Jemal et al., 2011). Similarly, gastric cancer mortality rates are decreasing in Japan, but remain the second most common cause of death, with 48,632 confirmed deaths in 2013 (Cancer Information Service, National Cancer Center, Japan, 2013). The standard of care for metastatic gastric cancer (MGC) is systemic chemotherapy, which improves survival and quality of life compared with best supportive care (Pyrhonen et al., 1995; Glimelius et al., 1997). The combination of fluoropyrimidines and platinum with or without docetaxel or epirubicin is the standard treatment option, and produces a median survival time of 8.6-13.0 months (Van Cutsem et al., 2006; Cunningham et al., 2008; Koizumi et al., 2008). However, systemic chemotherapy for MGC is generally palliative and survival beyond 2 years is rarely observed.

Several case reports and small case series have documented long-term survival (LTS) in selected patients with MGC (Saitoh et al., 2000; Tetzlaff et al., 2006; Hosokawa et al., 2007; Yamamoto et al., 2012; Kadowaki et al., 2014; Schildberg et al., 2014), and recent large phase III trials report 2-year survival rates of 9%-24% (Van Cutsem et al., 2006; Koizumi et al., 2008; Boku et al., 2009). In four phase II and 1 phase III trials for MGC conducted by the Japan Clinical Oncology Group (JCOG) from 1985 to 1997 (Yoshida et al., 2004), 39 (8%) and 11 (2%) of 497 patients survived beyond 2 and 5 years, respectively. However, these trials were conducted before the introduction of recent cytotoxic agents such as irinotecan and taxanes, and did not evaluate predictive factors for LTS. Furthermore, most recent trials of modern regimens failed to document long-term outcomes. Therefore, data showing clinical predictors of LTS and the possibility of cure for MGC patients after chemotherapy are limited. The aim of the present study was to clarify clinical characteristics that are predictive of LTS among MGC patients for whom irinotecan and taxanes treatments are available, and to estimate 5-year survival rates. The present data will be informative for evaluating changes in long-term outcomes of treatments with new agents including targeted drugs.

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## Materials and Methods

*Patients*

Between January 2001 and July 2008, 518 consecutive patients with MGC were treated with systemic chemotherapy at the Aichi Cancer Center Hospital. Inclusion criteria were as follows: at least 5-years of follow-up, histological confirmation of adenocarcinoma at the time of initial diagnosis, and demonstrated noncurative disease in imaging or histological examinations. Data were collected for age, gender, tumor differentiated type, the Eastern Cooperative Oncology Group (ECOG) performance status (PS), history of gastrectomy, metastatic sites, number of metastatic sites, adjuvant chemotherapy, serum alkaline phosphatase (ALP) levels at baseline, serum carcinoembryonic antigen (CEA) levels at baseline, first-line chemotherapy regimens, progression-free survival (PFS) at first-line therapy, tumor response after first-line therapy, and the use of second- or third-line chemotherapy. Responses of metastatic lesions to chemotherapy were assessed according to the Revised Evaluation Criteria in Solid Tumor (RECIST) version 1.0 (Therasse et al., 2000). We defined LTS as survival beyond 2 years after the initiation of systemic chemotherapy. The cutoff date for analysis was March 31, 2014. Approval was obtained from the Ethics Committee of the Aichi Cancer Center Hospital.

*Statistical analysis*

The aim of this study was to identify pretreatment characteristics that are predictive of survival beyond 2 years, and to estimate the 5-year survival rate among MGC patients treated with palliative chemotherapy. All clinical factors were converted into dichotomous variables including (1) age, <60 *vs* ≥60 years; (2) male *vs* female; (3) well or moderately differentiated *vs* poorly differentiated cancers; (4) PS of 0 or 1 *vs* PS ≥2; (5) a history of gastrectomy, yes *vs* no; (6) involved metastatic sites, yes *vs* no; (7) number of metastatic sites, 1 *vs* 2 or more; (8) adjuvant chemotherapy, yes *vs* no; (9) baseline ALP within upper limit normal (ULN) *vs* baseline ALP ≥ ULN, (10) baseline CEA, <ULN *vs* ≥ULN; and (11) treatment, fluoropyrimidines plus platinum-based therapy *vs* other regimens. Univariate analyses of categorical variables were performed using chi-square or Fisher's exact probability tests, as appropriate. Because all the patients were followed until their demise or for at least 5 years, this study was primarily descriptive in nature. Therefore, multivariate logistic regression analyses using forward selection methods were performed to identify clinical variables that are associated with LTS, and to calculate corresponding odds ratio (OR). Differences with p values of <0.20 in univariate analyses were included as covariates in multivariate analyses. PFS was recorded from the date of initial chemotherapy until the time of disease progression, death, or last contact. Overall survival (OS) was recorded from the date of the initial chemotherapy until death from any cause or last contact. PFS and OS curves were estimated using the Kaplan-Meier product limit method and were compared using the log-rank test. All data analyses were conducted using Dr. SPSS II software (SPSS Japan Inc., Tokyo, Japan), and a 2-sided p value of <0.05 was considered statistically significant.

## Results

*Patient characteristics and chemotherapy*

Of the 518 patients treated in this period, 514 (99%) were included in analyses and four were lost to follow-up prior to 5 years and were excluded from the analysis. First line chemotherapy regimens included fluoropyrimidine monotherapy in 269 patients (52%), fluoropyrimidine plus platinum-based therapy in 137 patients (27%), taxane monotherapy in 35 patients (7%), 5-fluorouracil plus methotrexate in 33 patients (6%), irinotecan plus cisplatin in 26 patients (5%), and other regimens in 14 patients (3%).

Patient characteristics and treatment outcomes are listed in Table 1. The median age was 62 years (range, 29-84). Among the 514 patients analyzed, 372 (72%) had poorly differentiated cancers and 78 (15%) had PS of two or more. Of the 246 (48%) patients who received resection of primary tumors before the start of chemotherapy, 57 (11%) received gastrectomy and had residual disease and 189 (37%) had recurrent disease after curative gastrectomy. The number of metastatic sites was only one in 273 patients (53%) and two or more in 241 (47%) patients. Abnormally high ALP and CEA levels were detected in 141 (27%) and 208 (41%) patients, respectively. A total of 376 (73%) and 192 (37%) patients received second- and third-line chemotherapy.

*Survival outcomes*

Median follow-up for living patients was 91 months (range, 71-119 months). Median OS was 11.7 months [95% confidence interval (CI), 10.7-12.7 months], and 1-, 2-, 3-, and 5-year survival rates were 49%, 19%, 8%, and 3%, respectively (Figure 1). A total of 96 (19%) and 16 (3%) patients survived for more than 2 and 5 years, respectively. Among 514 patients, 505 died of MGC or from unknown causes, six remained alive with no evidence of disease, and three were alive with disease at the cut-off date.

*Pretreatment factors associated with LTS*

As shown in Table 1, pretreatment factors that were predictive of LTS in univariate analyses included well or moderately differentiated cancer type (p=0.05), PS of 0 or 1 (p=0.003), prior gastrectomy (p<0.001), single metastatic sites (p<0.001), and normal ALP levels (p<0.001). The type of treatment was not a significant factor. In multivariate logistic regression analyses, a PS of 0 or 1 [odds ratio (OR)=3.47; p=0.01], history of gastrectomy (OR=1.85; p=0.01), single metastatic sites (OR=1.72; p=0.04), and normal ALP levels (OR=2.41; p<0.001) were significantly associated with LTS (Table 2). Of the 490 patients in which ALP levels were determined, patients with 0 (n=16), 1 (n=74), 2 (n=131), 3 (n=164), and 4 positive predictive factors (n=105) had 2-year survival rates of 0%, 5%, 12%, 20%, and 32%, respectively. Among the 342 patients with target lesions, 109 achieved

**Table 1. Patient Characteristics According to LTS and non-LTS**

| Characteristics | Total n=514 (%) | Non-LTSa n=418 (%) | LTSa n=96 (%) | p |
|---|---|---|---|---|
| Age (years) | | | | 0.76 |
| Median (range) | 62.0 (29-84) | 62.0 (29-84) | 63.0 (29-83) | |
| <60 | 216 (42) | 177 (42) | 39 (41) | |
| ≥60 | 299 (58) | 241 (58) | 57 (59) | |
| Gender | | | | 0.15 |
| Male | 337 (66) | 268 (64) | 69 (72) | |
| Female | 177 (34) | 150 (36) | 27 (28) | |
| Differentiation | | | | 0.05 |
| Well/moderate | 140 (27) | 106 (25) | 34 (35) | |
| Poor | 372 (72) | 310 (74) | 62 (65) | |
| Unknown | 2 (0.4) | 2 (0.5) | 0 (0) | |
| Performance status | | | | 0.003 |
| 0-1 | 436 (85) | 345 (82) | 91 (95) | |
| ≥2 | 78 (15) | 73 (18) | 5 (5) | |
| Target lesions | | | | 0.06 |
| No | 172 (33) | 132 (32) | 40 (42) | |
| Yes | 342 (67) | 286 (68) | 56 (58) | |
| Previous gastrectomy | | | | <0.001 |
| No | 268 (52) | 234 (56) | 34 (35) | |
| Yes | 246 (48) | 184 (44) | 62 (65) | |
| Metastatic site | | | | |
| Peritoneum | 310 (60) | 258 (62) | 52 (54) | 0.17 |
| Lymph node | 276 (54) | 231 (55) | 45 (47) | 0.14 |
| Liver | 141 (27) | 119 (29) | 22 (23) | 0.27 |
| Bone | 24 (5) | 23 (6) | 1 (1) | 0.06 |
| Number of metastatic sites | | | | <0.001 |
| 1 | 273 (53) | 205 (49) | 68 (71) | |
| ≥2 | 241 (47) | 213 (51) | 28 (29) | |
| Adjuvant chemotherapy | | | | 0.2 |
| No | 444 (86) | 365 (87) | 79 (82) | |
| Yes | 70 (14) | 53 (13) | 17 (18) | |
| Baseline ALP[b] | | | | <0.001 |
| <ULN[c] | 349 (68) | 273 (65) | 76 (79) | |
| ≥UNL[c] | 141 (27) | 130 (31) | 11 (11) | |
| Unknown | 24 (5) | 15 (4) | 9 (9) | |
| Baseline CEA[d] | | | | 0.13 |
| <ULN[c] | 290 (56) | 230 (55) | 60 (63) | |
| ≥UNL[c] | 208 (41) | 176 (42) | 32 (33) | |
| Unknown | 16 (3) | 12 (3) | 4 (4) | |
| First-line therapy | | | | 0.18 |
| FU+platinum-based | 137 (27) | 107 (26) | 31 (32) | |
| Others | 377 (73) | 311 (74) | 65 (68) | |
| First PFS[e] (months) | | | | <0.001 |
| Median (range) | 4.1 (0-110) | 3.6 (0-19.4) | 10.8 (0.5-110) | |
| Response† | | | | <0.001 |
| Responder (CRf+PR[g]) | 109 (32) | 73 (26) | 36 (64) | |
| Nonresponder | 233 (68) | 213 (74) | 20 (36) | |
| Second-line therapy | | | | <0.001 |
| No | 138 (27) | 128 (31) | 10 (10) | |
| Yes | 376 (73) | 290 (69) | 86 (90) | |
| Third-line therapy | | | | <0.001 |
| No | 322 (63) | 289 (69) | 33 (34) | |
| Yes | 192 (37) | 129 (31) | 63 (66) | |
| Taxanes dosing history | | | | 0.001 |
| No | 174 (34) | 156 (37) | 18 (19) | |
| Yes | 340 (66) | 262 (63) | 78 (81) | |
| Irinotecan dosing history | | | | <0.001 |
| No | 333 (65) | 291 (70) | 42 (44) | |
| Yes | 181 (35) | 127 (30) | 54 (56) | |

†Included patients with target lesions; LTS[a], long-term survivors; ALP[b], alkaline phosphatase; ULN[c], upper limit of normal; CEA[d], carcinoembryonic antigen; PFS[e], progression free survival; CR[f], complete response; PR[g], partial response

complete responses (CR) or partial responses (PR), and the response rate was 32%. The response rate in the LTS group (64%, 36 of 56) was higher than that in the non-LTS group (26%, 73 of 286; p<0.001). PFS at first-line treatment was significantly longer in the LTS group compared with that in the non-LTS group (median, 10.8 *vs* 3.6 months; log-rank p<0.001). The LTS group received taxanes and irinotecan and second- and third-line therapy more frequently than the non-LTS group.

*Long-term survivors beyond 5 years*

Patient characteristics of the 16 5-year survivors are shown in Table 3. Among these patients, survival times ranged from 60 to 119 months. The majority of patients had PS of 0 or 1 (94%, 15 of 16), received surgical resections of primary tumors (94%, 15 of 16), had normal ALP levels (100%, 12 of 12), and only one involved site (88%, 14 of 16). However, 10 patients received curative gastrectomy and five received noncurative gastrectomy before initial chemotherapy. Metastatic sites included the peritoneum alone in eight patients and abdominal lymph nodes alone in six. Fluoropyrimidine-based therapies were the first-line therapy for most patients, and comprised oral S-1 in nine patients and fluoropyrimidine plus platinum combination therapy in five. Despite cessation of treatment, six patients survived with no evidence of disease recurrence until the last follow-up. Among these, one patient (patients 6) achieved a CR after first-line therapy, although recurrence of lung metastasis was observed and curative resection was performed. Three patients (patients seven, 10, and 12) with residual disease

**Table 2. Multivariate Analysis of Clinical Factors Associated with LTS**

| Characteristics | Odds ratio | 95%CI | p |
|---|---|---|---|
| Performance status | | | |
| 0-1 *vs* ≥2 | 3.47 | 1.34-9.02 | 0.01 |
| Gastrectomy | | | |
| Yes *vs* No | 1.85 | 1.14-3.02 | 0.01 |
| Number of metastatic sites | | | |
| 1 *vs* ≥2 | 1.72 | 1.03-2.88 | 0.04 |
| Baseline alkaline phosphatase | | | |
| <ULN[a] *vs* ≥UNL[a] | 2.41 | 1.46-3.96 | <0.001 |

ULN[a], upper limit of normal

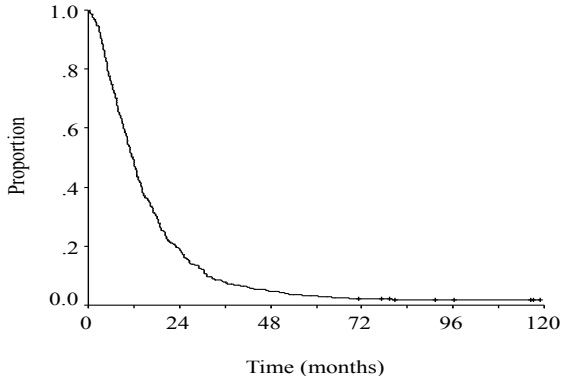


**Figure 1. Kaplan-Meier Survival Curves of Overall Survival (OS).** The median OS was 11.7 months (95% confidence interval, 10.7-12.7 months), and 2-, 3-, and 5-year survival rates were 19%, 8%, and 3%, respectively

**Table 3. Clinical Characteristics of 5-year Survivors**

| No. | Age | Sex | Performance status | Histology | Previous gastrectomy | Metastatic sites | ALP[a] | Regimen | Response | Survival (months) | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 63 | Male | 1 | Poorly-differentiated | No | Peritoneum | - | SP[c] | IR/SD[g] | 60 | Dead |
| 2 | 59 | Male | 1 | Poorly-differentiated | Curative | A-LN[b] | Normal | S-1 | SD | 63 | Dead |
| 3 | 51 | Male | 0 | Poorly-differentiated | Palliative | Peritoneum, A-LN[b] | Normal | XP[d] | CR | 64 | Dead |
| 4 | 48 | Female | 0 | Poorly-differentiated | Palliative | Peritoneum | Normal | S-1 | IR/SD[g] | 65 | Dead |
| 5 | 54 | Female | 1 | Poorly-differentiated | Curative | Peritoneum | - | Paclitaxel | IR/SD[g] | 69 | Dead |
| 6 | 71 | Male | 0 | Differentiated | Curative | A-LN[b], Lung | Normal | XP[d] | CR | 71 | Alive |
| 7 | 65 | Female | 0 | Poorly-differentiated | Palliative | Peritoneum | Normal | Paclitaxel | IR/SD[g] | 77 | Alive |
| 8 | 63 | Male | 0 | Poorly-differentiated | Palliative | A-LN[b] | Normal | SOX[e] | CR | 79 | Alive |
| 9 | 58 | Male | 1 | Differentiated | Curative | Peritoneum | Normal | S-1 | SD | 80 | Dead |
| 10 | 56 | Male | 0 | Differentiated | Curative | A-LN[b] | - | S-1 | SD | 81 | Dead |
| 11 | 55 | Female | 0 | Poorly-differentiated | Palliative | Peritoneum | Normal | SP[c] | IR/SD[g] | 81 | Alive |
| 12 | 66 | Male | 1 | Differentiated | Curative | A-LN[b] | Normal | S-1 | NE | 91 | Alive |
| 13 | 59 | Male | 1 | Poorly-differentiated | Curative | Peritoneum | Normal | FL[f] | IR/SD[g] | 96 | Alive |
| 14 | 67 | Male | 0 | Poorly-differentiated | Curative | A-LN[b] | - | 5-FU | CR | 117 | Alive |
| 15 | 32 | Male | 2 | Poorly-differentiated | Curative | A-LN[b] | Normal | S-1 | PR | 117 | Alive |
| 16 | 64 | Male | 0 | Poorly-differentiated | Curative | Peritoneum | Normal | S-1 | IR/SD[g] | 119 | Alive |

ALP[a], alkaline phosphatase; A-LN[b], abdominal lymph node; SP[c], S-1 plus cisplatin; XP[d], capecitabine plus cisplatin; SOX[e], S-1 plus oxaliplatin; FL[f], 5-fluorouracil plus leucovorin; IR/SD[g], incomplete response/stable disease

continued to receive systemic chemotherapy, and seven patients died of disease progression.

