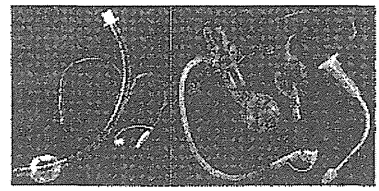
図7. カフ上部吸引ポートつきの気管チューブ



推奨カフ圧：25～30cm H_2O
低圧→VAP発生率の増加
高圧→気道局所の虚血性障害

※患者の体動 → 位置がずれカフ圧が変化 → 体位を交換した時はカフ圧測定を

http://www.covidien.co.jp より転載

6) 対策（多剤耐性菌が検出された場合）

- ✓ アウトブレイクの有無を確認する
- ✓ 伝搬防止のため、以下の点を再確認し、励行する
- ✓ 標準予防策の実施
- ✓ 手袋着用、手指衛生
- ✓ マスク着用
- ✓ ガウン着用

IV. 結核感染症

1) 概念および特徴

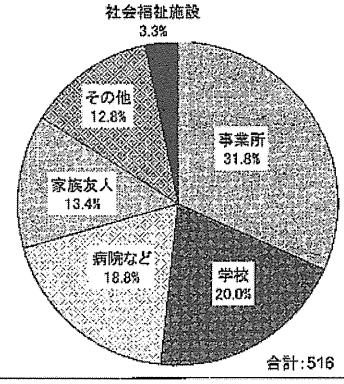
- ✓ 結核は空気感染で伝播する
- ✓ 結核は感染症法の2類感染症に分類され、感染症法第12条より医師は結核と診断した患者があった場合には直ちに最寄りの保健所に届け出なければならない。
- ✓ 感染症法第53条により病院管理者は結核患者が入院または退院した時は7日以内に保健所長に届出を行う
- ✓ 「感染＝発病」ではない（実際には結核菌に曝露した人のうち半分程度に感染が成立し、そのうち10～20%の人が発病する）
- ✓ 発病する人の約80%は感染後2年以内に、さらに残りは数ヶ月～数十年後になって発病する

2) 疫学

- ✓ わが国における2011年の全結核患者数は22,681人で、人口10万対罹患率は17.7、そのうち菌喀痰塗抹陽性肺結核者数は8,654人、喀痰塗抹陽性罹患率は人口10万対6.8[7]
- ✓ 患者数は低下傾向ではあるものの減少率は2%台と低い
- ✓ 70歳以上の高齢者結核が50%を占める
- ✓ 高齢者結核の約半数は呼吸器症状がなく、微熱、全身倦怠感、食欲不振、体重減少といった症状のみで、発見の遅れにつながる事がある

- ✓ わが国の医療従事者における結核罹患率は一般人口より高い（特に看護師、臨床検査技師の罹患率は他職種の同年齢層の罹患率と比較して高い）[8]
- ✓ 結核集団感染発生の場としては事業所、学校に続き、病院・診療所・老人保健施設などの医療施設が約20%を占めている（図8）[9]
- ✓ 結核集団感染の定義：同一の感染源が、2家族以上にまたがり、20人以上に結核を感染させた場合（ただし、発病者1人は6人が感染したものとして感染者を計算）

図8. 結核集団発生場所（1999年～2008年）
（厚生労働省健康局結核感染症課調査より）



3) 感染対策（早期発見）

- ✓ 感染源となる結核患者の診断を早期に発見することが院内伝播を予防する第一歩
- ✓ 2週間以上続く咳では積極的に胸部レントゲン写真を撮り、異常があれば必ず抗酸菌検査を行う
- ✓ 結核のハイリスクグループ（表4）では、咳の他にも痰、血痰、胸痛、全身倦怠感、発熱などを認めた場合には常に念頭に結核の存在を疑う[10]
- ✓ 外来患者で咳が激しい場合は外科用マスクを着用させ（そのような症状があれば申し出るよう表示も必要）、他の待合室に隔離し、他患者との接触をできるだけ避ける
- ✓ 救急外来や呼吸器内科外来などでは陰圧室が一つはあることがのぞましい
- ✓ 外来で結核が強く疑われ採痰する場合も陰圧室がない場合は採痰ブースの設置が望ましい

表4. 結核のハイリスクグループ

高齢者、高齢者収容施設入所者およびデイケアに通院するもの
ホームレス、特定結核高度蔓延地域(国)の住民
入国後3年以内の外国人
結核治癒所見を持っているもの
HIV感染者
珪肺、癌、人工透析、低栄養者
コントロール不良な糖尿病患者
免疫抑制薬、長期ステロイド、抗癌剤、TNF阻害剤などで治療中の者
BCG接種歴のない乳幼児(0～4歳)

（結核診療ガイドライン　日本結核病学会より一部改訂）

4) 感染対策（患者発生時の対応）

- ✓ 結核と診断された（または疑い）患者は陰圧の空調を備えた個室に隔離し、届出を行い治療を開始する
- ✓ 塗抹で抗酸菌陽性であるが、胸部レントゲン上は非結核性抗酸菌症が疑われる、肺外結核の診断はついているが、胸部の精査は行っていないなどといった場合も培養、PCR、胸部 CT 等で肺結核が除外されるまでは個室収容が安全である
- ✓ 陰圧個室がない場合は、転院可能な患者は転院まで、基礎疾患が重篤で転院できない者は転院できるようになるまで個室へ隔離する
- ✓ 陰圧でない個室のうち、空調設備が再循環式の場合（病室または特定区域からの排気の一部を循環させて、吸気の一部に用いる方式）で HEPA フィルターが備えられていないときは、空調を止め、簡易式の HEPA フィルター内蔵空気清浄器を使用する
- ✓ 結核病床のない一般病院でも 1～2 室の全排気方式の隔離室を備えていることが望ましい
- ✓ 病室に入る前には職員及び面会者はN95 マスクを着用する（図 9）
- ✓ 自分に合ったサイズのマスクを着用し、きちんと着用できているか定期的に確認を行う事が重要である（フィットテストの様子：図 10）

図9. 種々のN95マスク

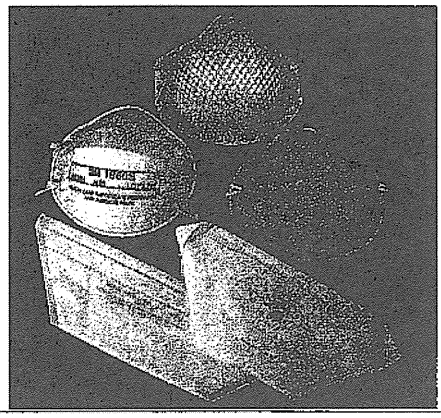


図10. フィットテストの様子

- ✓ やむなく病室外で検査等行わなければならない場合、患者には N95 マスクではなく外科用マスクを着用させる
- ✓ 関係部署には空気感染対策が必要である旨伝達しておく
- ✓ 医療関係者が病室に入る場合、空気感染対策中である事がわかる表示を院内共通の認識事項として周知しておくことも伝播を予防するためには重要である
- ✓ 可能な限り他の患者と接しないルートあるいは時間帯を設定しておく
- ✓ 患者が使用した食器は通常の処理を行い、リネン類も標準予防策に準じて洗濯を行う
- ✓ 血圧計、聴診器などは専用にする必要はない
- ✓ 喀痰は専用のごみ箱を設置し、室内でビニール袋などで密閉して感染性廃棄物として廃棄する
- ✓ 2 類感染症である結核の治療および検査で使用した後のものは感染性廃棄物となるが日常生活からでる一般のゴミは通常通り処理する

- ✓ 患者が退室した後は、部屋の扉を閉め、空気中の飛沫核が除去されるまで空室にする
- ✓ 除去されるまでの時間は1時間当たりの換気回数による（通常1時間当たり12回の換気では、約30分で99.9%の飛沫核を除去できる）

5) 感染対策（患者発生後の対応・接触者健診）

- ✓ 医療施設で発生した場合でも、自施設のみで事後対処せず、保健所長の指導のもと、協働して行う
- ✓ 濃厚接触者、非濃厚接触者、非接触者の判断をすることになるが、面会の家族を除く考えられる院内の濃厚接触者とは、同室者、主治医、担当看護師、気管支鏡検査や採痰を担当した医師、検査技師があげられる（同室者の主治医や担当看護師レントゲン技師などは非濃厚接触者に入る）

6) 感染対策（患者発生後の対応・結核早期発見のための職員健康管理）

- ✓ 日本結核病学会予防委員会では雇い入れ時の健康診断に際しては法令に定められた検査項目の他、クオンティフェロン（QFT）検査の実施を推奨している[11]
- ✓ 特に結核患者と常時接する職場（結核病棟など）で強く奨めている
- ✓ 雇い入れ時にQFT検査が行えない場合、明らかに結核患者との接触歴がない者はベースライン陰性として扱う
- ✓ 曝露後本検査で陽性化したものには予防投与を行うが、専門医に相談の上服用を開始するのが望ましい
- ✓ 毎年の健康診断は必ず受診する体制が必要である

7) 感染対策（その他）

- ✓ 痰や培養菌などを取り扱う際は安全キャビネット内で操作し、可能であれば検査室は陰圧の環境とするのがよい
- ✓ 結核が疑わしいがどうしても喀痰で診断がつかない場合、気管支鏡を施行することもありうるが、その際にはできるだけ最後に検査を組み入れ、陰圧の気管支鏡室が確保できなければ、簡易式のHEPAフィルター内蔵空気清浄器を設置するなどの工夫が必要である

V. ウイルス感染症

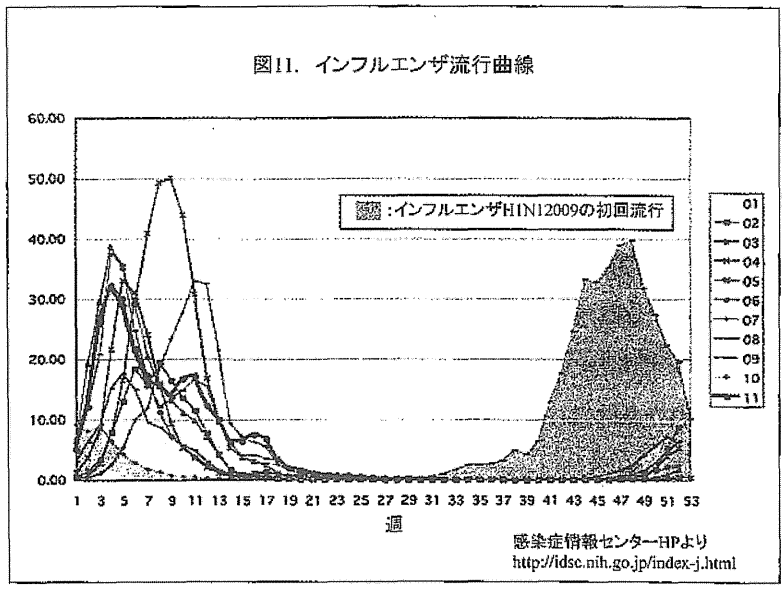
【インフルエンザウイルス】

1) 特徴

- ✓ RNAウイルスでA,B,Cの3つの型がある
- ✓ 世界的流行はA型ウイルスで認められ、国内や地域的な流行がB型ウイルスで認められる
- ✓ A型ウイルスの表面にはHemagglutinin (HA:赤血球凝集素)とNeuraminidase (NA:ノイラミニダーゼ)と呼ばれる特徴的なスパイクを認める
- ✓ HAと宿主の気道の細胞に存在するレセプターとの結合により感染する。この結合を防御することが感染予防の第一の標的となっており、インフルエンザワクチンはこれを目的として設計されている
- ✓ NAは宿主細胞から増殖したウイルス粒子が飛び出すときに必要な酵素である

- ✓ HAは16種類、NAは9種類の亜型があり、頭文字のあとに種類を示す数字を記載してH1N1のように示す
- ✓ 一般的には日本では、1月から2月にかけてA型インフルエンザの流行が認められる。2009-2010シーズンはインフルエンザH1N1 2009の流行により異なった分布となった（図11）
- ✓ 健康成人では、発症の1日前から発症後5－7日後まで感染力を有する
- ✓ 小児では、7日後以降も感染力を有している

図11. インフルエンザ流行曲線



2) インフルエンザと法律

- ✓ インフルエンザウイルス（鳥インフルエンザの原因となるA型インフルエンザウイルス及び新型インフルエンザ等感染症の原因となるインフルエンザウイルスを除く）による感染症は5類定点把握疾患となっている
- ✓ 鳥インフルエンザのうちH5N1によるものは2類、その他の鳥インフルエンザによるものは4類である
- ✓ 新型インフルエンザは1類～5類に属さない「新型インフルエンザ等感染症」の一つとして取り扱われる
- ✓ 学校保健安全法では、学校感染症の基準を示している（図12）。H5N1は第一種であり、その他の新型を含めたインフルエンザは第二種の感染症である
- ✓ 感染症予防法の指定感染症は第一種の感染症とみなす
- ✓ インフルエンザ脳炎を含む、急性脳炎は5類全数把握疾患である

図12. 学校感染症の基準

第一種	感染症法の第1類、第2類の疾患（結核を除く）に罹患している場合は、治癒まで出席停止である。ただし、これらはもともと都道府県知事の入院勧告、措置の対象である。
第二種	感染性が認められなくなるまで出席停止である。疾患によって基準が異なることに注意。
インフルエンザ	解熱後2日間経過するまで。
麻疹	解熱後3日間経過するまで。
流行性耳下腺炎	耳下腺の腫脹が消失するまで。
風疹	発疹の消失まで。
水痘	全ての発疹が痂皮化するまで。
結核	医師によって感染の恐れがないと認められるまで。
第三種	医師が感染の恐れがないと認めるまで出席停止となる。 腸管出血性大腸菌感染症、流行性角結膜炎、急性出血性結膜炎など。

3) 感染経路

- ✓ 感染経路は主として飛沫感染である
- ✓ 接触感染も重要な感染経路である
- ✓ 環境表面での生存は環境により異なるが、通常の飛沫では2-8時間までと考えられている
- ✓ 空気感染は限定された条件のもとでは起こりうる

4) ワクチン [12)]

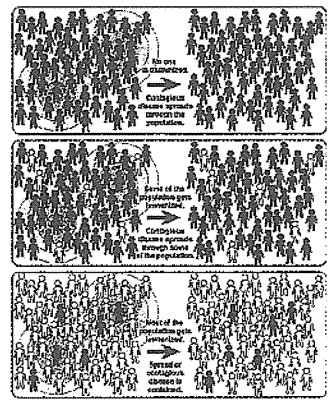
- ✓ 現在わが国で使用できるワクチンは不活化ワクチンである
- ✓ 授乳期や妊婦にも推奨されている
- ✓ リスク因子（図13に示す）を有するものおよび、同居、介護、看護、医療を行うものは、ワクチンを接種すべきである
- ✓ 年齢構成や基礎疾患などの背景因子によってバラつきはあるものの10-30%の発症予防効果が認められている
- ✓ 重症化の抑制に効果が期待できる

図13. インフルエンザ罹患後、重症化の代表的なリスク因子

- ・65歳以上の高齢者
- ・乳幼児から学童前まで
- ・慢性疾患罹患患者
- ・妊婦
- ・アスピリンを長期服用している若年者（Reye症候群のリスク）

- ✓ 集団内では約80%以上が接種している場合に、感染拡大が有意に抑制されると報告されている図14に示されるように、少数例のワクチン接種のみでは感染拡大を抑制できないことは直感的に支持できる。

図14.