## Discussion

To the best of our knowledge, this is the first study to evaluate LTS among patients with MGC for whom newer cytotoxic agents such as taxanes and irinotecan were available. The present study primarily focused on LTS among unselected patients and mortality data were rigorously obtained. MGC patients who achieved LTS had distinct clinical characteristics, including PS of 0 or 1, only one metastatic site, previous gastrectomy, and normal ALP levels, and a minority of patients survived beyond 5 years. Among the 514 patients analyzed, six (1.2%) were free of disease after cessation of chemotherapy, supporting curative chemotherapeutic intentions in highly selected patients.

Most previous studies fail to describe long-term outcomes or to identify factors that are predictive of LTS in MGC patients treated with palliative chemotherapy. A retrospective study of data from JCOG trials that were performed prior to marketing of taxane and irinotecan reported 2- and 5-year survival rates of 8% (39 of 497) and 2% (11 of 497) among MGC patients, respectively (Yoshida et al., 2004). This study showed that better PS, a small number of metastatic sites, and macroscopically nonscirrhous tumors are favorable prognostic factors for OS. However, in contrast with the present study, predictors of LTS were not identified. Recently, 2-year survival rates were reported from the S-1 Plus cisplatin versus S-1 In RCT In the Treatment for Stomach cancer (SPIRITS trial) (Koizumi et al., 2008), and were 24% for the S-1 plus cisplatin arm and 15% for the S-1 arm. In the JCOG9912 trial (Boku et al., 2009), 2-year survival rates were 14% in the continuous fluorouracil infusion arm, 18% in the irinotecan plus cisplatin arm, and 21% in the S-1 arm. Moreover, in a phase III trial (V325) comparing docetaxel, cisplatin, and 5-fluorouracil (DCF) with cisplatin and 5-fluorouracil (CF) (Van Cutsem et al., 2006), 2-year survival rates were 18% for DCF and 9% for CF. The 2-year survival rate (19%) in the present study was higher than that observed in the early JCOG trials (8%) and was comparable with data from prospective studies of modern regimens (Van Cutsem et al., 2006; Koizumi et al., 2008; Boku et al., 2009). In the SPIRITS and JCOG9912 trials, 74% to 83% of patients received second-line chemotherapy. Similarly, in the present study 73% of patients received second-line chemotherapy. Moreover, long-term survivors received multiple-line treatments with newer cytotoxic agents, including taxanes and irinotecan, more frequently than the other patients. In agreement with previous studies (Thuss-Patience et al., 2011; Kang et al., 2012; Ford et al., 2014), the present improvement in LTS may reflect the prevalence of second- and third-line chemotherapy with taxanes and irinotecan. Although recent trials of modern regimens suggest that improved overall survival with chemotherapy can lead to LTS, the present LTS data were not associated with types of chemotherapeutic regimens. However, few patients received fluoropyrimidine plus platinum-based regimens, and further investigations are warranted to evaluate the impact of first-line therapy on LTS.

In addition, previous retrospective studies (Hosokawa et al., 2007; Lee et al., 2007a; Lee et al., 2007c; Koo et al., 2011; Inal et al., 2012; Kadowaki et al., 2014) and a large prospective randomized trial (JCOG9912) (Takahari et al., 2014) show that better PS, single metastatic sites, previous gastrectomy, and normal ALP levels are associated with improved OS; Takahari et al. (Takahari et al., 2014) constructed the JCOG index using these prognostic factors. With the exception of the cut-off value for PS, the present LTS predictive factors were identical to the prognostic factors chosen for the JCOG index. Small numbers of metastatic sites and primary tumor resection successes rates are expected to reflect lower tumor volumes in patients with MGC. Patients with MGC who underwent gastrectomy but had residual disease often had minimal disease, including intraoperative peritoneal disseminations and other metastatic lesions. Recurrent

disease is often diagnosed with low tumor burdens during early follow-up and surveillance after curative surgery. Several previous studies report elevated ALP levels as a prognostic factor that generally reflects the presence of liver disease, bone metastases, and malignant disease (Lee et al., 2007a; Koo et al., 2011; Kadowaki et al., 2014; Takahari et al., 2014). In the present study, high ALP levels were associated with poor PS, liver metastasis, bone metastasis, target lesions, and multiple metastatic sites (data not shown). Thus, this serum marker may reflect increased tumor aggressiveness and burden.

In the present study, the observed 5-year survival rate (3%, 16 of 514) was equivalent to that (2%, 11 of 497) reported in early JCOG studies. These data do not indicate whether this extraordinary survival followed chemotherapy or reflected the underlying indolent nature of cancers in selected patients. However, most patients experienced durable responses or disease stabilization after first-line treatment, and six had no evidence of disease after cessation of therapy, suggesting that chemotherapy contributed to the present 5-year survival rate. Although specific sites of metastasis were not related to LTS, eight (50%) and six (38%) of the 16 5-year survivors had incurable peritoneal and abdominal lymph node metastasis, respectively. Similarly, Hosokawa et al. (Hosokawa et al., 2007) reported that 22 patients with only peritoneal metastasis survived for a median of 24 months, and 16 of these were diagnosed during laparotomy or laparoscopy; this suggests earlier detection of peritoneal disease. Furthermore, they found that six of nine 3-year survivors had only peritoneal disease. In the present study, most patients with peritoneal metastasis were diagnosed during open surgery, computed tomography imaging, or enema examinations, and had low tumor volumes. In a retrospective study from Korea (Park et al., 2011), patients with only para-aortic lymph node metastases had a higher response rates, longer times to progression, and improved OS compared with those with the other metastatic patterns. In the present cases, abdominal lymph node metastases were not histologically confirmed, although the majority of patients had evidence of tumor shrinkage and tumor marker decline after chemotherapy. Thus, patients with only lower peritoneal disease volumes or abdominal lymph node metastases may have improved chances of LTS following chemotherapy.

The present study was limited to retrospective analyses at a single center. Although consecutive patients were included in the study, intrinsic biases may have arisen from clinical practices and the patient population. Therefore, further investigations are required in larger cohorts of patients. Moreover, metastatic sites were not confirmed using pathological analyses in most cases. Thus, over classification of imaging abnormalities as metastatic disease may have occurred, although most patients had clinically convincing disease and died of disease progression. Finally, our analysis included patients for whom targeted agents such as trastuzumab and ramucirumab were not available as standard treatment options. Thus, future analyses of these agents may show improved long-term outcomes in MGC patients.

In conclusion, we assessed clinical characteristics of MGC patients who achieved LTS after systemic chemotherapy, and identified prognostic factors that indicate curative potential in highly selected MGC patients. These data may facilitate subsequent studies of changes in survival outcomes with new therapies such as molecular targeted agent.

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ORIGINAL ARTICLE

# Impact of docetaxel in addition to cisplatin and fluorouracil as neoadjuvant treatment for resectable stage III or T3 esophageal cancer: a propensity score-matched analysis

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**Abstract**
*Purpose* To investigate the influence of addition of docetaxel to neoadjuvant chemotherapy (NAC) with cisplatin plus 5-fluorouracil (CF) in patients with clinical stage III or T3 esophageal squamous cell carcinoma.
*Methods* Information about 209 esophageal cancer patients with stage III or T3 disease, who underwent NAC consisting of CF with or without docetaxel, was reviewed. The survival outcomes were analyzed using the Kaplan–Meier method and propensity score-adjusted Cox proportional hazards models. The relevant variables were included in the propensity score model.
*Results* NAC was administered to 149 patients in the CF group and 60 patients in the docetaxel plus CF (DCF) group. Overall, 129 patients treated with CF and 58 patients treated with DCF underwent surgery after NAC. The overall response rate was significantly higher in the DCF group compared with the CF group (61.0 vs. 43.2 %, $p = 0.021$). After matching, recurrence-free survival did not differ statistically between the CF and DCF groups [hazard ratio (HR) 0.83, 95 % confidence interval (CI) 0.50–1.37, $p = 0.46$]. After matching, the improvement in overall survival in the DCF group reached statistical significance (HR 0.49, 95 % CI 0.24–0.999, $p = 0.050$). No significant differences in rate of locoregional or distant recurrences were observed between the CF and DCF groups (53.0 vs. 48.3 %, $p = 0.54$).
*Conclusions* NAC with DCF is superior to CF in patients with clinical stage III or T3 esophageal squamous cell carcinoma.

**Keywords** Esophageal cancer · Neoadjuvant chemotherapy · Surgery · Propensity score analysis

## Introduction

Neoadjuvant chemotherapy (NAC) or chemoradiotherapy (CRT) followed by surgery is the standard treatment for resectable esophageal cancer. The Western world has seen a dramatic rise in adenocarcinomas of the esophagus in recent years. However, adenocarcinomas represent only 4.3 % of all esophageal cancers; the majority of esophageal carcinomas in Japan are squamous cell carcinomas [1]. The Japan Clinical Oncology Group (JCOG) has conducted several randomized phase III trials to establish new standard treatments for locally advanced esophageal cancer, defined as clinical stage IB–IIIC in the 7th edition of the Union for International Cancer Control (UICC) TNM classification. A randomized phase III trial (JCOG9204), which compared adjuvant chemotherapy with cisplatin plus 5-fluorouracil (CF) to surgery alone, showed the superiority of adjuvant chemotherapy with respect to disease-free

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survival [2]. A subsequent randomized phase III trial (JCOG9907) confirmed the survival benefit of NAC with CF compared to adjuvant CF chemotherapy [3]. The 5-year overall survival (OS) rate was 43 % in the adjuvant group and 55 % in the NAC group [hazard ratio (HR) 0.73, 95 % confidence interval (CI) 0.54–0.99, $p = 0.04$]. Therefore, NAC with CF is considered the current standard treatment for locally advanced esophageal cancer in Japan. However, JCOG9907 subgroup analyses revealed that NAC is not effective in patients with clinical stage III disease or T3 tumors. Thus, development of more intensive perioperative therapy is required for patients with clinical stage III disease or T3 tumors.

To further improve survival, a phase II trial that evaluated the addition of docetaxel to CF (DCF) in the NAC setting in patients with clinical stage II/III (UICC-TNM 6th edition) squamous cell carcinoma of the thoracic esophagus was conducted; this regimen yielded a good response rate (62.5–64.2 %) and 2-year OS rate (88.0 %), with no treatment-related deaths [4, 5]. NAC with DCF thus appears to be promising as preoperative treatment.

Only one retrospective report, which compared neoadjuvant CF with DCF in a small cohort of patients with clinical stage II–IV disease without distant metastases, has been published [6]. This report showed that neoadjuvant DCF provides significantly better OS compared with neoadjuvant CF. The objective of the present study was to investigate the influence of adding docetaxel to CF as neoadjuvant therapy in patients with clinical stage III or T3 esophageal squamous cell carcinoma.

## Patients and methods

### Patient population

This was a retrospective cohort study of esophageal cancer patients treated with NAC followed by surgery at Aichi Cancer Center Hospital between January 2003 and January 2013. A total of 209 patients met the following inclusion criteria: (1) carcinoma of the thoracic esophagus; (2) histological diagnosis of primary esophageal squamous cell carcinoma; (3) clinical stage III or T3 tumor (the 7th UICC-TNM classification); (4) no distant organ metastasis; (5) NAC consisting of CF or DCF; and (6) no previous thoracic radiotherapy (RT) or thoracic surgery. Patients who received neoadjuvant CRT followed by surgery were excluded from this analysis.

### Pretreatment staging

Pretreatment staging evaluation included physical examination, laboratory tests, esophagogastroduodenoscopy, barium esophagography, and contrast-enhanced computed tomography (CT) from the neck to the upper abdomen. Pretreatment stage, based on the 6th edition of the AJCC Cancer Staging Manual until 2009 and based on the 7th edition after 2010, was determined during a meeting of thoracic surgeons, radiologists, gastroenterologists, and medical oncologists. Treatment strategies were also determined at the meeting.

### Neoadjuvant chemotherapy and treatment assessments

The CF regimen consisted of intravenous cisplatin (80 mg/m$^2$) on day 1 followed by continuous infusion of 5-fluorouracil (800 mg/m$^2$/day) for 5 days, given every 3 weeks for two cycles. The DCF regimen was based on the previous phase II study [4, 5] and consisted of intravenous docetaxel (60–70 mg/m$^2$) and cisplatin (80 mg/m$^2$) on day 1 followed by continuous infusion of 5-fluorouracil (750–800 mg/m$^2$/day) for 5 days, given every 3–4 weeks for two to three cycles. Two DCF regimens were used: the lower-dose DCF regimen consisted of intravenous docetaxel (60 mg/m$^2$) and cisplatin (60 mg/m$^2$) on day 1 followed by continuous infusion of 5-fluorouracil (800 mg/m$^2$/day) for 5 days, while the higher-dose DCF regimen consisted of intravenous docetaxel (70 mg/m$^2$) and cisplatin (70 mg/m$^2$) on day 1 followed by continuous infusion of 5-fluorouracil (750 mg/m$^2$/day) for 5 days. Patients in the DCF group were given prophylactic antibiotics. Granulocyte colony-stimulating factor (G-CSF) was used if patients experienced grade 4 neutropenia or febrile neutropenia, but was not used for prophylaxis. Because few patients had measurable disease as determined by the Response Evaluation Criteria in Solid Tumors (RECIST), the treatment response of each primary esophageal lesion was endoscopically evaluated in patients without measurable disease and categorized as complete response (CR), partial response, stable disease, or progressive disease [7]. Partial response was defined as obvious morphological change, such as reduction in or flattening of the tumor or elevated lesion(s) around the ulcer, along with healing of the ulcer floor.

### Surgery and histopathological response evaluation

Patients were scheduled to undergo surgery 4–8 weeks after the last day of NAC, when esophageal resection was defined as potentially curative. All patients underwent subtotal esophagectomy with regional lymphadenectomy through right thoracotomy and laparotomy, and reconstruction was performed using the stomach via a retrosternal route with cervical anastomosis through a neck incision. The entire tumor bed was cut into slices containing the entire esophageal wall, and histological therapeutic effects were classified as follows: grade 3, complete disappearance

of viable cancer cells in the tumor bed; grade 2, disappearance of greater than two-thirds of viable cancer cells; and grade 1, disappearance of less than two-thirds of viable cancer cells [8].