ワクチンの効果

感受性宿主
免疫を有する宿主
感染者

米国NIHより

5) 環境整備

✓ エンベロープを有するため、56℃30 分の加熱、紫外線、エーテルなどの脂質を溶かす溶媒や界面活性剤、次亜塩素酸ナトリウム、エタノール等により容易に不活化される

✓ 湿度は限られた環境における伝播を一部阻止する可能性があるが、主たる感染防御手段ではない

6) 標準予防策と感染経路別予防策

✓ 呼吸器症状を有する患者で、感染症が否定されていない場合には、できるだけ早期に呼吸衛生・咳エチケットを実施する（**図15**）

図15. 呼吸器衛生／咳エチケット

ポスター等により掲示

✓ かぜの徴候があればすぐに申告
✓ 咳またはくしゃみの時は鼻や口を覆う
✓ 呼吸器分泌物に接触した後は手指衛生
✓ 呼吸器分泌物を封じ込めるためにはティッシュを使用
✓ 使用後のティッシュは最寄りのごみ箱に廃棄
✓ 口を覆うのは、病原菌の拡散を予防するためであることを示す
✓ マスクなど個人用保護具についての情報を示す

病院が用意すること

✓ 廃棄するためのノンタッチごみ箱を供給
✓ 速乾性手指消毒薬を使いやすい場所に設置
✓ 石けん及び使い捨てタオルをシンクの使いやすい場所に設置

✓ 飛沫感染が否定できない場合は、サージカルマスクによる防護を実施

✓ 手指衛生・飛沫感染対策を特に遵守する

✓ 特に流行期やリスクを有する疾患では、環境の整備をより十分に行う

7) 抗インフルエンザ薬による予防、アウトブレイクコントロール

✓ ザナミビル・オセルタミビルの予防内服の効果はそれぞれ、72－82%、68－89%と報告されている

- ✓ アマンタジン・リマンタジン等M2蛋白阻害剤は現在では耐性化のため使用されない
- ✓ 曝露後の予防内服の適応を図16に示す
- ✓ ワクチンを接種している医療従事者に対する曝露後予防内服については必ずしも推奨されていない
- ✓ 予期できない曝露によりアウトブレイクが起こった場合に、医療施設のように対象が限定された場合の予防内服によるアウトブレイクコントロールは可能と考えられている

図16. 曝露後の予防内服

- ✓ハイリスク者でワクチン未接種
- ✓ハイリスク者と同居、あるいは看護するものでワクチン未接種
- ✓小児でワクチン未接種
- ✓ワクチンが副作用等で利用できないもの
- ✓ワクチンによる免疫獲得が期待できないもの

【麻疹・水痘・風疹・ムンプスウイルス】

VPD（vaccine preventable diseases）は、4種（麻疹・水痘・風疹・ムンプスウイルス）、B型肝炎、インフルエンザなどのワクチン接種によって予防可能な疾患であり、医療従事者は基本的に自己の免疫状態を把握しておく必要があり、またできる限りワクチンなどによって抗体を有することを確認する必要がある。以下に特に4種ウイルスのそれぞれのウイルスの特徴とワクチンを含む感染予防策について記す[14]。

1）4種ウイルスの現状

① 抗体保有状況

- ✓ 麻疹・風疹は全数把握疾患である
- ✓ 麻疹・風疹の予防接種接種率は比較的高いが、水痘・ムンプスワクチンの接種率が約30%前後と低く、抗体保有率に影響してくる可能性がある
- ✓ 麻疹は、2008年11012名であったが、2009年732例、2010年455例と減少し、合併症例・死亡例も減少しているが、現在までに排除には至っていない
- ✓ 風疹は、2008年294例で、以後減少傾向にあったが、2011年371例、2012年は32週までで1016例と増加傾向にある。特に女性の患者に占める出産年齢世代の割合が72%と高く、注意が必要である
- ✓ 成人における水痘の抗体保有率調査では約95%、ムンプスでは約85%であった

② 共通の対策

- ✓ 個人と医療機関の両方が、すべての職員（外注を含む）・学生の罹患歴、予防接種歴を把握し、管理することが望ましい
- ✓ 抗体価の測定を行うことが望ましい
- ✓ 必要に応じて予防接種を実施する
- ✓ いずれの疾患も症状発現前から、感染力を有するため最も適切な対策はワクチン接種である

2) ワクチン接種[13)]

- ✓ 未接種・未罹患職員のワクチン接種は2回接種を原則とする
- ✓ 副作用等の理由で、ワクチン接種できない職員については配置等を考慮する
- ✓ 抗体価を測定した場合は判断基準に従い、陰性、陰性ではないが基準を満たさない、基準を満たす、の3群に分けてワクチン接種計画を作成する
- ✓ 陰性の場合は2回接種、陰性ではないが基準を満たさない場合と基準を満たすが既往歴・予防接種歴がはっきりしない場合は1回追加接種する対策が示されている
- ✓ ウイルスごとに判断基準に示された検査方法で検査を実施する（表5）

表5. 抗体価の判断基準

	麻疹	水痘・帯状疱疹	風疹	ムンプス
陰性	中和法で1:4未満 PA法で1:16未満 EIA法(IgG)で陰性	IAHA法で1:2未満 EIA法(IgG)で陰性 水痘抗原皮内テストで陰性	HI法で1:8未満 EIA法(IgG)で陰性	EIA法(IgG)で陰性
基準を満たさない（陰性ではない）	中和法で1:4 PA法で1:16～1:128 EIA 法(IgG)で±および16未満	IAHA法で1:2、1:4 EIA法(IgG)で±	HI法で1:8、1:16 EIA 法(IgG)で±および8未満	EIA法(IgG)で±
基準を満たす	中和法で1:8以上 PA法で1:256以上 EIA法(IgG)で16以上	IAHA法で1:8以上 EIA法(IgG)で陽性 水痘抗原皮内テストで陽性	HI法で1:32以上 EIA法(IgG)で8以上	EIA法(IgG)で陽性

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3) 4種ウイルスの特徴

① 麻疹

- ✓ 麻疹ウイルスはRNA型ウイルスで、エンベロープを有する
- ✓ ヒトが唯一の宿主である
- ✓ 伝播力がきわめて強い
- ✓ 春から夏にかけての流行が多い
- ✓ 潜伏期間は10日～12日間
- ✓ 前駆期（カタル期）として38度前後の熱、咳嗽、鼻漏、くしゃみなどの上気道症状と結膜炎症状を2～4日間発症し、カタル期後2～4日で発疹が顔面、体幹部、四肢に出現し、高熱も再発する

- ✓ 発疹出現前後の口腔内には白色小斑点（Koplik 斑）がみられる
- ✓ 発疹出現後4日で解熱し回復する
- ✓ 成人や移植患者では重症化することがある
- ✓ 麻疹ウイルスはカタル期に涙液、唾液中に大量に排出され、気道粘膜へ達することにより伝播する

② 水痘

- ✓ Varicella-Zoster virus（水痘・帯状疱疹ウイルス）はヘルペスウイルス科αヘルペス亜科に属するDNA型ウイルスで、エンベロープを有する
- ✓ ヒトを唯一の宿主とする
- ✓ 潜伏期間は約2週間（8日～21日）
- ✓ 発熱、全身倦怠感、発疹を発症し、発疹は体幹、顔、四肢に出現し、しばしば口腔や気道粘膜にも出現し、紅斑、丘疹、水疱の段階を経て痂皮となる
- ✓ 倦怠感、そう痒感を伴い38度前後の発熱が2～3日続く
- ✓ HIV患者や一般成人、とくに白血病患者や移植患者において重症化することがある
- ✓ 妊娠第1～2期妊婦が感染した場合、胎児に先天性水痘症候群をもたらすことがある
- ✓ 出産5日前～出産2日後に妊婦が水痘を発症した場合には新生児の水痘が重症となることがある
- ✓ 知覚神経節に持続的に潜伏感染する
- ✓ 加齢などの免疫力の低下要因により、末梢神経節に沿って帯状疱疹を回帰発症する
- ✓ 水痘・帯状疱疹ウイルスは接触感染および空気感染により拡大する
- ✓ 水痘症例では発疹出現の1～2日前から他者への感染性を持つようになり、約5日間感染性を保つ
- ✓ 抗ウイルス薬が存在する

③ 風疹

- ✓ 風疹ウイルスはRNA型ウイルスで、エンベロープを有す
- ✓ 潜伏期間は18日間前後（約14～21日間）
- ✓ 上部消化管からリンパ節へ移行した後、ウイルス血症となり、全身へと播種する
- ✓ 発熱、発疹、リンパ節腫脹（耳後部、後頭部、頚部）を引き起こす
- ✓ 発疹は顔などから現れ、数時間で拡大する
- ✓ 発疹は3日程度で消失する
- ✓ 妊娠前半期の妊婦の初感染による先天性風しん症候群の発生が危惧される
- ✓ 潜伏期間から鼻咽頭分泌物などにウイルスが含まれ、飛沫により伝播する
- ✓ 先天性風しん症候群の症例は、生後約1年程度は、咽頭、尿などからウイルスを排出する

④ ムンプス

- ✓ ムンプスウイルスはRNA型ウイルスでエンベロープを有す
- ✓ 約3分の1は、不顕性感染
- ✓ 潜伏期間は平均16～18日で約2～4週間の幅がある

- ✓ 前駆症状として微熱、食欲不振、倦怠感などが現れることがある
- ✓ 呼吸器症状から全身症状を伴ったウイルス血症へと移行し、耳下腺、唾液腺の腫脹などを起こす
- ✓ 成人男性の約25%において睾丸炎、成人女性の約5%において卵巣炎がみられる
- ✓ 妊婦においては流産や早産の危険性を高める
- ✓ 約10%の頻度で無菌性髄膜炎が起こるが、おおむね軽症
- ✓ まれに永続的な難聴となることがある
- ✓ まれに脳炎に至り死因となることもある
- ✓ ムンプスウイルスは潜伏期から唾液などに含まれており、飛沫により伝播する

4) 感染予防策

それぞれのウイルスの特徴を表6に示す

表6. 4種 ウイルスの特徴

	麻疹	水痘・帯状疱疹	風疹	ムンプス
原因ウイルス名	Measles virus	Varicella-Zoster virus	Rubella virus	Mumps virus
抗体保有率	比較的高い	比較的高い	比較的低い	比較的低い
主なハイリスク者	妊婦・胎児、新生児、白血病患者、移植患者、HIV 患者など			
感染経路	空気感染	接触・空気感染	飛沫感染	飛沫感染
感染症例に必要な感染対策	空気予防策	接触・空気予防策	飛沫予防策	飛沫予防策
エンベロープ	エンベロープ有			
アルコールに対する感受性	良好			
持続感染	通常なし	あり	通常なし	通常なし
感染症法	5類全数把握疾患	5類定点	5類全数把握疾患	5類定点

- ✓ 麻疹・水痘では空気感染予防策が必要であり、N95 規格以上のマスクの使用と患者隔離、空調管理された部屋の使用が求められる
- ✓ 空気感染対策のための空調管理は、陰圧であることが望ましいが、少なくとも独立換気が必要である
- ✓ 水痘はさらに接触感染予防策が必要である
- ✓ 風疹・ムンプスは飛沫感染予防策が必要である
- ✓ いずれの場合も疑われる段階から予防策を実施し、否定されてから解除する
- ✓ 疑い患者・確定診断患者に対しては、医療従事者の免疫状態・抗体保有状況などから担当を判断する

5) 就業停止期間

- ✓ 就業停止期間は感染者、感受性を有するものが曝露された場合に分けて示している（表7）
- ✓ 就業停止期間は長期にわたるため、業務に支障を来たすなど経済的な負担が増加する。ワクチンによる予防や各医療従事者（学生を含む）の既往歴・抗体価管理を適切に行うほうが、医療経済的に優れていることが示されている

表7. 就業停止期間

	麻疹	水痘	風疹	ムンプス
感染者	発疹出現後 7 日間か急性疾患が続く期間のいずれか長い方の期間	全ての発疹が乾燥し痂皮化するまで	発疹出現後 5 日間	耳下腺炎発症後 9 日間
ウイルス曝露感受性者	曝露後 5 日目から最後の曝露後 21 日目まで	曝露後 10 日目から最後の曝露後 21 日目まで（グロブリン投与後は 28 日目まで）	曝露後 7 日目から最後の曝露後 21 日目まで	曝露後 12 日から最後の曝露後 26 日目まで

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Correlation between Triazole Treatment History and Susceptibility in Clinically Isolated *Aspergillus fumigatus*

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This is the first report of a detailed relationship between triazole treatment history and triazole MICs for 154 *Aspergillus fumigatus* clinical isolates. The duration of itraconazole dosage increased as the itraconazole MIC increased, and a positive correlation was observed ($r = 0.5700$, $P < 0.0001$). The number of itraconazole-naïve isolates dramatically decreased as the itraconazole MIC increased, particularly for MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$). We also examined the relationship between cumulative itraconazole usage and the MICs of other azoles. A positive correlation existed between itraconazole dosage period and posaconazole MIC ($r = 0.5237$, $P < 0.0001$). The number of itraconazole-naïve isolates also decreased as the posaconazole MIC increased, particularly for MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$). Conversely, the correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$). Susceptibility to three triazole agents did not change as the duration of voriconazole exposure changed. In addition, we carried out detailed analysis, including microsatellite genotyping, for isolates obtained from patients infected with azole-resistant *A. fumigatus*. We confirmed the presence of acquired resistance to itraconazole and posaconazole due to a G54 substitution in the *cyp51A* gene for a patient with chronic pulmonary aspergillosis after oral itraconazole therapy. We should consider the possible appearance of azole-resistant *A. fumigatus* if itraconazole is used for extended periods.

Aspergillosis has become an increasingly important fungal infection, because the number of immunocompromised patients has increased (21, 29). However, antifungal drugs for treating different types of aspergillosis such as invasive pulmonary aspergillosis or chronic pulmonary aspergillosis have insufficient efficacy (18–20, 32). Among the few types of drugs with anti-*Aspergillus* activity, triazoles hold a prominent position because they are the only licensed class of oral drugs for treating aspergillosis (32).

Recently, the appearance of azole-resistant *Aspergillus fumigatus* has come under scrutiny in several countries (1, 2, 7, 14, 17, 23–27, 30). Reports from some countries have raised concerns over the increased prevalence of azole-resistant *A. fumigatus* (7, 17, 27). Therefore, it is important to elucidate the mechanism of resistance to prevent the spread of azole-resistant *A. fumigatus* and subsequent outbreaks. The possible origins of these azole-resistant isolates include the environment and the patient's own body (31). Some cases of acquired resistance in *A. fumigatus* have been reported in patients with aspergilloma during treatment with azoles (3, 6, 8, 9, 11, 22). Environments such as farms are especially suspected of promoting the production of azole-resistant isolates harboring the TR/L98H mutation in the *cyp51A* gene, which encodes cytochrome P450 14-α sterol demethylase, the primary target for azole compounds (23, 31).

Despite the presence of case reports on the development of azole resistance during azole therapy, little information is available on the amount of azole needed for the development of azole resistance (8, 17, 22). Howard et al. reported that the first azole-resistant isolate was identified after using azole for 1 to 30 months (17). A recent study by Camps et al. warned of a rapid induction of resistance for which the median time between isolation of the last cultured wild-type isolate and isolation of the first azole-resistant isolate was 4 months (8). Such data are important, because long-term, perhaps lifelong, antifungal treatment is required for some chronic pulmonary aspergillosis cases (32).

Recently, we reported the antifungal MIC distribution of 196 *A. fumigatus* clinical isolates with a *cyp51A* gene mutation in Nagasaki, Japan (28). Of those, we analyzed 154 isolates from 64 patients retrospectively in this study, and we evaluated the cumulative amount of azoles administered to patients at the time of isolation of each *A. fumigatus* clinical isolate. Moreover, we investigated the backgrounds of patients from whom azole-resistant *A. fumigatus* was isolated and conducted microsatellite genotyping of the isolates to analyze their genetic relationships. This is the first report to analyze the correlation between azole usage and azole susceptibility of *A. fumigatus* clinical isolates.