History and physical examination, complete blood cell count, gastrointestinal endoscopy, chest X-ray, and CT scanning of the neck, chest, and abdomen were performed approximately every 3–6 months after initiation of treatment until death or until patients were lost to follow-up.

### Data collection

The following information was recorded from the medical records and radiological images of each patient: treatment initiation date, age, gender, Eastern Cooperative Oncology Group performance status (ECOG PS), cancer site, primary tumor length, histopathological grade, clinical stage according to the AJCC 7th edition, serum albumin at pretreatment, clinical response, pathological stage, pathological margin, histological therapeutic effects, and final date of survival assessment.

### Statistical analysis

All patient characteristics were considered categorical variables, with the exception of age and primary tumor length, which were treated as continuous data. Specific comparisons between groups were made using Chi-square and Mann–Whitney tests. OS was calculated from treatment initiation date to the time of death from any cause, or to time of last follow-up. Relapse-free survival (RFS), locoregional recurrence-free survival (LRFS), and distant recurrence-free survival (DRFS) were determined from treatment initiation date to documented date of first recurrence, to the time of death from any cause, or to time of last follow-up. Unmatched survival analyses were performed using the Kaplan–Meier method comparing survival curves with the log-rank test and unadjusted Cox proportional hazard models.

Multivariable adjusted Cox proportional hazards regression analyses were performed that included age, gender, PS, cancer site, primary tumor length, histopathological grade, clinical T stage (cT), number of lymph node metastases, serum albumin, and year of treatment (three groups). The propensity score was calculated using a multivariable logistic regression model with NAC regimen as the dependent variable and age, gender, PS, cancer site, primary tumor length, histopathological grade, cT, number of lymph node metastases, serum albumin, and year of treatment (three groups) as independent variables. The Cox proportional hazards models for OS and RFS were then adjusted using propensity score matching together with the aforementioned a priori-defined covariates. Therefore, the measure of association in this study was HR plus the 95 % CI. Statistical analyses were performed using STATA version 13 (Stata Corp LP, College Station, TX, USA) and R version 3.1.0 (R Project for Statistical Computing, Vienna, Austria). A $p$ value less than 0.05 was considered statistically significant.

## Results

### Patient characteristics

Patient characteristics are summarized in Table 1. Of 209 consecutive esophageal cancer patients, 149 patients were treated with NAC with CF and 60 patients were treated with DCF. Most of the primary tumors were located in the mid-lower thoracic esophagus. The histological diagnosis of all tumors was squamous cell carcinoma. The histopathological grade values were significantly different among all patients ($p = 0.020$). In the DCF group, 28 patients received lower-dose DCF and 32 patients received higher-dose DCF. The median follow-up periods were 2.7 years (range 0.46–10.1) in all patients. The follow-up period was significantly longer in the CF group ($p = 0.0026$).

### Response to chemotherapy

The overall response rate was significantly better in the DCF group than in the CF group (61.0 vs. 43.2 %, $p = 0.021$) (Table 2), but no significant difference in CR rate was observed between the two groups (3.4 vs. 4.7 %, $p = 0.64$). Overall, 129 (87 %) patients in the CF group and 58 (97 %) patients in the DCF group underwent surgery after NAC. In the CF group, 20 (13 %) patients underwent CRT instead of esophagectomy for the following reasons: Six patients were hopeful after achieving a CR, three patients refused to undergo surgery, and 11 patients had incurable disease. In the DCF group, two (3 %) patients underwent CRT instead of esophagectomy for the following reasons: one patient was hopeful after achieving a PR, and one patient had incurable disease.

No significant difference in R0 resection rate was observed between the CF and DCF group [80 % (119 of 149) vs. 80 % (48 of 60), $p = 0.98$]. Histological examination of primary lesions revealed that eight (6.2 %) of 129 patients in the CF group and seven (12.1 %) of 58 patients in the DCF group achieved a grade 3 histological postchemotherapeutic effect (Table 3, $p = 0.23$). Two (7.1 %) and five (16.7 %) patients who received lower- and higher-dose DCF, respectively, achieved a grade 3 histological postchemotherapeutic effect.

**Table 1** Patient and tumor characteristics

| Characteristic | CF $n = 149$ | DCF $n = 60$ | $p$ |
|---|---|---|---|
| Age (years) | | | 0.24 |
| Median | 62 | 61 | |
| Range | 44–79 | 46–74 | |
| | | | 0.99 |
| <65 | 87 | 35 | |
| ≥65 | 62 | 25 | |
| Gender | | | 0.33 |
| Male | 118 | 51 | |
| Female | 31 | 9 | |
| Performance status | | | 0.61 |
| 0 | 23 | 11 | |
| 1 | 126 | 49 | |
| Cancer site | | | 0.78 |
| Upper thoracic | 15 | 8 | |
| Mid-thoracic | 75 | 30 | |
| Lower thoracic | 59 | 22 | |
| Primary tumor length (cm) | | | 0.082 |
| Median | 5 | 5.5 | |
| Range | 2.0–10 | 2.0–10 | |
| cT stage | | | 0.67 |
| 1 | 2 | 0 | |
| 2 | 5 | 2 | |
| 3 | 142 | 58 | |
| cN stage | | | 0.26 |
| 0 | 25 | 4 | |
| 1 | 79 | 36 | |
| 2 | 44 | 19 | |
| 3 | 1 | 1 | |
| Histopathological grade | | | 0.020 |
| 1 | 17 | 1 | |
| 2 | 93 | 36 | |
| 3 | 20 | 7 | |
| X | 19 | 16 | |
| cStage | | | 0.056 |
| II | 25 | 4 | |
| III | 124 | 56 | |
| | | | 0.84 |
| IIIA | 86 | 38 | |
| IIIB | 37 | 17 | |
| IIIC | 1 | 1 | |
| Serum albumin (g/dL) | | | 0.59 |
| Median | 4.1 | 4.2 | |
| Range | 3.0-4.9 | 2.7-4.8 | |
| <4.0 | 41 | 18 | 0.72 |
| ≥4.0 | 108 | 42 | |
| Follow-up period (years) | | | 0.0026 |
| Median | 3.6 | 2.2 | |
| Range | 0.46–10.1 | 0.65–5.7 | |

*CF* cisplatin plus 5-fluorouracil, *DCF* docetaxel plus cisplatin plus 5-fluorouracil

### Survival

In the unadjusted situation, no statistically significant difference in median RFS was observed between the CF (2.1 years) and DCF (2.8 years) groups. The following RFS rates were observed: 1-year RFS rate, 60.9 versus 71.7 %, respectively; 2-year RFS rate, 51.1 versus 51.8 %, respectively; and 3-year RFS rate, 45.5 versus 39.9 %, respectively (HR 0.91, 95 % CI 0.60–1.37, $p = 0.66$). After matching, RFS did not differ statistically between the CF and DCF groups (HR 0.83, 95 % CI 0.50–1.37, $p = 0.46$) (Fig. 1a).

The OS difference also did not reach statistical significance in the unadjusted situation, but was higher in the DCF group compared with the CF group: median OS, not reached versus 4.7 years, respectively; 1-year OS rate, 93.1 versus 88.3 %, respectively; 2-year OS rate, 85.4 versus 70.6 %, respectively; and 3-year OS rate, 70.2 versus 59.9 %, respectively (HR 0.60, 95 % CI 0.33–1.07, $p = 0.088$). After matching, the higher OS in the DCF group reached statistical significance (HR 0.49, 95 % CI 0.24–0.999, $p = 0.050$) (Fig. 1b).

### Patterns of postoperative recurrence

No significant differences in rates of locoregional or distant recurrences were observed between the CF and DCF groups (53.0 vs. 48.3 %, $p = 0.54$). The LRFS did not differ statistically between the CF and DCF groups (HR 0.80, 95 % CI 0.50–1.30, $p = 0.385$). Moreover, estimated LRFS rates at 1 and 2 years were 79.4 and 63.9 %, respectively, in the CF group, whereas they were 84.5 and 64.7 %, respectively, in the DCF group.

The DRFS did not differ statistically between the CF and DCF groups (HR 0.83, 95 % CI 0.49–1.43, $p = 0.515$). Moreover, estimated DRFS rates at 1 and 2 years were 73.6 and 68.2 %, respectively, in the CF group, whereas they were 81.6 and 74.9 %, respectively, in the DCF group.

With respect to treatment modality after recurrence, no significant differences were observed between the CF and DCF groups (Table 4).

## Discussion

Although NAC or CRT followed by esophagectomy has been standard therapies for resectable esophageal cancer [9–13], the prognosis of esophageal cancer patients with advanced squamous cell carcinoma remains poor. The JCOG9907 study showed the superiority of NAC with CF with respect to OS compared with adjuvant chemotherapy in patients with resectable (non-T4) esophageal

**Table 2** Response to chemotherapy

| | CF $n = 149$ | DCF $n = 60$ | $p$ |
|---|---|---|---|
| Overall response | | | |
| CR | 7 | 2 | |
| PR | 57 | 34 | |
| SD | 71 | 21 | |
| PD | 13 | 2 | |
| Negative | 1 | 1 | |
| Response rate | | | |
| | | | 0.021 |
| CR + PR (%) | 43.2 | 61.0 | |

*CF* cisplatin plus 5-fluorouracil, *DCF* docetaxel plus cisplatin plus 5-fluorouracil, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

**Table 3** Postoperative pathological data

| | CF $n = 129$ | DCF $n = 58$ | $p$ |
|---|---|---|---|
| ypT stage | | | 0.6 |
| 0 | 8 | 7 | |
| 1 | 13 | 6 | |
| 2 | 24 | 8 | |
| 3 | 81 | 34 | |
| 4 | 3 | 3 | |
| ypN stage | | | 0.73 |
| 0 | 30 | 17 | |
| 1 | 50 | 19 | |
| 2 | 30 | 15 | |
| 3 | 19 | 7 | |
| ypStage | | | 0.52 |
| 0 | 6 | 6 | |
| I | 10 | 5 | |
| II | 29 | 12 | |
| III | 84 | 35 | |
| Surgical margin | | | 0.23 |
| Negative | 115 | 48 | |
| Positive | 14 | 10 | |
| Histological therapeutic effects | | | 0.12 |
| Grade 3 (100 %) | 8 | 7 | |
| Grade 2 (67–99 %) | 27 | 14 | |
| Grade 1 (1–66 %) | 82 | 37 | |
| Grade 0 (0 %) | 4 | 0 | |
| Unknown | 8 | 0 | |

*CF* cisplatin plus 5-fluorouracil, *DCF* docetaxel plus cisplatin plus 5-fluorouracil

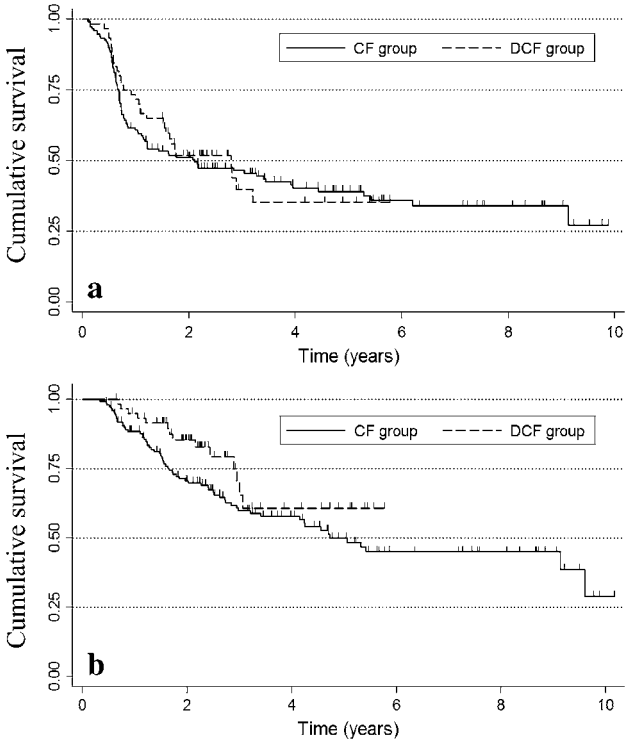


**Fig. 1** **a** Comparison of the Kaplan–Meier curves for RFS between the CF and DCF groups in a matched situation. **b** Comparison of the Kaplan–Meier curves for OS between the CF and DCF groups in a matched situation

cancer [3]. Furthermore, subgroup analyses of the JCOG 9907 study suggested that NAC does not provide a survival benefit in patients with stage III disease or T3 tumors. We hypothesized that intensive preoperative chemotherapy could improve survival outcomes in patients with stage III disease or T3 tumors. The present results show that the RFS survival curve in the DCF group almost overlapped with the curve in the CF group, and that the DCF group had a favorable OS curve compared with that of the CF group. The 2-year RFS and LRFS rates were similar between groups, but the DCF group had a favorable 2-year DRFS rate compared with that of the CF group.

Ui et al. [6] reported that NAC with DCF improves histological response, progression-free survival, and OS compared with CF. Because patients with stage IV disease were included in this study analysis, the outcomes of patients treated with CF were poorer than in those who received CF in the JCOG9907 study. In contrast, the survival outcomes of patients treated with CF in the present study were similar to those of patients in the JCOG9907 study: The 3-year OS rate in the present study was 59.9 %, compared to 62.6 % in the JCOG9907 study.

**Table 4** Postoperative recurrence

| | CF $n = 149$ | DCF $n = 60$ | $p$ | Lower-dose DCF $n = 28$ | Higher-dose DCF $n = 32$ | $p$ |
|---|---|---|---|---|---|---|
| Pattern of recurrence | | | 0.91 | | | 0.81 |
| None | 68 | 30 | | 14 | 16 | |
| Locoregional | 32 | 13 | | 6 | 7 | |
| Distant | 47 | 16 | | 8 | 8 | |
| Unknown | 2 | 1 | | 0 | 1 | |
| | $n = 81$ | $n = 30$ | $p$ | $n = 14$ | $n = 16$ | $p$ |
| Treatment after recurrence | | | 0.94 | | | 0.64 |
| None | 6 | 2 | | 2 | 0 | |
| Chemoradiotherapy | 28 | 11 | | 5 | 6 | |
| Chemotherapy alone | 36 | 11 | | 5 | 6 | |
| Radiotherapy alone | 5 | 3 | | 1 | 2 | |
| Surgery | 3 | 1 | | 0 | 1 | |
| Unknown | 3 | 2 | | 1 | 1 | |

*CF* cisplatin plus 5-fluorouracil, *DCF* docetaxel plus cisplatin plus 5-fluorouracil

Patients who receive neoadjuvant CRT and have a complete pathologic response experienced significantly improved survival [14]. In the present study, although the overall response rate was significantly better in the DCF group, histopathological findings revealed that pathological CR rate was similar between groups. An additional subgroup analysis showed that pathological CR rate was slightly higher in patients who received higher-dose DCF compared with those who received CF or lower-dose DCF. Higher-dose DCF therefore appears to improve survival outcomes.

In the Western world, neoadjuvant CRT followed by surgery is the standard treatment for resectable esophageal cancer [9]. We also hypothesized that reinforcement of systemic control with more intensive NAC is an additional strategy to improve survival of patients with locally advanced esophageal cancer. The addition of docetaxel to CF might lead to improvement in local control and reduce distant metastasis compared with neoadjuvant CRT. A slightly lower rate of distant metastasis was observed in patients treated with DCF; this result may be associated with the better OS observed in the matched analysis.

We recognize that the present study has several limitations. First, only squamous cell carcinomas were evaluated. A second limitation is that this was a retrospective study using a small number of patients. A third limitation is that the median follow-up period in the DCF group was 2.2 years. A fourth limitation is that propensity score adjustment can only be based on measured covariates and lacks inclusion of unmeasured potential confounders.

In conclusion, the results of the present study indicate that NAC with DCF is superior to CF in patients with clinical stage III or T3 esophageal squamous cell carcinoma. A three-arm randomized controlled trial comparing CF versus DCF versus CRT as NAC therapy for locally advanced esophageal cancer (JCOG1109, NExT study) is ongoing [15].

**Conflict of interest** None of the authors have identified a conflict of interest.

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CHEST

# Asbestosis and other pulmonary fibrosis in asbestos-exposed workers: high-resolution CT features with pathological correlations

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**Abstract**

*Objective* The purpose was to identify distinguishing CT features of pathologically diagnosed asbestosis, and correlate diagnostic confidence with asbestos body burden.

*Methods* Thirty-three workers (mean age at CT: 73 years) with clinical diagnoses of asbestosis, who were autopsied (n=30) or underwent lobectomy (n=3), were collected. Two radiologists independently scored high-resolution CT images for various CT findings and the likelihood of asbestosis was scored. Two pathologists reviewed the pathology specimens and scored the confidence of their diagnoses. Asbestos body count was correlated with CT and pathology scores.

*Results* Pathologically, 15 cases were diagnosed as asbestosis and 18 cases with various lung fibroses other than asbestosis. On CT, only the score of the subpleural curvilinear lines was significantly higher in asbestosis ($p$=0.03). Accuracy of CT diagnosis of asbestosis with a high confidence ranged from 0.73 to 0.79. Asbestos body count positively correlated with CT likelihood of asbestosis (r=0.503, $p$=0.003), and with the confidence level of pathological diagnosis (r=0.637, $p$<0.001).

*Conclusions* Subpleural curvilinear lines were the only clue for the diagnosis of asbestosis. However, this was complicated by other lung fibrosis, especially at low asbestos body burden.

*Key points*

- *Various patterns of pulmonary fibrosis occurred in asbestos-exposed workers.*
- *The fibre burden in lungs paralleled confident CT diagnosis of asbestosis.*
- *The fibre burden in lungs paralleled confident pathological diagnosis of asbestosis.*
- *Subpleural curvilinear lines were an important CT finding favouring asbestosis.*

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**Keywords** Computed tomography, radiography · Asbestos · Pulmonary fibrosis · Asbestosis · Chronic interstitial pneumonia

## Introduction

Asbestosis is suspected when diffuse lung fibrosis is identified in patients with clinical evidence of vast amounts of asbestos exposure. However, it is not only asbestosis that presents with diffuse lung fibrosis in such patients, but also idiopathic pulmonary fibrosis (IPF), and this is the important differential diagnosis. Pathologically, asbestosis is characterized by the fibrosis of alveolar walls adjacent to the respiratory bronchioles, which extend to involve the surrounding lung in the centrifugal direction [1]. In contrast, usual interstitial pneumonia (UIP), which is the pathologic counterpart of IPF, begins at the periphery of the secondary pulmonary lobule and progresses in the centripetal direction. These anatomical differences of lung fibrosis could be appreciated by high-resolution computed tomography (CT) images to some extent; however, the story is not straightforward. There are three studies that dealt with the imaging differences between asbestosis and IPF; they have yielded conflicting results [2–4]. Two reports found some important differences that facilitated the diagnosis; however, the other report found no differences between asbestosis and IPF. The drawback of these previous researches is that the diagnosis was made clinically without pathological diagnosis, and the IPF patients were not always exposed to asbestos.

In this retrospective study, we collected high-resolution CT and lung tissue obtained from autopsy or lobectomy from a nationwide network that cares for asbestos-exposed patients in our country. The purpose of this study was to find CT differences between asbestosis and other pulmonary fibrosis (non-asbestosis) in asbestos-exposed workers based on pathological diagnosis, and to elucidate diagnostic feasibility of computed tomography and pathology in comparison to the degree of asbestos exposure.

## Materials and methods

This retrospective study was approved by the institutional review boards of the six participating hospitals. Informed consent from the patients who were alive was obtained; it was waived for deceased patients.

### Patients

We collected cases of possible asbestosis from the nationwide hospital network that cares for asbestos workers. Those cases with a pathologic specimen (either lobectomy or autopsy) were identified, and the CT images, during life and pathology specimens, were collected. Patients were followed up for pulmonary fibrosis with known occupational asbestos exposure. Fifty-six cases were collected from six hospitals. Twenty-three cases were excluded because of the lack of high-resolution CT or absence of lung fibrosis on CT and pathological analysis. Thus, 33 patients (31 men, two women, mean age at CT: 73 years) who underwent chest CT between May 2000 and July 2011 were enrolled in the study group. Sixteen patients, in whom subsequent pathological diagnosis revealed four asbestosis and 12 non-asbestosis, as described below, were included in the previous study [5]. Thirty cases underwent autopsy, and three cases had lobectomies for lung cancer (two from right lower lobe, and one from left upper lobe). The interval between CT scan and autopsy ranged from 1 month to 68 months (mean=16 months). In the autopsied cases, those CT images were avoided that showed complications such as pneumonia, acute exacerbation of chronic interstitial pneumonia or advanced lung cancer. Only those images of patients in stable condition were evaluated. For lobectomy cases, CT images were obtained within 2 months of lobectomy. Occupational histories included: asbestos-manufacturer (n=9), shipyard workers (n=8), asbestos-spraying (n=4), repairing boiler (n=2), insulation worker (n=2), plumbing (n=2), and others (n=5). Working years ranged from 10 to 42 years (mean=24 years).

### High-resolution CT technique

CT images were obtained by various CT systems. Patients were imaged in the supine position. Lung window images were provided with 1–2 mm thickness with 10 mm intervals of the whole lung in all but one case, in which lung and mediastinal window images were provided with 3 mm thickness without gap. Additional contiguous images with 5–7 mm thickness of the whole lung were also available in most cases. Images were provided with the DICOM format and reviewed on monitors.

### Image analysis

Two radiologists (K. A. and K. K., with 24 and 20 years of experience, respectively) independently reviewed the images without knowledge of pathological diagnosis and occupational history, but with knowledge of age and sex of the patient. Disagreements about the presence or absence of each CT finding were resolved by the decision of the third radiologist (H. A., 22 years of experience).

CT images for lung disease were scored by the nearest of 10 % of the cross-sectional area in each zone. The zone consisted of upper, middle and lower; the tracheal carina and the confluence of inferior pulmonary vein served as the boundaries. The extent of ground-glass opacity, reticulation,

honeycombing, consolidation and emphysema were scored. The presence of dot-like opacity [6], subpleural curvilinear line [7, 8], parenchymal band and mosaic perfusion were evaluated (score range, 0–6). The coarseness of fibrosis was scored as: 0=ground-glass opacity only, 1=ground-glass opacity with reticulation, 2=honeycomb cysts less than 5 mm, 3=honeycomb cysts more than 5 mm (score range, 0–18) [3]. If no interstitial opacity was identified in the zone, the zone was excluded for scoring coarseness. The number of segments with traction bronchiectasis was scored in the upper, middle/lingual and lower lobes (score range, 0–18).

The pleural disease, comprising both pleural plaque and diffuse pleural thickening, was scored in each zone by the maximum extent compared to the circumference of hemithorax at the level of tracheal carina as: 1=less than one-quarter, 2=more than one-quarter and less than one-half, 3=more than one-half and less than three-quarters, 4=more than three quarters (score range, 0–24). The presence of diffuse pleural thickening and rounded atelectasis were also evaluated. Pleural calcification was not evaluated.

CT scores determined by the two radiologists were averaged, which yielded the final scores.

Finally, the likelihood of asbestosis was given to each case on a four-point scale: 0=not asbestosis, 1=possible asbestosis, 2=probable asbestosis, 3=definite asbestosis. The summation of the two scores provided the CT-asbestosis score. The CT diagnosis of asbestosis was made based on the previous report [2, 4]. The presence of subpleural dot-like opacity, subpleural curvilinear lines only a few millimetres from the pleural surface, subpleural consolidation without traction bronchiectasis (atelectatic induration) [8], and mosaic perfusion were CT findings favouring asbestosis, while extensive honeycomb cysts, severe traction bronchiectasis with architectural distortion, absence of pleural disease were regarded as favouring an alternative diagnosis. In the case with conflicting CT findings, the diagnosis and confidence level depended on the experience of each reviewer.

### Pathological diagnosis

Two pulmonary pathologists (K. H. and K. O., with 31 and 34 years of experience, respectively) independently reviewed the same tissue specimens without knowledge of occupational history, made diagnoses and suggested a confidence level for each case based on the recently published criteria of asbestosis [1]. It should be noted that fibrosis in asbestosis is accompanied by very little inflammation, and fibroblastic foci are infrequent [1]. In early asbestosis, the fibrosing process is limited to the walls of alveoli immediately around the bronchioles. In the advanced stage, however, a variety of morphologic patterns may be seen, such as UIP, nonspecific interstitial pneumonia (NSIP), and even an unclassifiable pattern can be identified [1]. The pathological diagnosis was scored on a three-point scale: 0=fibrosis other than asbestosis, 1=possible asbestosis, 2=definite asbestosis. The summation of the score given by each pathologist provided the pathological asbestosis score. Because the pathological specimens were obtained from autopsy or lobectomy, we reviewed multiple specimens from different sites. In the autopsy cases, several samples were obtained from different lobes. In 16 cases, we re-excised the specimen in order to correlate with CT findings and confirm the diagnosis.

### Asbestos body count

Asbestos body count was performed by experienced technicians in one of the institutions participating in this study. The method of counting asbestos bodies is detailed elsewhere [9]. Briefly, one specimen was sampled from each lobe and trimmed so that the weight of the wet lung totalled to between 1 and 2 g. In the autopsy cases, specimens were sampled from each lobe, excluding the heavily damaged ones. In regard to the lobectomy cases, one sample was excised, avoiding the tumour. The specimens were mixed and allowed to react with laboratory bleach. The digested solution was centrifuged two times, followed by filtering through the membrane filter. The filter was then fixed on the glass slide, and the ferruginous bodies were counted using polarized light microscopy.

### Statistical analysis

Agreement of CT scores were evaluated by single determination standard deviation [10]. Agreement of CT and pathological scores were calculated by weighted kappa statistics. The difference in CT scores between asbestosis and non-asbestosis were evaluated by a non-parametric test (IBM SPSS Statistics ver. 22, Tokyo, Japan). The correlations of asbestos body count and CT/pathological scores were evaluated with the Spearman rank correlation coefficient. A value of $p<0.05$ was considered significant.

## Results

The inter-observer agreement of pathological diagnosis was excellent (weighted kappa, 0.80). Each pathologist diagnosed 14 and 12 cases as definite asbestosis, one and five cases as possible asbestosis, and 18 and 16 cases as non-asbestosis, respectively. When the pathological asbestosis score of 2 or more was considered asbestosis, there were 15 asbestosis and 18 non-asbestosis cases. Non-asbestosis cases included UIP (n=5), chronic interstitial pneumonia that cannot be classified in the current classification (unclassifiable) (n=4) and mixed dust fibrosis (n=2) (Fig. 1). In two cases where one pathologist suggested an asbestosis (score 1), the other pathologist made the diagnosis of UIP and NSIP, respectively (Fig. 2).

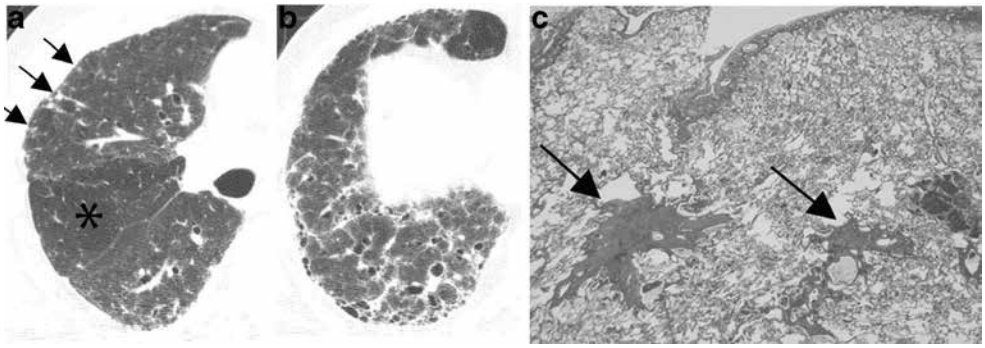


**Fig. 1** A 72-year-old previous asbestos textile male worker with mixed dust fibrosis. **a**. HRCT of right upper lobe shows multiple subpleural dot-like opacities that are relatively well defined and show high density in spite of their small size. Note that there is a lower attenuation area indicating mosaic perfusion (*asterisk*). **b**. HRCT of right lower lobe shows septal line thickening and subpleural ground-glass opacity with traction bronchiectasis resembling UIP. **c**. Low-power view of pathological specimen obtained from right upper lobe corresponding to *a* shows centrilobular stellate fibrosis typical of mixed dust fibrosis, which differs from asbestosis. Multiple asbestos bodies were identified in the specimen (not shown). The asbestos body count was 67,406/g (dry lung)

In these cases, asbestos body count was low (263,480/g and 86,560/g, respectively). In the other five cases, pathological diagnosis was either UIP, NSIP or unclassifiable pattern, and they were discordant.

In terms of CT scores, there were good inter-observer agreements with a single determination standard deviation. They were less than 5 % for all the parenchymal opacities, 2.7 for traction bronchiectasis, 1.2 for both dot-like and subpleural curvilinear opacities, 0.5 for coarseness, and less than 2 for others including pleural diseases. The inter-observer agreement in the diagnosis of asbestosis by two radiologists was 0.56 by weighted kappa statistics. Each radiologist diagnosed 19 and 16 cases as asbestosis, respectively. With a CT score of 2 or more considered as a high likelihood of asbestosis (i.e., probable and definite asbestosis) and the pathological diagnosis as a gold standard, sensitivity, specificity and accuracy by two radiologists were 0.67, 0.78, 0.73 and 0.73, 0.83, 0.79, respectively.

The mean asbestos body count was 1,464,711 and 98,745 for asbestosis and non-asbestosis, respectively ($p<0.001$) (Table 1). Age and work period were comparable between the two groups. Among the various CT findings, only the scores for subpleural curvilinear lines were significantly different between asbestosis and non-asbestosis (2.9 and 1.7, respectively, $p=0.03$) (Table 1) (Fig. 3). They were equally identified in each lung zones (summation of scores in all patients were 20, 23 and 23, in the upper, middle and lower lung zones, respectively). The other CT scores showed no significant differences between the two groups. The frequencies of CT findings considered important in the diagnosis of asbestosis from previous study are indicated in Table 2 [2]. Again, the prevalence of subpleural curvilinear lines was significantly higher in asbestosis than in non-asbestosis (86.7 % vs. 50 %, $p=0.034$). Honeycombing was observed less frequently in asbestosis than in non-asbestosis; however, the difference was not significant (40 % vs. 66.7 %, $p=0.17$). The other CT findings were observed in the comparable frequencies in both groups. Of note, two patients with asbestosis did not show pleural plaque. The likelihood of asbestosis by CT was significantly higher for pathologically diagnosed asbestosis than for pathologically diagnosed non-asbestosis (mean=3.5 vs. 1.0; $p<0.001$).