MATERIALS AND METHODS

***A. fumigatus* isolates.** The isolates were collected in the Pneumology Department of Nagasaki University Hospital, Nagasaki, Japan, between February 1994 and April 2010. We identified all isolates as *A. fumigatus*

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TABLE 1 Characteristics of patients and isolates

Parameter	Value(s)[a]
No. of isolates	154
No. of patients	64
Sample origin	
Sputum	96/154 (62)
Bronchoalveolar lavage fluid	36/154 (23)
Lung tissue	9/154 (5.8)
Other[b]	2/154 (1.3)
Unknown	11/154 (7.1)
Clinical diagnoses[c]	
Invasive pulmonary aspergillosis[d]	9/64 (14)
Chronic pulmonary aspergillosis except simple aspergilloma	27/64 (42)
Simple aspergilloma	12/64 (19)
Allergic bronchopulmonary aspergillosis	4/64 (6.3)
Colonization	12/64 (19)

[a] Other than patient and isolate data, all values represent number of positive results/total number of results (percent).
[b] "Other" includes lung abscess and bone marrow.
[c] Diagnoses of 23 other patients were unknown.
[d] All diagnoses were classified as "probable."

according to the macroscopic colony morphological and micromorphological characteristics and the ability to grow at 48°C (4). Azole-resistant isolates were subjected to additional molecular identification by amplification of ribosomal internal transcribed spacers and ribosomal large-subunit D1-D2 sequencing as described previously (16).

Patients. Clinical information was extracted from the clinical records on the type of aspergillosis and history of azole antifungal use. The periods of triazole administration were cumulatively determined until the time of *A. fumigatus* isolation; therefore, the periods were different for each isolate and even for isolates obtained from the same patient. In patients infected with azole-resistant *A. fumigatus*, we examined the underlying diseases and characteristics of therapeutic failure. Patient 1 (a 48-year-old man) had chronic cavitary pulmonary aspergillosis (CCPA) (see Table 2). Both his lungs were damaged by multiple partial lobectomies because of repeated refractory pneumothorax, and multiple cavities and bullas with pleural thickness were observed in both the lungs. *A. fumigatus* was frequently cultured from his sputum despite oral itraconazole treatment (200 to 400 mg/day). After the isolation of itraconazole-resistant *A. fumigatus*, the patient was treated with oral voriconazole. Since then, his symptoms such as productive cough or hemosputum have improved, and no fungus has been subsequently isolated from his sputum. Patient 2 (a 70-year-old woman) was clinically diagnosed as having aspergilloma in the upper lobes of both the lungs (see Table 2). She had a history of pulmonary tuberculosis and had several cavities in both the lungs. Patients 3 (an 80-year-old woman) and 5 (a 63-year-old man) were diagnosed with simple aspergilloma. Patient 4 (a 56-year-old woman) was diagnosed with CCPA (see Table 2).

Antifungal susceptibility testing and *cyp51A* sequencing. We previously reported results for antifungal susceptibility analysis and *cyp51A* sequencing (28). The breakpoints used for resistance were ≥4 μg/ml for itraconazole and voriconazole and ≥1 μg/ml for posaconazole (30).

Genotyping. Sixteen isolates (including both azole-susceptible and azole-resistant isolates) were obtained from 5 patients infected with azole-resistant *A. fumigatus*. DNA was extracted from these isolates by using a MasterPure yeast DNA purification kit (Epicentre Biotechnologies, Madison, WI), and 9 short tandem-repeat regions (2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, and 4C) were amplified by PCR as described previously (12). The repeat numbers were determined by sequencing analysis, and we compared the patterns of repeat numbers. DNA sequences were determined using a BigDye Terminator version 1.1 cycle sequencing kit (ABI) and an ABI 3100xl DNA analyzer.

Statistics. Statistical analyses of azole usage and azole susceptibility were performed using Pearson's correlation and Fisher's exact tests with Prism version 5.0 (GraphPad). Differences were considered significant when $P < 0.05$.

RESULTS

Correlation between azole usage (duration and amount) and azole susceptibility. A total of 154 *A. fumigatus* clinical isolates obtained from 64 patients were analyzed. Most of the specimens were isolated from the lungs (Table 1). Chronic pulmonary aspergillosis (including simple aspergilloma) accounted for 61% of the clinical diagnoses (Table 1).

The scatter plot of the itraconazole dosage period and itraconazole MICs is shown in Fig. 1A. Patients infected by *A. fumigatus* with itraconazole MICs < 2 μg/ml had been treated with itraconazole for <1 year. All isolates with itraconazole MICs ≥ 4 μg/ml (MF-452, MF-460, MF-468, MF-469, MF-329, and MF-357) had been exposed to itraconazole for >115 days (Table 2). The itraconazole dosage duration increased as the itraconazole MIC increased, and the dosage duration was positively correlated with the itraconazole MIC ($r = 0.5700$, $P < 0.0001$) (Fig. 1A). The number of itraconazole-naïve isolates dramatically decreased as the MIC increased, particularly for MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$) (Fig. 1B). These results indicated that long-term itraconazole treatment could induce azole-resistant *A. fumigatus*.

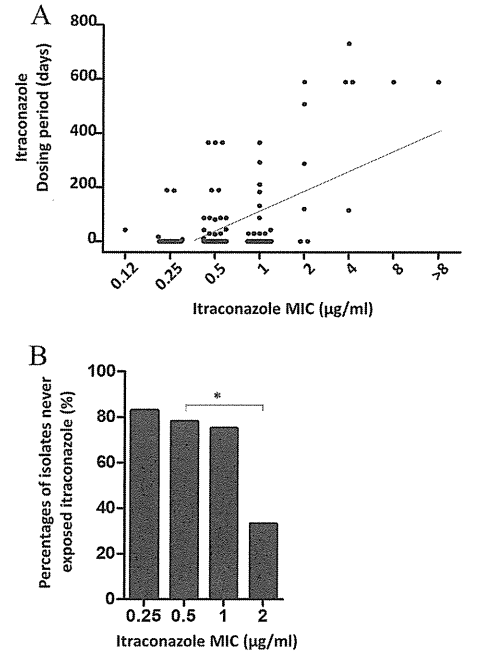

FIG 1 Relationship between itraconazole MICs and the history of itraconazole usage for 154 *A. fumigatus* clinical isolates. (A) The itraconazole dosage duration increased as the itraconazole MIC increased, and a positive correlation was observed between the itraconazole dosage duration and the itraconazole MIC ($r = 0.5700$, $P < 0.0001$). (B) The number of itraconazole-naïve isolates dramatically decreased as the itraconazole MIC increased, particularly for itraconazole MICs exceeding 2 μg/ml (0.5 μg/ml versus 2 μg/ml, $P = 0.03$). *, $P < 0.05$ (Fisher's exact test).

TABLE 2 Characteristics of the 16 isolates obtained from patients infected with azole-resistant *A. fumigatus*[a]

Patient no.	Isolate no.	Date of isolation (day-mo-yr)	ITC exposure[b]		Time from end of ITC therapy (days)	MIC (μg/ml)[c]			Cyp51A substitution[d]
			Period (days)	Amt (mg)		ITC	POS	VRC	
1	MF-368	16-08-2000	189	37,800	252	0.5	0.06	0.5	No substitution
	MF-367	16-08-2000	189	37,800	252	0.5	0.06	0.25	No substitution
	MF-370	07-09-2000	189	37,800	274	0.25	0.06	0.25	No substitution
	MF-439	19-10-2001	507	144,850	0	2	0.5	0.25	G54E
	MF-452	03-04-2002	589	161,650	84	>8	0.5	0.5	No substitution
	MF-454	17-04-2002	589	161,650	98	2	0.5	0.125	G54E
	MF-460	08-05-2002	589	161,650	119	4	2	0.25	G54E
	MF-468	22-05-2002	589	161,650	133	4	0.5	0.25	G54E
	MF-469	29-05-2002	589	161,650	140	8	1	0.25	G54E
2	MF-329	24-08-1998	115	23,000	0	4	0.5	0.25	No substitution
	MF-331	29-08-1998	120	24,000	0	2	>8	0.25	G54W
	MF-336	10-09-1998	132	26,400	0	1	0.25	2	No substitution
3	MF-357	09-02-2000	731	146,200	1223	4	0.5	0.5	No substitution
4	MF-933	11-03-2008	0	0		0.5	0.25	0.25	No substitution
	MF-1011	09-10-2008	210	42,000	0	1	2	0.125	G54W
5	MF-327	16-07-1998	287	43,050	435	2	2	0.125	G54R

[a] Azole-resistant *A. fumigatus* had itraconazole MIC ≥ 4μg/ml or posaconazole MIC ≥ 1μg/ml. Voriconazole resistant isolates (voriconazole MIC ≥ 4μg/ml) were not found.
[b] Accumulated periods and amounts before isolation.
[c] ITC, itraconazole; POS, posaconazole; VRC, voriconazole.
[d] Only substitution associated with azole resistance.

A positive correlation was also observed between the itraconazole dosage period and the posaconazole MIC ($r = 0.5237$, $P < 0.0001$) (Fig. 2A). The number of itraconazole-naïve isolates decreased as the posaconazole MIC increased, particularly for posaconazole MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$) (Fig. 2C). The correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$) (Fig. 2B). The voriconazole MIC did not increase with increasing itraconazole usage. In addition, the numbers of itraconazole-naïve isolates were not correlated with the voriconazole MIC (Fig. 2D). These results suggested the possibility of inducing resistance to posaconazole but not to voriconazole by long-term itraconazole therapy.

A. fumigatus was isolated after voriconazole treatment from only a few patients; therefore, analysis of the relationship between voriconazole usage histories before *A. fumigatus* isolation and azole susceptibilities was limited. Only 10 isolates were exposed to voriconazole therapy before isolation, and the average duration of the therapy was 8.3 ± 6.3 days. Voriconazole exposure did not alter the susceptibility to the 3 triazole agents.

In this study, we counted the duration of azole exposure as the cumulative time of treatment. *A. fumigatus* was not always clinically isolated from patients during therapy; it was also isolated after the cessation of azole therapy. Because the selection pressure on azole-resistant *A. fumigatus* might be at its highest during the treatment, azole resistance might dissipate over time after therapy. Hence, we examined the relationship between the itraconazole MIC and the time from the end of itraconazole therapy to isolation. Of the 154 isolates, 42 had been exposed to itraconazole therapy before isolation. The time from the end of itraconazole treatment to isolation had no relationship with itraconazole susceptibility ($r = -0.1302$, $P = 0.4110$) (Fig. 3). Azole-resistant *A. fumigatus* was isolated even after azole treatment had been discontinued.

Clinical analysis of patients infected with azole-resistant *A. fumigatus*. Five patients were infected with azole-resistant *A. fumigatus*, and 16 isolates (including susceptible isolates) were obtained from these patients (Table 2). To analyze the genetic relationships among these 16 isolates, a panel of nine short tandem repeats for exact and high-resolution fingerprinting of *A. fumigatus* isolates was examined in this study. The 16 isolates obtained from the 5 patients were divided into 6 genotypes via microsatellite typing (Table 3).

Nine isolates were cultured from patient 1 (Table 2). *A. fumigatus* isolated in earlier periods was susceptible to azole, and it harbored the I266N mutation in the *cyp51A* gene; however, later isolates showed itraconazole or posaconazole resistance and new mutations such as G54E. Despite the discontinuation of itraconazole treatment, azole-resistant isolates were cultured from sputum of the patient 140 days after the end of the treatment (Table 2). All isolates were confirmed to be genetically homogeneous (Table 3).

From patient 2, three *A. fumigatus* isolates were cultured during days 115 to 132 of the itraconazole dosage period. The isolates were homogeneous; however, the itraconazole or posaconazole MICs and *cyp51A* mutations in the three isolates were significantly different (Tables 2 and 3). *A. fumigatus* isolates from patient 4 were heterogeneous.

DISCUSSION

In this study, we showed a correlation between the duration of clinical itraconazole exposure and the MICs of triazoles for *A. fumigatus*. It has already been reported that itraconazole exposure can induce the formation of azole-resistant *A. fumigatus* carrying a G54 mutation in the *cyp51A* gene *in vitro* (13). As expected,

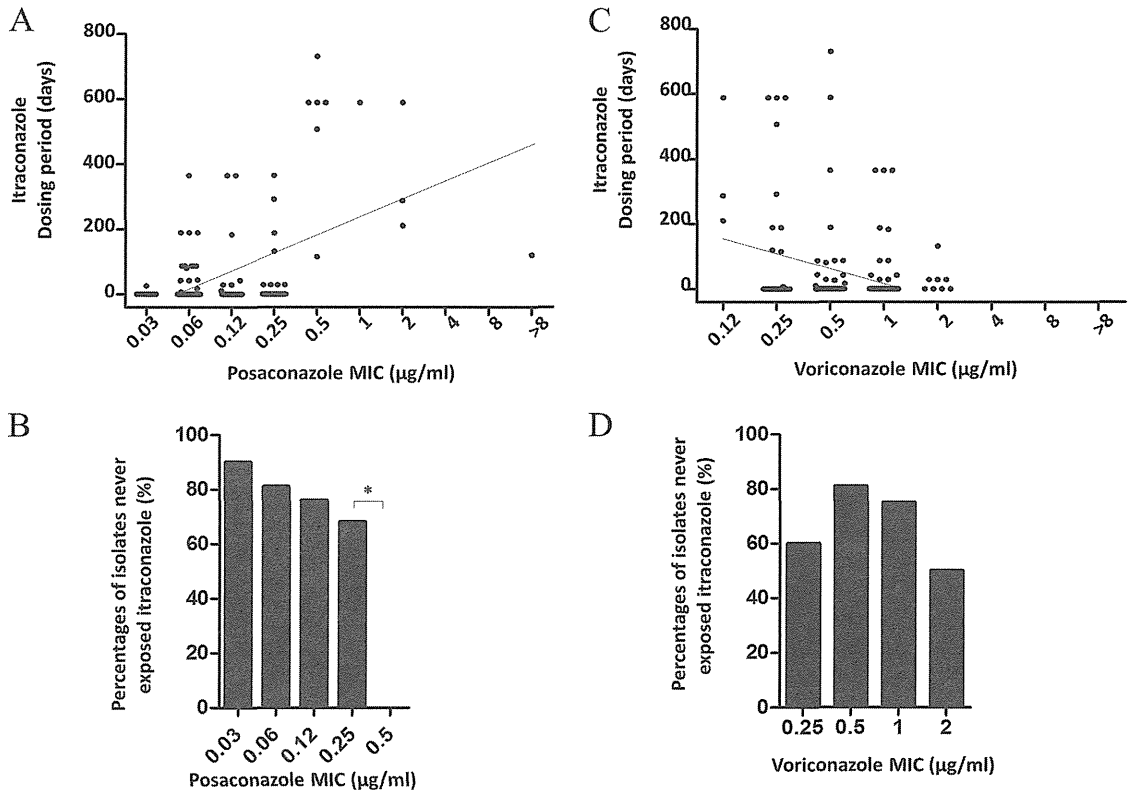

FIG 2 Relationship between the MICs of other triazoles and the history of itraconazole usage for the 154 *A. fumigatus* clinical isolates. (A) A positive correlation was observed between the itraconazole dosage period and the posaconazole MIC ($r = 0.5237$, $P < 0.0001$). (B) The number of itraconazole-naïve isolates decreased as the posaconazole MIC increased, particularly for posaconazole MICs exceeding 0.5 μg/ml (0.25 μg/ml versus 0.5 μg/ml, $P = 0.004$). (C) The correlation coefficient obtained from the scattergram of itraconazole usage and voriconazole MICs was small ($r = -0.2627$, $P = 0.001$). (D) No significant difference was observed in the percentages of itraconazole-naïve isolates and the individual MICs of voriconazole. *, $P < 0.05$ (Fisher's exact test).

increased use of itraconazole was associated with decreased itraconazole susceptibility among the *A. fumigatus* clinical isolates. The posaconazole susceptibility of the isolates was also decreased, presumably because of the appearance of G54 substitution in the *cyp51A* gene, indicating that clinicians should be careful when selecting posaconazole as an antifungal agent for the treatment of patients who had previously received long-term itraconazole therapy. If long-term itraconazole therapy induces voriconazole resistance in *A. fumigatus*, then this will have a significant impact on the treatment of aspergillosis. Our study indicated that itraconazole treatment did not induce voriconazole cross-resistance. These results were consistent with previous reports (15, 25). The reason for the lack of cross-resistance between itraconazole and voriconazole in this study was that the G54 mutation in azole-resistant isolates resulted in a resistance to itraconazole and posaconazole but not to voriconazole.