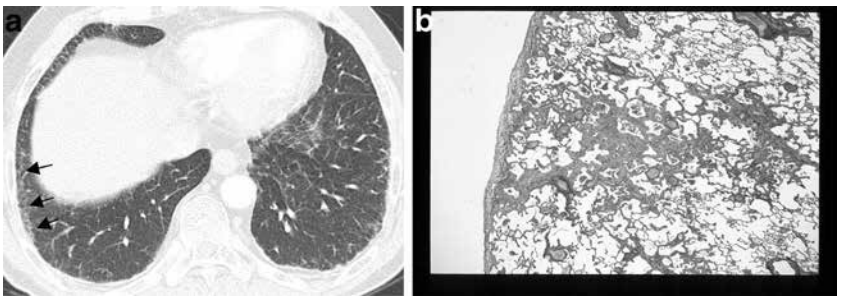


**Fig. 2** A 60-year-old male carpenter with lung cancer and pulmonary fibrosis other than asbestosis. **a**. HRCT at lower lobes before right lower lobe resection shows peripheral ground-glass and fine reticular opacities without obvious traction bronchiectasis. Note there are coalescent dot-like opacities in the midst of ground-glass opacity of right lung base, simulating subpleural curvilinear line (*arrows*). **b**. Pathological specimen (Elastic-Goldner stain) obtained from the right lung base corresponding CT image in *a* shows subpleural fibrosis as well as fibrosis in the centrilobular area. No linear fibrosis corresponding to that of CT image was observed. Asbestos body was identified (not shown) and one pathologist suggested possible asbestosis, while the other diagnosed fibrotic NSIP. Asbestos body count was 86,560/g (dry lung)

**Table 1** Patients' demographics, asbestos body count and CT scores of asbestosis vs. non-asbestosis

| | Asbestosis (n=15) | | Non-Asbestosis (n=18) | | *p* Value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| CT-Asbestosis Score | 3.5 | 1.7 | 1.0 | 1.5 | **0** |
| Asbestos Body | 1,464,711 | 1,974,822 | 98,745 | 174,492 | **0** |
| Age at CT | 74 | 5 | 72 | 8 | 0.274 |
| Work Period (years) | 24 | 11 | 29 | 15 | 0.35 |
| Ground-Glass Opacity | 10.1 | 6.1 | 9.7 | 6.2 | 0.682 |
| Reticular Opacity | 9.4 | 6.0 | 10.8 | 6.5 | 0.464 |
| Honeycombing | 4.9 | 6.3 | 6.2 | 7.3 | 0.656 |
| Coarseness | 1.8 | 0.5 | 1.8 | 0.7 | 0.985 |
| Consolidation | 9.3 | 10.5 | 4.1 | 3.8 | 0.135 |
| Emphysema | 4.0 | 4.4 | 11.8 | 17.9 | 0.117 |
| Dot-Like Opacity | 4.4 | 1.4 | 3.8 | 2.1 | 0.58 |
| Subpleural Curvilinear Lines | 2.9 | 1.7 | 1.7 | 1.6 | **0.04** |
| Septal Lines | 4.5 | 2.0 | 4.9 | 1.3 | 0.656 |
| Parenchymal Band | 1.9 | 2.3 | 1.8 | 2.1 | 0.957 |
| Traction Bronchiectasis | 13.5 | 6.5 | 12.2 | 6.3 | 0.486 |
| Bronchial Wall Thickening | 4.7 | 1.9 | 3.9 | 1.9 | 0.117 |
| Mosaic Perfusion | 0.5 | 1.1 | 0.2 | 0.7 | 0.656 |
| Pleural Plaque | 7.5 | 5.3 | 7.5 | 4.0 | 0.929 |
| Diffuse Pleural Thickening | 1.1 | 1.2 | 1.1 | 1.5 | 0.817 |

*p* values less than 0.05 were indicated with bold

We found a significant positive correlation between asbestos body count and CT-asbestosis score (r=0.503, $p$=0.003) and between asbestos body count and pathological asbestosis score (r=0.637, $p$<0.001) (Figs. 4 and 5). CT-asbestosis score and pathological asbestosis score also showed a significant positive correlation (r=0.656, $p$<0.001).

## Discussion

There have been a few reports discussing the imaging differences of asbestosis and chronic interstitial pneumonia, especially IPF [2–4]. Akira et al. reported the significant differences of CT findings between asbestosis and IPF [2]. In their report, subpleural dot-like opacities and subpleural curvilinear lines were the relatively specific CT findings of asbestosis seen in 81 % and 69 % of cases as opposed to 25 % and 28 % in IPF, respectively. Other highly specific CT findings included parenchymal band and mosaic perfusion in 48 % and 49 %, compared to 4 % and 11 % in IPF. Notably, honeycombing, the hallmark of UIP pattern, was seen in only 34 % of asbestosis patients as compared to 76 % in IPF. The paucity of honeycombing in asbestosis has also been suggested by pathologists [8, 11]. Al-Jarad et al., in their earlier comparison of CT findings between asbestosis and IPF, gained

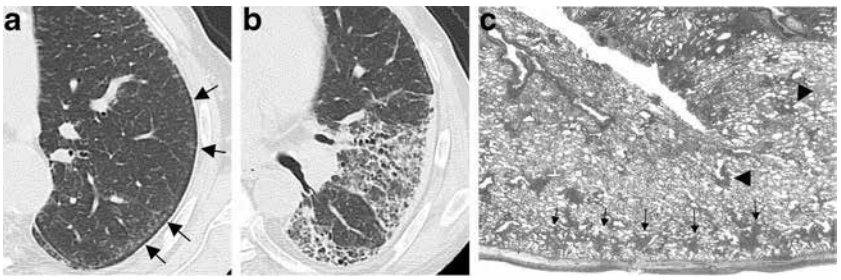


**Fig. 3** A 76-year-old previous asbestos insulation male worker with asbestosis. **a**. HRCT of left upper lobe shows a typical subpleural curvilinear line. Note that the line is formed by the coalescence of dot-like opacities and is identified along the lateral chest wall as well as in the dependent lung. **b**. HRCT of the left lower lobe shows ground-glass opacity with fine reticulation and strong traction bronchiectasis in segmental distribution, precluding the diagnosis of idiopathic pulmonary fibrosis. **c**. Low-power view of pathological specimen shows alveolar wall fibrosis of respiratory bronchioles typical of asbestosis in the subpleural area (*arrows*), which corresponds to subpleural curvilinear lines. Note that there are alveolar wall fibroses adjacent to respiratory bronchioles in the more inner side of the lung (*arrowheads*) (Hematoxylin-eosin staining). Asbestos body count was 2,711,807/g (dry lung)

**Table 2** Frequencies of CT findings in asbestosis and non-asbestosis

| | Asbestosis (n=15) | Non-asbestosis (n=18) | *p* Value |
|---|---|---|---|
| Subpleural Dot-like Opacity | 15 (100) | 16 (88.9) | 0.489 |
| Subpleural Curvilinear Lines | 13 (86.7) | 9 (50) | **0.034** |
| Honeycomb Lung | 6 (40) | 12 (66.7) | 0.17 |
| Mosaic Perfusion | 2 (13.3) | 2 (11.1) | 1 |
| Parenchymal Band | 8 (53.3) | 5 (27.8) | 0.169 |
| Pleural Plaque | 13 (86.7) | 17 (94.4) | 0.579 |
| Diffuse Pleural Thickening | 6 (40) | 5 (27.8) | 0.488 |

Note: The numbers indicate how many cases, with percentage in parentheses

the qualitative impression that fibrosis of IPF was more distorting than that of asbestosis [4]. Copley et al., however, reported no significant differences between asbestosis and IPF, and concluded that clinically diagnosed asbestosis closely resembled biopsy-confirmed IPF [3]. No pathological confirmation was obtained in these three reports, and the discrepancy is considered to be due to the selection bias of their asbestosis cases. In the era of strict asbestos regulation, cases with radiological pleural plaque and pulmonary fibrosis do not necessarily equal asbestosis, because pleural plaque occurs at much lower exposure levels, and lung fibrosis other than asbestosis can be incidental. Furthermore, several epidemiological studies have shown that workers exposed to various kinds of dust including smoking are more inclined to have IPF than those without such exposure [12–15].

Our asbestos-exposed workers were pathologically confirmed for lung fibrosis by either autopsy or surgical lobectomy. Our study confirmed the significance of subpleural curvilinear line as the sole high-resolution CT difference between asbestosis and other chronic interstitial pneumonia. Other imaging findings considered important in discriminating asbestosis from IPF in previous study did not differ in our series [2]. One reason is that non-asbestosis cases in our series included workers exposed to asbestos and other kinds of dust, and their lung conditions were complicated with various kinds of lung fibrosis. Non-asbestosis cases included not only IPF, but also mixed dust fibrosis and chronic interstitial pneumonia of unclassifiable histopathology. Mixed dust fibrosis and some unclassifiable interstitial pneumonia were airway-centred, and on that point, resembled asbestosis. This is an important difference from the previous reports, and at the same time, made differentiation by imaging difficult. Coexistence of various kinds of fibrosis in dust-exposed

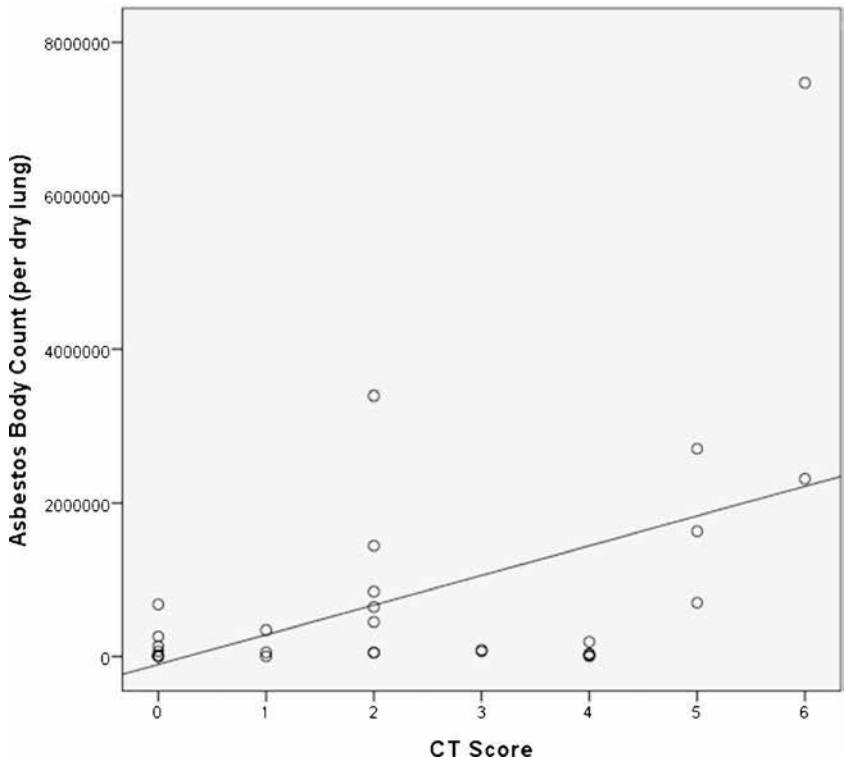


**Fig. 4** Scattered plot of asbestos body burden in lungs against CT score. There was a significant positive correlation between asbestos body count and CT-asbestosis score (r=0.503, *p*=0.003)

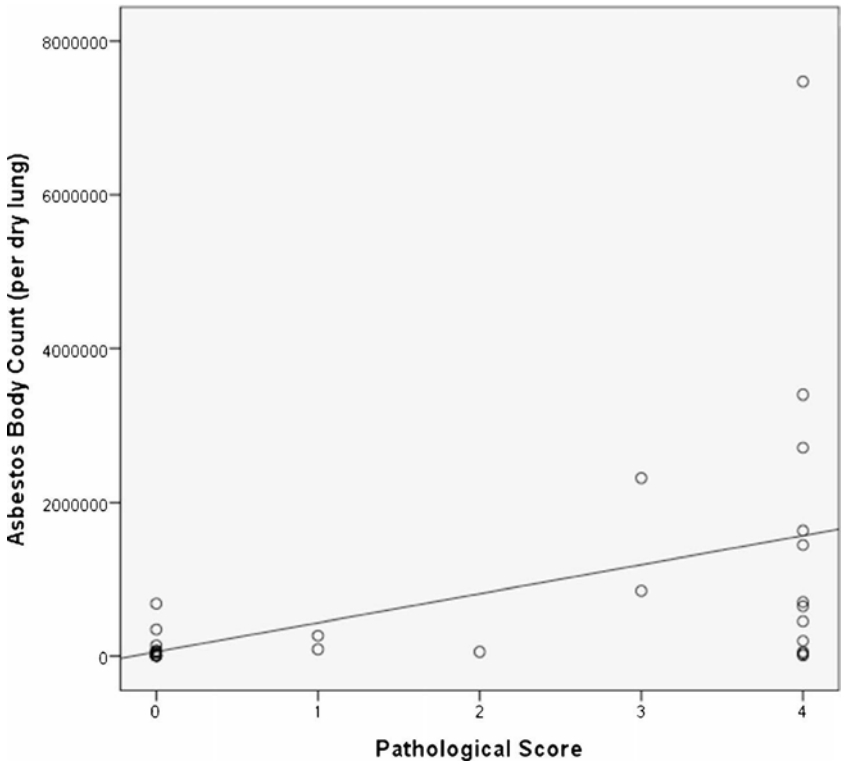


**Fig. 5** Scattered plot of asbestos body burden in lungs against pathological score. There was a significant positive correlation between asbestos body count and pathological asbestosis score ($r=0.637$, $p<0.001$)

patients was one of the problems in the pathological diagnosis of these patients; this was indicated by the fact that discordant pathological diagnoses were made in seven of 18 non-asbestosis cases, and this must have been reflected in the CT diagnosis as well.

Interesting observations were obtained in our series that found significant positive correlations of likelihood of asbestosis on CT, pathology and quantity of asbestos bodies; in other words, the more asbestos bodies in the lungs, the more the radiographic and pathological appearance of asbestosis becomes obvious. Asbestosis is a pneumoconiosis, creating a dose-response relationship, and thus the associated lung fibrosis is the direct consequence of deposited asbestos fibres. It is quite expected that the greater the fibre burden in the lungs, the more easily the patient will develop asbestosis. Our results indicate that while lungs with heavily deposited asbestos show typical appearance of asbestosis, lungs with a lesser degree of deposition may show rather atypical radiographic findings or may be complicated with other kinds of lung fibrosis that can obscure the typical findings of asbestosis, if any, resulting in atypical CT findings.

There are several limitations in our study. First, CT images were obtained in supine position, which might cause difficulty in the analyses of ground-glass opacity and subpleural curvilinear lines. Our two radiologists are well-experienced chest radiologists, and the inter-observer agreements were 4.9 and 1.2 for ground-glass opacity and subpleural curvilinear lines by single determination standard deviation, respectively. Subpleural curvilinear lines were observed as often in the upper lobes as in lower lobes, and they were not only in the dependent, but also in the lateral zone of the lungs (Fig. 3). We suppose that some inaccuracy remains in the evaluation of these findings; however, it is not so big as to alter our conclusion. Second, the series included a total of 33 cases, 15 asbestosis and 18 non-asbestosis, which is rather small in number. Third, our series included only patients with pathological diagnosis, mostly autopsy, which could be the selection bias. Fourth, the interval between CT imaging and pathology diagnosis was long, with a mean of 16 months. However, interval progression of lung fibrosis in our series is indolent and the pathology diagnosis could not change. Finally, we did not count the number of asbestos fibres, but rather counted only asbestos bodies. It has been reported that rare cases showing significant numbers of asbestos fibres with small asbestos body counts have been found [16]. While such cases could possibly be found in our series, we assume that such cases are rare and might not affect the results.

In summary, in a series of 33 asbestos-exposed patients with pathologically confirmed pulmonary fibrosis, more than half were not asbestosis and included various kinds of fibrosis, which was often discordant between pathologists. We found subpleural curvilinear lines to be the sole CT finding that differed between asbestosis and non-asbestosis cases. The CT likelihood and pathological confidence of asbestosis paralleled the asbestos body count. These findings may indicate the difficulty of discriminating asbestosis from other

types of lung fibrosis in the era of low asbestos exposure. Careful enquiry of working conditions as well as detailed observation of HRCT are mandatory.

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ORIGINAL ARTICLE

# Impact of body surface area on survival in EGFR-mutant non-small cell lung cancer patients treated with gefitinib monotherapy: observational study of the Okayama Lung Cancer Study Group 0703

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**Abstract**
*Background* The approved dose of gefitinib is fixed, without adjustment for physical size. We demonstrated previously that its efficacy was affected by body surface area (BSA) in patients with EGFR-mutant non-small cell lung cancer (NSCLC). To validate these observations, we assessed the association between BSA and the efficacy of gefitinib using a different patient cohort.
*Methods* Prospective cohort data from 115 NSCLC patients with EGFR-mutant tumours, who received gefitinib monotherapy between 2007 and 2012, were analysed.
*Results* Gefitinib was less effective in individuals with a high BSA ($\geq$1.5 $m^2$) in EGFR-mutant NSCLC compared with those with a low BSA (<1.5 $m^2$). The median progression-free survival (PFS) in the high- and low-BSA groups was 4.2 and 8.5 months, respectively, although there was no difference in survival among the whole NSCLC cohort. Multivariate analysis also showed a significant effect of BSA on PFS (hazard ratio 1.72; 95 % confidence interval 1.08–2.74; $p = 0.021$). Sensitivity analysis revealed that the use of the BSA cut-off level around 1.50 $m^2$ was robust for detecting subpopulations that would benefit less from gefitinib monotherapy.
*Conclusion* We found in the prospective cohort data that BSA could affect the efficacy of gefitinib monotherapy in patients with EGFR-mutant NSCLC, suggesting that

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BSA-based dose setting of gefitinib monotherapy might be further investigated, despite the fact that no molecular-targeted agent described to date undergoes dose adjustment according to BSA.

**Keywords** Lung cancer · Gefitinib · Body surface area · EGFR-TKI

## Introduction

The epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy because EGFR signalling is associated with the proliferation and survival of cancer cells and is overexpressed frequently in a variety of tumours, including non-small cell lung cancer (NSCLC). Activating mutations in *EGFR* were identified in a subset of NSCLC patients; the tumours with *EGFR* mutations were highly sensitive to EGFR tyrosine kinase inhibitors (TKI), including gefitinib, erlotinib and afatinib [1–3]. As a result of randomised phase III trials, the use of EGFR-TKIs has become standard for the treatment of advanced NSCLC patients harbouring activating EGFR mutations [4–6].

The dosing of most chemotherapeutic agents is based traditionally on an estimated body surface area (BSA) to reduce the pharmacokinetic inter-patient variability. In contrast, the approved dose of gefitinib is fixed at 250 mg/day based on two dose-finding randomised studies: IDEAL-1 and 2 [7, 8]. However, to date only limited data regarding the clinical significance of BSA-based dosing for gefitinib monotherapy are available.

We previously performed a small retrospective study showing a potential effect of BSA on progression-free survival (PFS) in patients treated with gefitinib monotherapy for advanced NSCLC with *EGFR* mutations [9]. Here, we performed an additional study to validate these findings and determine whether BSA affects PFS in individuals treated using gefitinib monotherapy for EGFR-mutated NSCLC using data from a prospective cohort of NSCLC patients receiving gefitinib.

## Patients and methods

### Patients

We used the prospective cohort data of consecutive EGFR-TKI-naïve NSCLC patients treated with gefitinib monotherapy at the Okayama Lung Cancer Study Group (OLCSG) affiliated hospitals between July 2007 and March 2012. The baseline BSA was estimated according to the height and weight of each patient as follows: BSA ($m^2$) = (body weight [kg]) 0.425 × (height [cm]) 0.725 × a 0.007184 [10]. Each patient provided written informed consent. The ethics committee of Okayama Lung Cancer Study Group (OLCSG) affiliated hospital approved the study protocol.

### Detection of EGFR mutations

The *EGFR* mutation status of all patients was assessed using direct sequencing or the polymerase chain reaction (PCR) clamp method. PCR mutation analyses were performed using the primary tumour, metastatic lesions, such as lymph node metastasis, or pleural effusion.

### Response assessment

Imaging tests such as chest X-ray or computed tomography (CT) were performed at ≤2-month intervals by the attending physicians. Two investigators (K. K. and K. H.) re-evaluated the tumour responses according to the response evaluation criteria in solid tumours (RECIST), version 1.1 [11].

### Statistical analysis

Overall survival (OS) and PFS were defined as the period from the beginning of gefitinib treatment to the day of disease progression or death from any cause, and the day of death from any cause, respectively, using the Kaplan–Meier method. The association between BSA and survival time were assessed using the log-rank test and multivariate Cox proportional hazards model. The latter was built using a step-wise method, with threshold $p$ values for entering and removing variables (age, pack-year, gender, stage, serum KL-6 level, Eastern Cooperative Oncology Group [ECOG] performance status [PS], histology and line of gefitinib treatment) from the model of 0.15 and 0.20, respectively. Variations and uncertainty regarding the cut-off level of BSA were assessed using sensitivity analyses. Differences were deemed statistically significant if $p < 0.05$. All statistical analyses were performed using the STATA software package (version 11.0).

## Results

### Patients' characteristics

A total of 115 patients were enrolled in the study from 2007 to 2012. This patient cohort did not overlap with the patient population assessed in the previous retrospective study [12]. The patient characteristics are summarised in Table 1. The median BSA was 1.45 $m^2$ (range 0.98–1.95 $m^2$). BSA was distributed normally at the centre peak of the curve around 1.50 $m^2$ (Supplemental Figure 1). The cohorts were divided into two groups using a BSA cut-off level of 1.5 $m^2$

**Table 1** Patient characteristics

| | EGFR-mutant cohort ($n = 115$) | | |
|---|---|---|---|
| | Total | BSA <1.5 | BSA ≥1.5 |
| *EGFR* mutation status (Exon 19/Exon 21/Exon 18/others) | 49/56/4/6 | 30/34/3/2 | 19/22/1/4 |
| Median BSA, $m^2$ | 1.45 | 1.34 | 1.61 |
| Median age, years (range)* | 71 (43–92) | 75 (43–91) | 63 (43–92) |
| Median pack-years (range)* | 0 (0–122.5) | 0 (0–122.5) | 30 (0–96) |
| Smoking status (current/former/never) | 29/26/60 | 11/8/50 | 18/18/10 |
| Gender (male/female)* | 55/60 | 20/49 | 35/11 |
| Staging (recurrence/others) | 21/94 | 11/58 | 10/36 |
| KL-6 (≥500/<500) | 47/68 | 24/45 | 23/23 |
| ECOG PS (0–1/2–4) | 85/30 | 47/22 | 38/8 |
| Histology (Ad/non-ad) | 98/17 | 57/12 | 41/5 |
| Line of gefitinib monotherapy† | 44/71 | 33/36 | 11/35 |

Major, exon 19 deletion mutation or exon 21 L858R; none, includes unknown status; recurrence, postoperative recurrence; ECOG PS, Eastern Cooperative Oncology Group performance status

*EGFR* epidermal growth factor receptor, *BSA* body surface area, *PS* performance status, *Ad* adenocarcinoma

* $p < 0.01$ and † $p = 0.01$ in the *EGFR*-mutant cohort

(BSA ≥ 1.5 vs. <1.5 $m^2$) based on the results of our previous study (Table 1) [12]. Several pre-treatment clinical demographic parameters, such as the line of gefitinib treatment, age, gender and pack-years, were different between the high- and low-BSA groups (Table 1). Sixty-seven (58 %) patients received the post-study treatment including cytotoxic agents and erlotinib. Regarding the drug delivery, forty-one (36 %) patients interrupted the gefitinib treatment mainly because of hepatic dysfunction and rash. Interstitial lung disease occurred in four (9 %) of 46 patients and four (6 %) of 69 patients in higher and lower BSA groups, respectively. At the time of this analysis, 103 patients discontinued the treatment; the reasons for the discontinuation were as follows: disease progression ($n = 82$), toxicity ($n = 10$) and others ($n = 11$).

### Association between BSA and survival

The survival analysis was available in the whole patients ($n = 115$). The median OS time and PFS time in this cohort were 14.9 months and 7.1 months, respectively. These were somewhat shorter than those in the EGFR-TKI phase 3 trials [6, 13], possibly because those with gefitinib therapy both in the first-line and salvage setting were included. Interestingly, individuals with a higher BSA tended to have worse PFS than those with a lower BSA (median 4.2 vs. 8.5 months, respectively, $p = 0.132$ log-rank test; Fig. 1a).

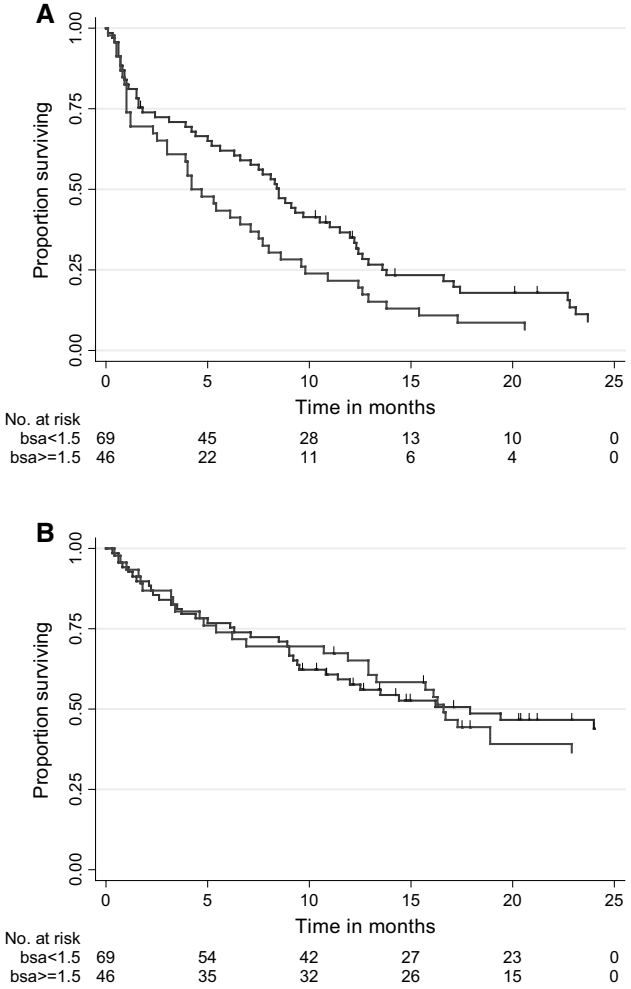


**Fig. 1** Survival curves in *EGFR*-mutant patients. The *red* and *blue lines* represent patients with a body surface area of ≥1.5 and <1.5 $m^2$, respectively. **a** Progression-free survival curve. **b** Overall survival curve. *EGFR* epidermal growth factor receptor

In multivariate analysis, BSA affected PFS significantly: patients with a higher BSA had worse PFS (HR 1.72, 95 % CI 1.08–2.74; $p = 0.021$; Table 2). Poor PS and pack-year ≥30 were also associated with a worse PFS significantly, whereas adenocarcinoma histology and postoperative recurrent stage correlated with a better PFS. In contrast, a similar OS was observed in patients with low and high BSA (HR 0.76, 95 % CI 0.44–1.32; $p = 0.335$; Fig. 1b; Table 2).

Because we assumed that gender could obscure the causal relationship between BSA and survival, we investigated the association between BSA and PFS after stratifying patients with *EGFR*-mutant tumours according to gender. In males, we obtained similar observations to those described above, whereby patients with a high BSA did not benefit significantly but marginally from gefitinib monotherapy (HR 1.98, 95 % CI 0.99–3.97; $p = 0.054$;

**Table 2** Multivariate analysis of survival in patients with EGFR-mutant tumours ($n = 115$)

| | Overall survival | | Progression-free survival | |
|---|---|---|---|---|
| | HR (95 % CI) | *p* | HR (95 % CI) | *p* |
| Body surface area | | | | |
| ≥1.5 versus <1.5 $m^2$ | 0.76 (0.44–1.32) | 0.335 | 1.72 (1.08–2.74) | 0.021 |
| ECOG PS | | | | |
| 0–1 versus ≥2 | 0.38 (0.20–0.71) | 0.003 | 0.51 (0.29–0.91) | 0.022 |
| Gender | | | | |
| Female versus male | 1.49 (0.83–2.65) | 0.179 | Excluded | |
| Age (years) | | | | |
| ≥70 versus <70 | Excluded | | 1.42 (0.91–2.22) | 0.125 |
| Histology | | | | |
| Ad versus non-Ad | 0.64 (0.34–1.21) | 0.173 | 0.51 (0.27–0.94) | 0.032 |
| Staging | | | | |
| Rec versus others | 0.52 (0.25–1.08) | 0.079 | 0.48 (0.27–0.86) | 0.013 |
| KL-6 | | | | |
| ≥500 versus <500 | 1.48 (0.92–2.37) | 0.104 | Excluded | |
| Pack-year | | | | |
| ≥30 versus <30 | 2.05 (1.08–3.89) | 0.027 | 2.62 (1.59–4.30) | <0.001 |
| Line of gefitinib monotherapy | | | | |
| First line (no vs. yes) | Excluded | | Excluded | |

*HR* hazard ratio, *CI* confidence interval, *ECOG PS* Eastern Cooperative Oncology Group performance status, *Ad* adenocarcinoma, *EGFR* epidermal growth factor receptor, *rec* postoperative recurrence

Supplementary Table 1). In contrast, we failed to detect any significant difference in female patients, possibly due to the small number of female patients with a high BSA (HR 0.80, 95 % CI 0.37–1.72; $p = 0.566$; Supplementary Table 2). Smoking status was the significant prognostic factor.

### Potential effect of the BSA cut-off level on PFS

We performed sensitivity analysis to assess potential variations and uncertainty regarding the BSA cut-off level (Table 3). Among various BSA cut-off scores around the median, a BSA of 1.55 $m^2$, as well as 1.50 $m^2$, could explain the difference in PFS among patients with high and low BSA (HR 1.98, 95 % CI 1.24–3.15 and HR 1.72, 95 % CI 1.08–2.74, respectively). This suggests that the use of the BSA cut-off level around 1.50 $m^2$ in our previous and current studies [12] is robust for detecting subpopulations that would benefit less from gefitinib monotherapy.

**Table 3** Sensitivity analysis for the potential impact of the BSA cut-off level on progression-free survival in patients with *EGFR*-mutant tumours

| | Adjusted HR (95 % CI)[a] | *p* |
|---|---|---|
| BSA | | |
| ≥1.40 versus <1.40 $m^2$ | 0.89 (0.55–1.46) | 0.656 |
| ≥1.45 versus <1.45 $m^2$ | 1.27 (0.83–1.93) | 0.270 |
| ≥1.50 versus <1.50 $m^2$ | 1.72 (1.08–2.74)* | 0.021 |
| ≥1.55 versus <1.55 $m^2$ | 1.98 (1.24–3.15)* | 0.004 |
| ≥1.60 versus <1.60 $m^2$ | 1.62 (0.96–2.72) | 0.068 |

Variations and uncertainty regarding the cut-off level of BSA were investigated using sensitivity analyses

*EGFR* epidermal growth factor receptor, *BSA* body surface area, *HR* hazard ratio, *CI* confidence interval

* $p < 0.05$

[a] Adjusted according to potential confounding factors: Eastern Cooperative Oncology Group performance status, serum KL-6 level, gender, age, histology, stage, pack-year and line of gefitinib treatment

## Discussion

Although *EGFR*-mutant NSCLC tumours could be sensitive to EGFR-TKIs, we here identified subpopulations among patients with *EGFR*-mutant tumours who might benefit less from gefitinib monotherapy. When stratified according to BSA score, patients with a high BSA tended to have a poor PFS compared with those with a lower BSA (HR 1.72, 95 % CI 1.08–2.74; $p = 0.021$; Table 2).

It would be interesting to understand why gefitinib was less effective in individuals with a high BSA in the prospective cohort data (Fig. 1a). Importantly, the current findings reproduced the observations of our previous retrospective study (HR 2.34; 95 % CI, 1.78–2.89; $p = 0.002$) [12, 14]. It is possible that the approved dose of gefitinib in

the high-BSA group might not be sufficient to achieve the clinically meaningful pharmacokinetic profiles that were obtained in patients with a low BSA. Regarding imatinib monotherapy, one of the targeted agents in chronic myelogenous leukaemia, BSA was correlated inversely with the blood concentration of imatinib [15]. In addition, patients with a low BSA could achieve clinically effective drug concentrations using lower doses of imatinib than the approved dose [16]. This suggests that BSA might affect the pharmacokinetics (PK) of TKIs, as well as existing cytotoxic chemotherapeutic agents.

The trough blood concentration of gefitinib on day 8 in patients with advanced NSCLC is strongly associated with PFS individuals with lower concentrations had a shorter PFS, whereas those with higher levels had a longer PFS (median 2.4 vs. 11.2 months, respectively, $p = 0.009$) [17], despite the lack of appropriate patient selection according to *EGFR* mutation status. The potential association between BSA and the PK of TKI [15] as well as that between the PK of TKI and PFS [17] suggests that further investigations to clarify whether BSA affects PK and pharmacodynamics during gefitinib monotherapy for *EGFR*-mutant NSCLC are warranted.