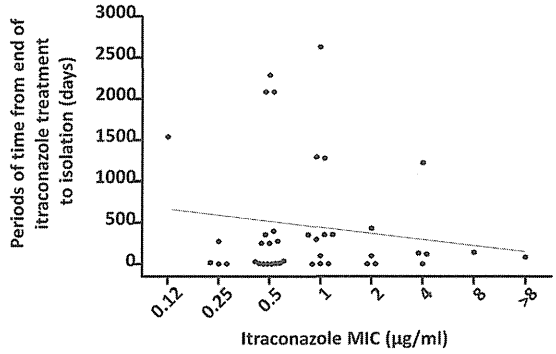

FIG 3 We examined the relationship between itraconazole MICs and the time from the end of itraconazole therapy to *A. fumigatus* isolation. Of the 154 isolates, 42 had been exposed to itraconazole before isolation. These isolates were analyzed for the relationship; however, the relationship could not be confirmed by the scatter plot ($r = -0.1302$, $P = 0.4110$).

The most important limitation of this study was that no data could be obtained regarding the serum concentration of itraconazole during its usage. Itraconazole has a relatively low bioavailability after oral administration, especially when given in capsule form (33). Of the 42 isolates exposed to itraconazole before isolation, 39 had been exposed to itraconazole capsules, and the remaining 3 isolates had been exposed to the oral solution, which has a greater bioavailability than the capsule form (5). Most patients who were administered the capsule form of itraconazole were prescribed a dose of 200 mg/day, which is the approved dose in Japan. Despite the lack of a report examining the presence of a mutation selection window for itraconazole by *A. fumigatus*, both the low bioavailability and blood concentration of itraconazole in capsule form might be risk factors for azole resistance. The solution form may overcome these disadvantages; however, patient 4, who was infected with posaconazole-resistant *A. fumigatus* carrying the G54W *cyp51A* mutation, had been administered the itraconazole oral solution at a dose of 200 mg/day for 210 days.

TABLE 3 Genotypes of the 16 *A. fumigatus* isolates by STR*Af*

Patient no.	Isolate no.	No. of tandem repeats at indicated microsatellite by STR*Af*[a]								
		2A	2B	2C	3A	3B	3C	4A	4B	4C
1	MF-368	23	15	10	25	11	32	8	10	7
	MF-367	23	15	10	25	11	32	8	10	7
	MF-370	23	15	10	25	11	32	8	10	7
	MF-439	23	15	10	25	11	32	8	10	7
	MF-452	23	15	10	25	11	32	8	10	7
	MF-454	23	15	10	25	11	32	8	10	7
	MF-460	23	15	10	25	11	32	8	10	7
	MF-468	23	15	10	25	11	32	8	10	7
	MF-469	23	15	10	25	11	32	8	10	7
2	MF-329	19	21	14	18	10	16	7	13	5
	MF-331	19	21	14	18	10	16	7	13	5
	MF-336	19	21	14	18	10	16	7	13	5
3	MF-357	18	19	23	34	13	20	18	9	8
4	MF-933	20	12	20	24	22	36	13	9	5
	MF-1011	11	21	11	28	12	31	18	9	10
5	MF-327	21	21	10	23	11	27	8	9	8

[a] Data represent the number of tandem repeats at the given microsatellite number.

Itraconazole oral therapy is often administered long-term for the treatment of chronic pulmonary aspergillosis (32). The judgment of treatment failure is still difficult; therefore, we need more information to decide whether itraconazole treatment should be continued. Despite the importance of the duration of itraconazole treatment with respect to the induction of azole resistance, few studies have investigated the relationship between azole resistance and azole exposure. Howard et al. reported that the duration of azole exposure before the identification of the first resistant isolate was 1 to 30 months, and the most commonly administered azole was itraconazole (17). Mortensen et al. also reported that patients with azole-resistant *A. fumigatus* isolates had received mold-active azoles for 11.5 to 69.5 months before the detection of resistant isolates (22). In our study, patients with azole-resistant *A. fumigatus* had been administered itraconazole for 3.8 to 24.3 months. These data are similar to those described above. Moreover, patients infected by *A. fumigatus* with itraconazole MICs < 2 μg/ml had been administered itraconazole for <1 year. Clinicians should be careful of the potential appearance of itraconazole-resistant isolates during long-term sequential itraconazole therapy administered for several months to more than 1 year.

Recently, Camps et al. reported that median time between collection of the last cultured wild-type isolate and the first azole-resistant isolate was 4 months (range, 3 weeks to 23 months) (8). In our study, the times between the last isolation of an azole-sensitive strain and the first appearance of an azole-resistant strain were about 10 and 7 months in patients 1 and 4, respectively (Table 2). These periods were longer than the median time reported by Camps et al. but fell within the reported range (3 weeks to 23 months).

We confirmed that long-term itraconazole therapy induced azole resistance in *A. fumigatus*. Even if azole-resistant mutants were dominant during treatment, their dominance could dissipate after cessation of the therapy because of the differences in the growth rates of the resistant and susceptible specimens (3). However, resistant isolates were still cultured 140 days after the cessation of azole therapy in patient 1. In patients 3 and 5, the times from the end of treatment to isolation were 1,223 and 435 days, respectively, which might indicate the possibility of the presence of resistant isolates for years after the end of azole therapy or the possibility of new infection. There were no differences in the growth rates of azole-resistant and azole-susceptible *A. fumigatus* isolates *in vitro* (data not shown). When patients receive long-term itraconazole therapy, clinicians should aggressively culture *A. fumigatus* from the patients and perform susceptibility tests even long after the cessation of itraconazole therapy.

We isolated azole-resistant *A. fumigatus* from clinical samples, such as sputum, but we did not isolate *A. fumigatus* from the environment or detect a TR/L98H mutant (28). It is interesting that the most common mechanism of resistance detected in this study was G54 substitution, because the selection pressure of itraconazole induces G54 mutation (13). Moreover, most resistant isolates detected in the environments around the world carry the TR/L98H substitution and no other mutation such as an G54 substitution (10, 23). These facts suggest that different azoles select different mutations. Itraconazole might selectively induce mutations such as G54 substitution, whereas some azoles used in agriculture may tend to select the TR/L98H mutation. The mechanisms of these differences remain to be completely elucidated. Further investigation is needed to clarify these mechanisms, and this knowledge may enable us to prevent the induction of the TR/L98H mutation in the environment.

In conclusion, this is the first report to show a detailed relationship between azole usage and azole MICs for *A. fumigatus*. Furthermore, we confirmed the presence of acquired resistance to itraconazole and posaconazole in a patient with chronic pulmonary aspergillosis after consecutive oral itraconazole treatments in Japan. The possibility of the presence of azole-resistant *A. fumigatus* should be considered during long-term itraconazole therapy in patients with chronic pulmonary aspergillosis.

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□ CASE REPORT □

A Case of Refractory Chronic Respiratory Tract Infection due to *Pseudomonas aeruginosa* Successfully Controlled by Combination of Clarithromycin and Azithromycin

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Abstract

The prognosis of patients with chronic respiratory tract infections, especially diffuse panbronchiolitis, is remarkably improved by long-term administration of low-dose macrolides. However, in some cases, patients are refractory to macrolide treatment and show a low or no response; therefore, new treatment strategies are required. Here we present a patient refractory to either single low-dose clarithromycin or azithromycin but responded remarkably to the combination usage of both macrolides.