In the current study, several factors might have affected the true causal relationship between BSA and gefitinib efficacy. First, the patient population is very heterogeneous, and there were various lines of gefitinib treatment, despite use of the prospective cohort data. Also, gender, one of the most important factors, might affect the BSA score: the higher scores were more common in males, whereas lower scores were present in more females. Therefore, we also performed analyses after stratification by sex. However, the statistical analyses were not robust because of the small number of individuals in each subpopulation, particularly in female patients. In addition, some of the pre-treatment clinical demographic parameters differed between the high- and low-BSA groups (Table 1). Although we adjusted for this during multivariate analyses, these parameters or other undetectable factors might have affected the original association. Finally, since the cut-off value of BSA we used here was different from that commonly used in the Western countries (i.e. 1.7 $m^2$) [18], further assessments would be needed if our results are to be applied for Europeans and North Americans. All of these issues should be interpreted with caution.

In conclusion, this study revealed that gefitinib was less effective in individuals with a high BSA in our prospective cohort of patients with EGFR-mutant NSCLC, consistent with our previous retrospective study [12]. To date, dose adjustment according to BSA has not been approved for any molecular-targeted agent. Our findings suggest that the potential influence of BSA on the pharmacokinetic and pharmacodynamic variability of gefitinib would be considered the next step in investigating the clinical meaningfulness of BSA-based dosing during gefitinib monotherapy.

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**Conflict of interest** K.H. has received honoraria from Pfizer, Eli Lilly Japan, Sanofi, Daiichi-Sankyo Pharmaceutical and Chugai Pharmaceutical. NT received honoraria from AstraZeneca, Chugai Pharmaceutical Company and Boehringer-Ingelheim in Japan. KK received honoraria from Eli Lilly Japan, Nihon Kayaku, AstraZeneca, Daiichi-Sankyo Pharmaceutical, Chugai Pharmaceutical, Taiho Pharmaceutical and Sanofi-Aventis. All other authors declared no conflicts of interest regarding this study.

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RESEARCH ARTICLE

# Magnitude of the Benefit of Progression-Free Survival as a Potential Surrogate Marker in Phase 3 Trials Assessing Targeted Agents in Molecularly Selected Patients with Advanced Non-Small Cell Lung Cancer: Systematic Review

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## Abstract

### Background

In evaluation of the clinical benefit of a new targeted agent in a phase 3 trial enrolling molecularly selected patients with advanced non-small cell lung cancer (NSCLC), overall survival (OS) as an endpoint seems to be of limited use because of a high level of treatment crossover for ethical reasons. A more efficient and useful indicator for assessing efficacy is needed.

### Methods and Findings

We identified 18 phase 3 trials in the literature investigating EGFR-tyrosine kinase inhibitor (TKIs) or ALK-TKIs, now approved for use to treat NSCLC, compared with standard cytotoxic chemotherapy (eight trials were performed in molecularly selected patients and ten using an "all-comer" design). Receiver operating characteristic analysis was used to identify the best threshold by which to divide the groups. Although trials enrolling molecularly selected patients and all-comer trials had similar OS-hazard ratios (OS-HRs) (0.99 vs. 1.04), the former exhibited greater progression-free survival-hazard ratios (PFS-HR) (mean, 0.40 vs. 1.01; $P<0.01$). A PFS-HR of 0.60 successfully distinguished between the two types of trials (sensitivity 100%, specificity 100%). The odds ratio for overall response was higher in trials with molecularly selected patients than in all-comer trials (mean: 6.10 vs. 1.64; $P<0.01$). An odds ratio of 3.40 for response afforded a sensitivity of 88% and a specificity of 90%.

## Conclusion

The notably enhanced PFS benefit was quite specific to trials with molecularly selected patients. A PFS-HR cutoff of ~0.6 may help detect clinical benefit of molecular targeted agents in which OS is of limited use, although desired threshold might differ in an individual trial.

## Introduction

Platinum-based and single-agent cytotoxic chemotherapies have been the standard treatments for advanced non-small cell lung cancer (NSCLC) patients in first-line and salvage settings, respectively [1–5]. Unfortunately, even upon application of such standard therapies, nearly all patients with advanced NSCLC experience disease progression, and thus, the ultimate goals of palliative chemotherapy are to prolong overall survival (OS) and to improve symptoms and the quality of life, rather than to cure. To date, evaluation of the efficacies of treatment strategies and the approval of most new agents used to treat advanced NSCLC have been based principally on OS prolongation in randomized clinical trials [6]. OS, the time from randomization to death from any cause, represents a direct measure of clinical benefit to the patient, and to date, no other endpoint has been shown statistically to serve as a suitable surrogate for OS in advanced NSCLC [7–12].

Many molecular targeted agents, including epidermal growth factor receptor (EGFR)- and anaplastic lymphoma kinase (ALK)-tyrosine kinase inhibitors (TKIs), have been assessed in phase 3 trials, compared with standard cytotoxic chemotherapy. Several trials have failed to demonstrate significant improvement in OS and/or progression-free survival (PFS), mainly because appropriate patient selection was not applied, i.e. the trials used molecularly unselected or all-comer designs. However, even in trials using only patients selected after evaluation of EGFR-mutant or ALK-fusion status (thus, molecularly selected patients) [13–21], EGFR- and ALK-TKIs failed to demonstrate any significant advantage in terms of OS, although these drugs are now widely approved in the U.S., E.U., and Japan. This would be explained by the inevitable high levels of crossover, essential from an ethical viewpoint, that allow control-arm patients to access these highly active investigational agents [13,18]. Thus, the data do not reflect inappropriate patient numbers or inadequate efficacy of the tested agents.

Currently, medical oncologists have strong views that in trials with crossover designs in molecularly selected patients, 1) the lack of any observed effect on OS does not necessarily mean that the agent is not efficacious; 2) the use of OS as the primary endpoint is limited; and 3) other endpoints are now urgently required to evaluate the efficacy of molecular targeted agents. If a significantly impressive benefit in PFS or overall response is evident specifically in molecularly-selected but not all-comer trials, we hypothesized that the PFS-hazard ratio (HR) or the odds ratio for the overall response would serve as a useful novel indicator in the former trial setting [10]. In order to propose novel efficient and useful markers of efficacy in this setting, we reviewed published phase 3 trials that compared EGFR- or ALK-TKIs (gefitinib, erlotinib, afatinib, or crizotinib) with traditional cytotoxic chemotherapy. We next identified differences in the magnitudes of PFS-HRs or odds ratios for the overall response in the two types of trials, those conducted in molecularly selected populations versus those conducted in all-comers.

## Methods

### Literature search

We performed a literature search of trials published between January 2003 and June 2014. To avoid publication bias, both published and unpublished trials were identified using a computer-based search of the PubMed database and of abstracts from conferences of the American Society of Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), and the International Association for the Study of Lung Cancer (WCLC). The following search terms were used: "lung cancer AND advanced AND phase III study OR phase 3 study OR phase 3 trial OR phase III trial OR randomized controlled trial OR clinical trial OR controlled clinical trial". Our search was also guided by a thorough examination of the reference lists of original and review articles, books, and meeting abstracts (ASCO, ESMO, and WCLC), and of the Physician Data Query registry of clinical trials.

### Trial selection

Eligible phase 3 trials were those that evaluated EGFR-TKIs or ALK-TKIs in the treatment of advanced NSCLC (Fig. 1), provided data on PFS, the overall response rate, and OS. Drugs acting on known specific molecular targets were defined as molecular targeted agents [22,23]. Trials designed to assess combined modality treatment, including radiotherapy and/or surgery, were excluded. We selected phase 3 trials that compared EGFR- or ALK-TKIs with existing cytotoxic chemotherapy.

### Data extraction

To avoid bias, two certified medical oncologists (K.H. and Y.K.) independently abstracted the trial data and compared their results, as described previously [5,24]. The following information was obtained from each report: year of trial initiation, number of patients randomized, treatment regimens, line of treatment, publication type, primary endpoint, PFS- and OS-HRs, and the number of responders. All data were verified for internal consistency, and disagreements were resolved by discussion between the investigators. The principal investigators of the trials were contacted and invited to confirm or update published data.

### Statistical analysis

We performed linear regression analysis to investigate associations after assigning weights determined by sample size to each trial. The strength of each association was defined *a priori* using the commonly accepted criterion of the coefficient of determination (the R-square value; $r^2$) [25], which ranges from $0 < r^2 < 1$, with a higher score indicating a stronger association [24,25].

Any influence of trial design (molecularly selected patients vs. all-comers) on the PFS-HR or the odds ratio of the overall response was evaluated by multiple stepwise regression analysis using the following stepping criteria: *P*-value allowing model entry, $\leq 0.05$; *P*-value compelling removal from the model, $\geq 0.20$, with adjustment for several confounders including the year of trial initiation, line of treatment, primary endpoint, number of randomized patients, and type of reporting.

The significance of differences between groups was assessed using *t*-tests. Receiver operating characteristic (ROC) analysis was used to identify the most accurate discrimination thresholds dividing the groups. The most suitable cutoff level was defined as that closest to the top-left corner. The odds ratio for the overall response was calculated as follows: ([number of patients in the investigational arm who achieved a complete or partial response: A]/[number of randomized patients allocated to the investigational arm—A])/([number of patients in the control arm who achieved a complete or partial response: B]/[number of randomized patients allocated to the control arm—B]).

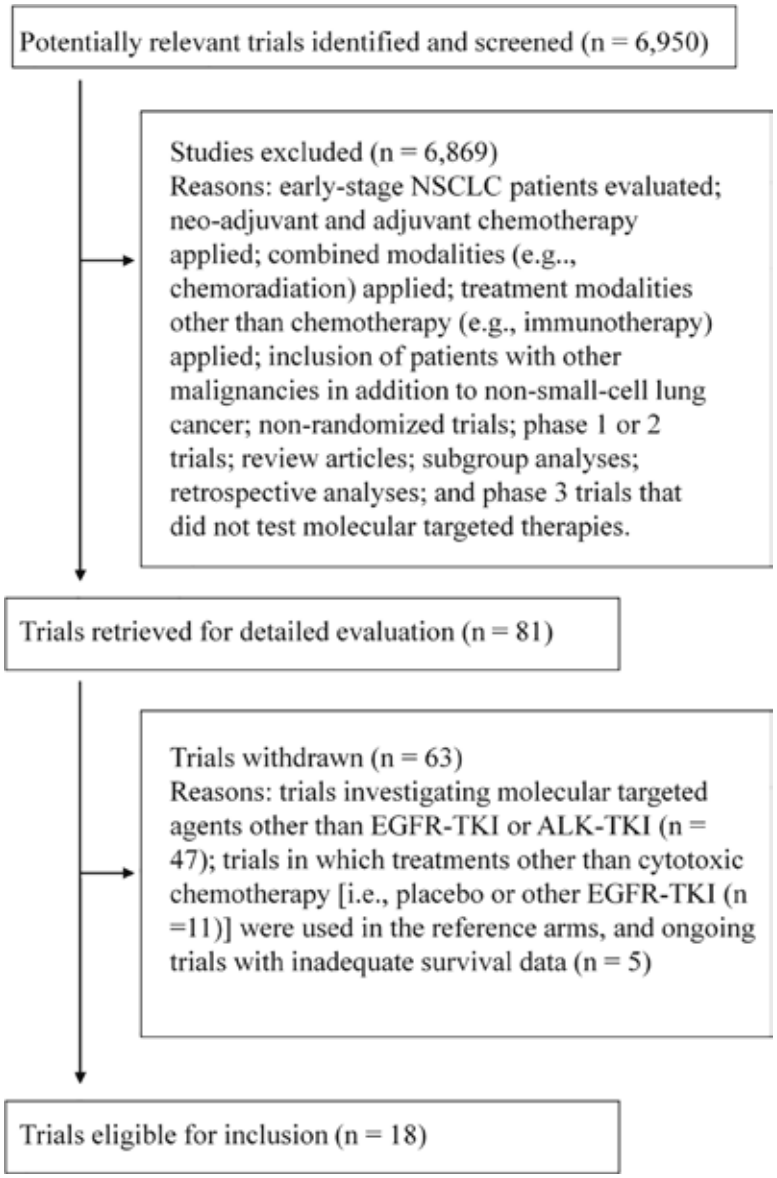


**Fig 1. A flow chart demonstrating the selection process of the trials analyzed.** NSCLC = non-small cell lung cancer, EGFR-TKI = epidermal growth factor receptor-tyrosine kinase inhibitor, ALK-TKI = anaplastic lymphoma kinase-tyrosine kinase inhibitor.

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All *P* values were calculated using two-sided tests, and the level of significance was set at $P < 0.05$. Statistical analyses were performed using STATA (Version 11; StataCorp, Dallas, TX, USA).

## Results

### Trial demographics

Of the 6,950 trials screened, 18 phase 3 trials that investigated four molecular targeted agents (gefitinib, erlotinib, afatinib, and crizotinib) in patients with advanced NSCLC were identified

(S1 Table). The trials included a total of 7,633 randomized patients (Fig. 1). The trial characteristics are listed in Table 1. We found eight trials enrolling molecularly selected patients and 10 all-comer trials. Sixteen trials evaluated EGFR-tyrosine kinase inhibitors (TKIs) in patients with EGFR-mutant NSCLC, and the remaining two trials assessed the use of crizotinib, an ALK-TKI, to treat ALK-rearranged NSCLC.

## Correlation between the OS-HR and PFS-HR and between the OS-HR and the odds ratio of the overall response

First, we examined the strength of the correlation between the PFS-HR and the OS-HR. As shown in Fig. 2A, the PFS-HR had no meaningful association with OS-HR (overall R-squared value = 0.233), suggesting that the PFS-HR explained only 23.3% of the overall variability in OS-HR (Fig. 2A). This weak association was especially apparent in molecularly selected patient trials compared with all-comer design trials (R-squared values = 0.002 vs. 0.409, respectively; Fig. 2B). Similar observations were made when the association between the odds ratio of the overall response and the OS-HR were compared (overall R-squared value = 0.101, Fig. 2C). The association was more marked in trials with molecularly selected patients (R-squared values = 0.039 vs. 0.429, respectively; Fig. 2D).

Neither the PFS-HR nor the odds ratio of the overall response accurately predicted OS when a linear regression model was used to analyze data from molecularly selected patient trials.

## OS-HRs in trials with molecularly selected patients and all-comers designs

We found no significant difference in the OS-HRs between the two trial types (mean, 0.99 vs. 1.04 in molecularly selected patient trials vs. all-comer trials, respectively; $P = 0.50$) (Fig. 3A). In contrast, median survival time in molecularly selected patient trials was approximately double that in all-comer trials (median 23.1 and 26.6 months in the investigational and control arms of molecularly selected trials, respectively, compared with 11.9 and 12.2 months, respectively, in all-comer trials).

**Table 1. Trial demographics (n = 18).**

| | | |
|---|---|---|
| *Trial characteristics* | | |
| No. of randomly assigned patients per trial [(median (range)] | | 328 (161–1,466) |
| Year of trial initiation [(median (range)] | | 2007 (2003–2011) |
| Publication type (full text/abstract only) | | 16/2 |
| Primary endpoint (OS/PFS/TTP) | | 5/12/1 |
| First-line setting (y/n) | | 10/8 |
| *Type of molecular targeted agent investigated* | | |
| EGFR-tyrosine kinase inhibitor | | |
| | gefitinib | 7 |
| | erlotinib | 6 |
| | afatinib | 3 |
| ALK-tyrosine kinase inhibitor | | |
| | crizotinib | 2 |

OS, overall survival; PFS, progression-free survival; TTP, time to progression; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase

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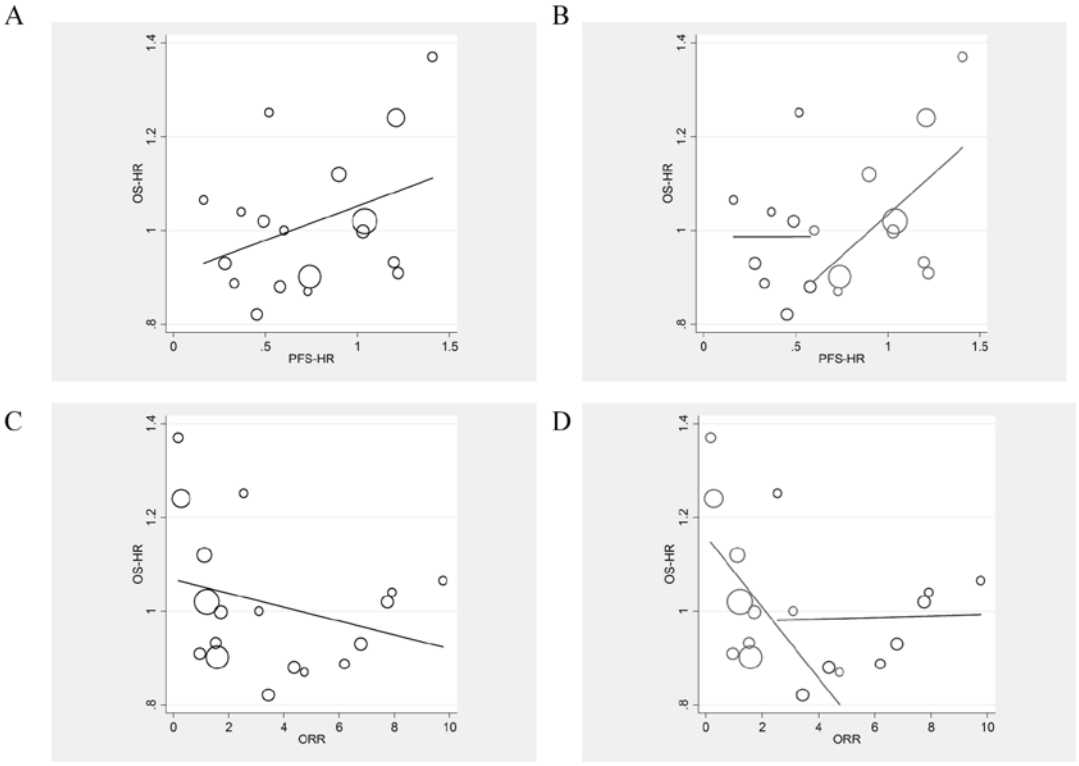


**Fig 2. Associations between the progression-free survival-hazard ratio (PFS-HR) and overall survival (OS)-HR (R-squared = 0.233) (A), and that after stratification by trial design (B) (the molecularly selected patient design [blue], R-squared = 0.002, vs.** the all-comer design [pink], R-squared = 0.409; *P*-value for interaction = 0.34). Associations between the odds ratio of the overall response and OS-HR (R-squared = 0.101) (C), and that after stratification by trial design (D) (the molecularly selected patient design [blue], R-squared = 0.039, vs. the all-comer design [pink], R-squared = 0.429; *P*-value for interaction = 0.03). All analyses were weighted by trial size.

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The left and right columns in each panel represent data from molecularly selected patient trials and all-comer trials, respectively. The diameter of each circle is representative of the size of the trial.

A, Trials with molecularly selected patients had almost identical OS-HRs, compared with those of all-comer trials (mean, 0.99 vs. 1.04, $P = 0.50$). B, The PFS-HRs were 0.40 vs. 1.01 in the two trial types ($P < 0.01$). C, Trials with molecularly selected patients had significantly greater odds ratios in terms of the overall response (mean; 6.10 vs. 1.64, $P < 0.01$).

## PFS-HRs in both molecularly selected patient and all-comer trials

We next investigated differences in the PFR-HRs between the two trial groups. Molecularly selected patient trials had a greater PFS-HR than did all-comer trials (mean, 0.40 vs. 1.01; $P < 0.01$; Fig. 3A). This significant influence of trial design on PFS-HR was observed even when several potential confounders were adjusted upon multivariate analysis; trials using molecular selection had a PFS-HR score 0.42 points lower than that of the all-comer trials; $P < 0.01$; Table 2).

ROC analysis revealed that a PFS-HR of 0.60 was a useful cutoff point to distinguish the two types of trial designs, with a sensitivity and specificity of 100% and 100%, respectively, and an area under the ROC curve (AUC) of 1.00 (Table 3, Fig. 4A).

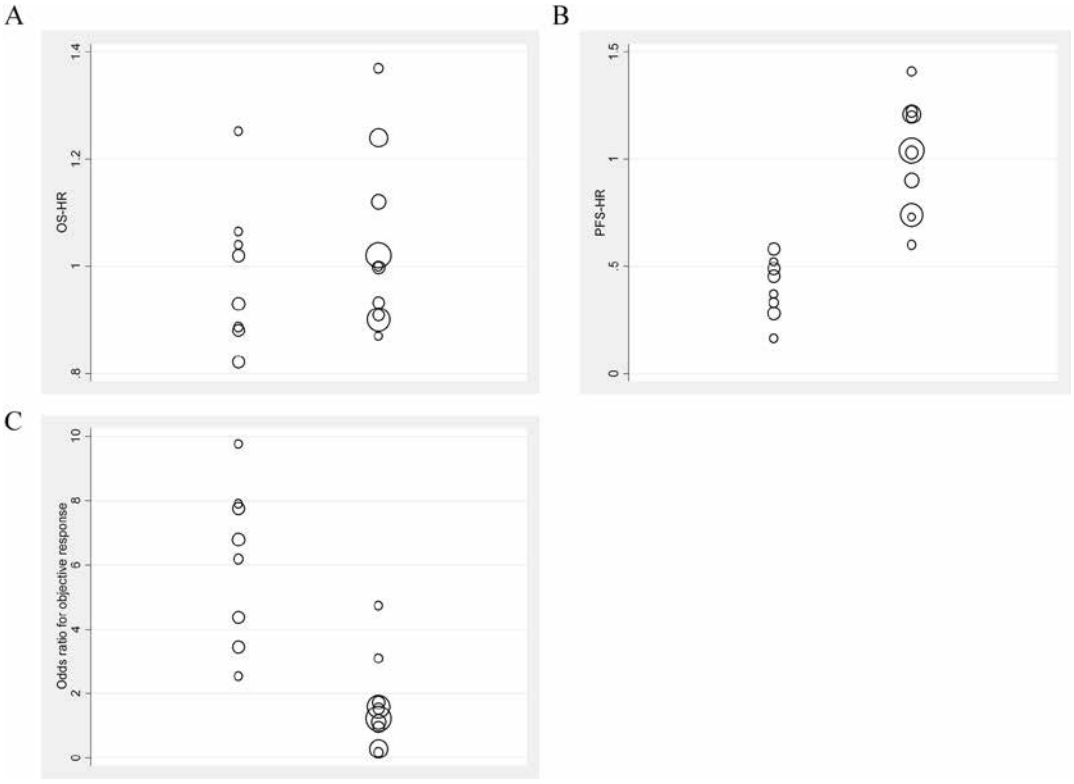


**Fig 3. Distributions of hazard ratios (HRs) for overall survival (OS) (A) and progression-free survival (PFS) (B) and odds ratios for the overall response (C), stratified according to the two types of trials.**

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## Odds ratios of the overall response both in molecularly selected patient and all-comer trials

The odds ratio of the overall response was higher in trials with molecularly selected patients than in all-comer trials (mean: 6.10 vs. 1.64, respectively; $P < 0.01$, Fig. 3C). This was maintained upon multivariate analysis; the former trial type had a 4.46-point greater odds ratio than that of the all-comer trial; $P < 0.001$; Table 2).

**Table 2. Multiple stepwise linear regression analysis of PFS-HR and the odds ratio for overall response.**

| | PFS-HR | | Odds ratio for overall response | |
|---|---|---|---|---|
| **Factor** | Regression coefficient (95% CI) | *P* | Regression coefficient (95% CI) | *P* |
| Trial design (molecularly-selected vs. all-comer) | −0.42 (−0.67 to −0.18) | < 0.01 | 4.46 (2.52–6.39) | < 0.01 |
| Primary endpoint (PFS vs. other) | −0.31 (−0.56 to −0.53) | 0.02 | excluded | |
| Line of treatment (1st vs. other) | Excluded | | excluded | |
| Year of trial initiation (before or in 2006 vs. 2007 or later)† | Excluded | | excluded | |
| No. of randomized patients | Excluded | | excluded | |
| Type of reporting (full text vs. abstract only) | Excluded | | excluded | |

The threshold p values for entry into and removal from the model were 0.05 and 0.20, respectively.

†The cutoff level was set as the median year of trial initiation. PFS-HR, progression-free survival-hazard ratio; CI, confidence interval.

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**Table 3. The accuracy by which molecularly selected patient trials can be distinguished from all-comer trials using two alternative endpoints.**

| Endpoint with the most accurate cutoff level | | Molecularly selected patient trials (*n* = 8) | All-comer trials (*n* = 10) | Sensitivity | Specificity | AUC |
|---|---|---|---|---|---|---|
| PFS-HR | | | | 100% | 100% | 1.00 |
| | $< 0.60$ | 8 | 0 | | | |
| | $\geq 0.60$ | 0 | 10 | | | |
| Odds ratio for the overall response | | | | 88% | 90% | 0.95 |
| | $\geq 3.40$ | 7 | 1 | | | |
| | $< 3.40$ | 1 | 9 | | | |
| Both the PFS-HR and odds ratio for the overall response | | | | | | |
| | $< 0.60$ and $\geq 3.40$ | 7 | 0 | | | |
| | Other | 1 | 10 | | | |
| Either the PFS-HR or the odds ratio for the overall response | | | | | | |
| | $< 0.60$ or $\geq 3.40$ | 8 | 1 | | | |
| | Other | 0 | 9 | | | |

AUC, area under the receiver operating characteristic curve.

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The ROC curve indicated that an odds ratio of 3.40 for the overall response was a potentially useful cutoff point to identify trials with molecularly selected patients, affording a sensitivity of 88%, a specificity of 90%, and an AUC of 0.95 (Table 3, Fig. 4B). The odds ratio, even in combination with the PFS-HR, did not increase the probability of detecting trials of molecularly selected patients (Table 3).

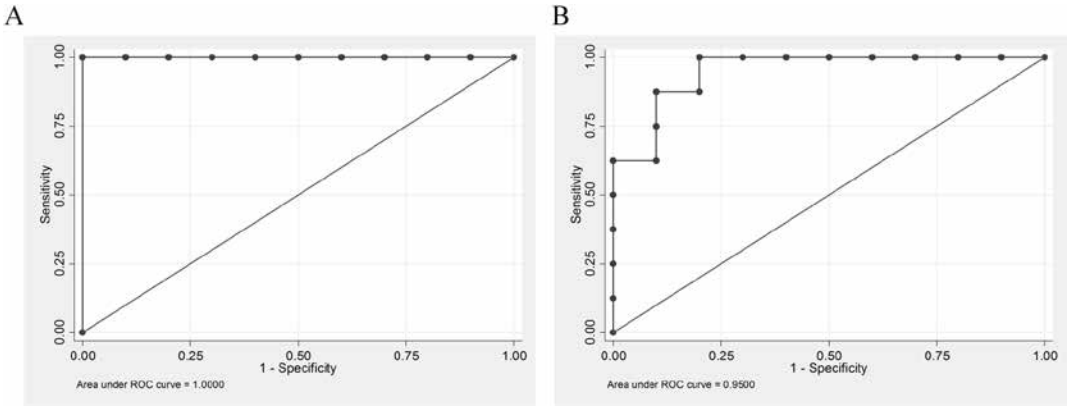


**Fig 4. Receiver operating characteristic (ROC) curve defining cutoff levels predicting outcomes in eligible trials with molecularly selected patients in terms of the progression-free survival-hazard ratio (PFS-HR) (A) and the odds ratio for the overall response (B).** The most suitable cutoff was defined as that closest to the upper left corner. A, A PFS-HR of 0.60 was the optimal cutoff for distinguishing molecularly selected patient trials from all-comer trials (sensitivity 100%, specificity 100%, and AUC [area under the receiver operating characteristic curve], 1.00). B, The odds ratio for an overall response of 3.40 was a potentially useful cutoff to distinguish trials with molecularly selected patients from all-comer trials (sensitivity 88%, specificity 90%, and AUC = 0.95).

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## Discussion

We noted robust benefits in terms of both the PFS and overall response in trials evaluating approved EGFR-TKIs or ALK-TKIs in molecularly selected patients (Fig. 3B and 3C, Table 3). In particular, a PFS-HR of approximately 0.6 was a useful cutoff for distinguishing molecularly selected patient trials from all-comer trials, with a sensitivity of 100% and a specificity of 100% (Fig. 4A, Table 3). To date, PFS has not been shown to be a statistically acceptable surrogate for OS because of the lack of a strong association between PFS and OS in advanced NSCLC patients [7,9]. Thus, PFS is not a formal surrogate endpoint but rather a potential future indicator of the clinical benefit of molecular targeted agents in trial designs in which an OS endpoint is of limited utility.

The principal result of our study was that of the 18 phase 3 trials assessing EGFR-TKIs and an ALK-TKI, we found that the PFS-HR yielded by the approved molecular targeted agents in molecularly selected patient trials was sufficiently large to allow distinction between the two trial types, with high accuracy, at a PFS-HR cutoff level of 0.6 (Fig. 3A and Table 3). Assuming that trials with molecularly selected patients have shown and will continue to show only small differences in OS, caused by high levels of crossover treatment, regardless of the effectiveness of the investigational agent [10], the extent of PFS benefit could serve as an important measure of the clinical benefit (Fig. 3A and Table 3). The U.S. Federal Drug Administration (FDA) considers that PFS is a valid clinical endpoint for advanced NSCLC when regulatory decisions on drug approval based on the substantial magnitudes of their effects are to be made [6]. However, "substantial magnitude" remains poorly defined. Here, we describe a cutoff level that will be of potential use in future trials using molecularly selected patients; use of this cutoff will help resolve this long-standing issue.

In contrast, the odds ratio for the overall response seemed less useful for distinguishing trials using molecularly selected patients (Fig. 3C, and Table 3), possibly because the overall response did not accurately reflect dramatic tumor shrinkage; rather, it reflected the proportion of patients in whom the tumor diameter was reduced by $\geq$ 30%, thus ignoring profound shrinkage [26]. A novel concept is required to establish surrogacy of the overall response; the proportion of patients exhibiting "dramatic responses", as revealed by a waterfall plot might suffice.

Neither recent randomized trial of LUX-Lung 3 nor-6 revealed any significant OS advantage of afatinib, one of the existing EGFR-TKIs, over the platinum-based chemotherapy, although both combined analysis of these two trials and some subgroup analyses showed an OS benefit of the investigational agent [20]. Our current result would also be applied even in such situation, as long as a trial demonstrates a large PFS benefit but no OS benefit.

A limitation of our study was that all analyses were performed in the absence of any detailed individual patient information, and thus future patient-based data analyses may be necessary to confirm our present findings [27,28]. In addition, we included a limited number of clinical trial analyses that were retrospective in design, and we only analyzed trials evaluating EGFR- or ALK-TKIs. Furthermore, PFS might be a more useful endpoint if it were combined with other endpoints such as quality of life, but no relevant data on this were available to us. Therefore, our work is still at the stage of hypothesis generation; we believe further studies are strongly warranted.

In conclusion, OS is no longer of utility in trials using molecularly selected patients that allow subsequent crossover to active investigational agents. In this situation, these molecularly targeted trials using PFS would be considered positive if their HR is less than or equal to 0.6 for PFS. Although desired threshold might differ in an individual trial, we have contributed critical information to the long-standing debate on potential endpoints alternative to the traditional OS endpoint used in trial design.

## Supporting Information

**S1 PRISMA Checklist.**
(DOC)

**S1 Table.**
(DOC)

## Author Contributions

Conceived and designed the experiments: KH YK. Performed the experiments: KH YK. Analyzed the data: KH YK. Contributed reagents/materials/analysis tools: KH YK. Wrote the paper: KH YK NL NT RMG HK TH KD TK M. Tabata M. Tanimoto KK.

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