Key words: chronic respiratory tract infection, *Pseudomonas aeruginosa*, clarithromycin, azithromycin

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Introduction

The prognosis of patients with refractory chronic respiratory tract infections such as diffuse panbronchiolitis, has been dramatically improved owing to the long-term administration of low-dose macrolides. There are some cases, however, that are refractory to such treatment, and newer or improved strategy of treatment is urgently required in clinical settings. We here report a case of refractory chronic respiratory infection caused by *Pseudomonas aeruginosa* that was not well controlled by administration of either single low-dose clarithromycin (CAM) or azithromycin (AZM) but good control was achieved by combined therapy with both macrolides.

Case Report

A 60-year-old woman, previously diagnosed with systemic lupus erythematosus, interstitial pneumonia due to collagen diseases, Sjögren syndrome, and antiphospholipid syndrome, in 1992, 1994, 2003, and 2008, respectively, was followed up clinically at the Department of Dermatology and Respiratory Medicine of Nagasaki University Hospital (NUH), Nagasaki, Japan. She had been taking oral prednisolone (5 mg/day) continuously for 16 years. Interstitial pneumonia gradually progressed, and the lungs showed a honeycomb appearance. The severity of clinical symptoms such as cough and sputum production had gradually increased since 2008. Moreover, the frequency of recurrences of chronic respiratory tract infection due to *P. aeruginosa* had increased since May 2008. Administration of oral CAM (200 mg/day) was initiated in August 2008. The patient was admitted to NUH on January 28, 2009, because the cough and sputum production worsened and she had a persistent high fever.

On admission, she was alert and vital signs were as follows: body temperature, 39.2℃; heart rate, 136 beats/min with a regular rhythm; SpO_2, 89% (on room air); respiratory rate, 24 breaths/min with regular rhythm; and blood pressure, 106/71 mmHg. Physical examination revealed emaciation (height =156.0 cm and body weight =42.5 kg) and diminished respiratory sounds with moist rales in both the

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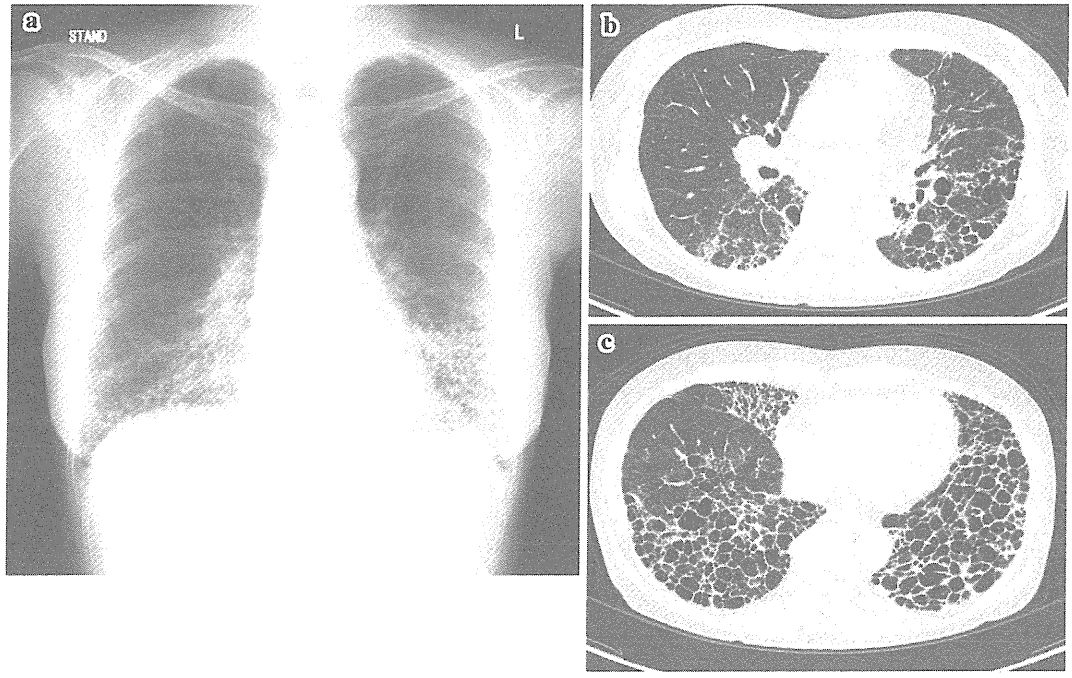

Figure 1. **Radiological findings on admission. a) Chest radiograph showing severe cystic and reticular shadows in both the right and left lower lung fields. b) and c) Chest computed tomography scan images showing the honeycomb appearance of the lungs.**

right and left lower lung fields. No signs of systemic lymphadenopathy, hepatosplenomegaly, or pre-tibial edema were observed. On admission, her white blood cell count was $11.1\times10^3/\mu L$ with a shift to the left (neutrophils, 83%) and C-reactive protein level was 10.1 mg/dL. The findings of the blood gas analysis were as follows: pH, 7.488; PCO_2, 34.9 torr; PO_2, 91.5 torr; and HCO_3^-, 25.9 mmol/L (O_2 nasal, 1.5 L/min). A microbiological test of the sputum revealed the presence of *P. aeruginosa* at 1×10^5 CFU/mL, and a drug susceptibility test indicated that the minimum inhibitory concentrations (MICs) of gentamycin, ciprofloxacin, and meropenem were 2.0, <0.25, and <0.25 μg/mL, respectively. Chest radiographs showed severe cystic shadows in both the right and left lower lung fields. Computed tomography scans showed a honeycomb appearance of the lungs (Fig. 1). Fig. 2 illustrates the clinical course of this case. Recurrence of chronic respiratory infection was diagnosed on admission, and the administration of tazobactam/piperacillin (4.5g×4/day) was started. Her clinical symptoms and fever were rapidly recovered and tazobactam/piperacillin was continued for 14 days then the patient was discharged. Three days after the discharge, however, the patient was re-admitted to NUH because of high fever. Refractory chronic pulmonary infection due to *P. aeruginosa* was diagnosed again. Although inhaled tobramycin with intravenous ciprofloxacin, followed by tazobactam/piperacillin with intravenous amikacin, and colistin were administered, she did not recover completely. As for long-term macrolide treatment, the previously administered CAM at 200 mg/day was switched to AZM at 250 mg/day every other day; however, this treatment was not effective at all. A single treatment with AZM was not effective; therefore, in August 2009, we initiated combined therapy with CAM at 400 mg/day and AZM at 250 mg/day once daily. This combined treatment reduced the event of high fever but low grade fever continued occasionally after December, 2010. The patient has been receiving both CAM and AZM for 2 years, and only 1 apparent episode of recurrence of chronic respiratory infection which required hospitalization and intravenous antibiotics administration (meropenem and ciprofloxacin for a week in January 2010) has been observed. Combined treatment with CAM and AZM is currently administered at the outpatient clinic. Additionally, an increase in the sensitivity of *P. aeruginosa* to almost all anti-*Pseudomonas* antimicrobial agents was observed after combined administration of anti-pseudomonas antimicrobial agents (Table 1).

Discussion

Long-term administration of low-dose erythromycin treatment, established by Kudoh et al., has remarkably improved the prognosis of patients with diffuse panbronchiolitis (1, 2). Apart from their anti-microbial activity, macrolides were found to have immunomodulatory effects, and these effects were extensively studied in Japan. These macrolides have been found to be highly effective in (a) reducing the amount of sputum produced via suppression of mucin secretion by blocking chloride channels of bronchial epithelial cells (3, 4); (b) blocking and inhibiting the accumulation of neutrophils and lymphocytes, neutrophil elastase activity, cytokine production, and adherence to cells (5-7); (c) decreasing and disrupting biofilm formation by *P. aeruginosa* (8); and (d) suppressing *P. aeruginosa* quorum-sensing systems as cell-to-cell communication (9). In fact, these effects work