E．結論

現在は、オセルタミビル耐性 H274Y 変異をもつ A/H1N1pdm09 ウイルスの頻度は低いが、季節性 A/H1N1 のようにオセルタミビル耐性 H274Y 変異が流行する可能性がある。したがって、今後も H274Y 変異を持つ耐性株の薬剤耐性サーベイランスを続ける必要があると考えられる。また、H274Y 以外の変異によるオセルタミビル耐性や、他の NA 阻害剤への耐性の獲得についても、一部の地域で頻度の上昇が報告されていることや、複数のアミノ酸変異による $IC_{50}$ 値の上昇が報告されており、薬剤耐性サーベイランスを継続して行う必要があると考えられる。

F．補足

2011-2012 年シーズンにおいて、北海道、新潟、群馬、東京、京都、大阪、兵庫、長崎の８都道府県 16 病院、医院から採取された合計 1192 件の臨床検体から分離されたインフルエンザ陽性検体中 A 型が 323 件で全て H3N2 亜型であった。B 型は 317 件で、そのうち 299 件では HA 遺伝子がビクトリア系統、NA 遺伝子が山形系統のリアソータントであり、18 件が HA, NA 遺伝子共に山形系統のウイルスであった。また、培養検体のみの検査結果ではあるが、A 型/H3N2 と B 型の両方で陽性の培養検体が大阪を除く全ての県で確認された。各都道府県のインフルエンザ陽性検体総数における A 型/H3N2、B 型両方陽性の培養検体の割合は 1-16%と地域差があったが（平均 5%）、東京では 16%(4/35)、北海道では 15%(3/20) と比較的高く、逆に兵庫では 2% (1/49)、京都では 1% (2/140) であった（2012 年 3 月 29 日現在）。

G．研究発表

１．論文発表

なし

２．学会発表

なし

H．知的所有権の取得状況

１． 特許取得

なし

２．実用新案登録

なし

３．その他

*日本サーベイランスグループ

北海道：

| | |
|---|---|
| 松前町立松前病院 | 木村 眞司先生 |

新潟：

| | |
|---|---|
| よいこの小児科さとう | 佐藤 勇先生 |

群馬：

| | |
|---|---|
| 川島内科クリニック | 川島 崇先生 |

京都：

| | |
|---|---|
| 日比小児科クリニック | 日比 成美先生 |
| ふじわら小児科内科医院 | 藤原 史博先生 |
| 生嶋こどもクリニック | 生嶋 聡先生 |
| つなもと医院 | 綱本 健太郎先生 |

大阪：

| | |
|---|---|
| よしはら小児科クリニック | 吉原 隆夫先生 |

兵庫：

| | |
|---|---|
| はしだ小児科 | 橋田 哲夫先生 |

表1　2009/2010年シーズン,2010/2011年シーズンのインフルエンザウイルス分離株

| | シーズン | | | | | |
|---|---|---|---|---|---|---|
| | 2009/2010 | | | 2010/2011 | | |
| | 検体数(IC50試行数) | | | 検体数(IC50試行数) | | |
| 調査地域 | A/H1N1pdm | A/H3N2 | B | A/H1N1pdm | A/H3N2 | B |
| 北海道 | 0 | 0 | 0 | 6(1) | 38(8) | 0 |
| 福島 | 51(9) | 0 | 0 | 0 | 0 | 0 |
| 新潟 | 74(7) | 0 | 0 | 65(12) | 295(18) | 15(11) |
| 群馬 | 27(5) | 0 | 0 | 5(3) | 358(8) | 0 |
| 京都 | 295(18) | 0 | 0 | 217(26) | 59(7) | 14(4) |
| 兵庫 | 60(7) | 0 | 0 | 59(8) | 33(6) | 3(3) |
| 大阪 | 0 | 0 | 0 | 21 | 6 | 0 |
| 長崎 | 94(11) | 0 | 0 | 41(10) | 59(12) | 1 |
| 合計 | 601(57) | 0 | 0 | 414(60) | 525(59) | 33(18) |

表2　2009/2010年シーズン,2010/2011年シーズンの各型・亜型対するペラミビル三水和物、オセルタミビルカルボン酸、ザナミビル水和物、ラニナミビルの$IC_{50}$値

| | | シーズン | | | |
|---|---|---|---|---|---|
| | | $IC_{50}$ (nM) | | | |
| | | 2009/2010 | | 2010/2011 | |
| 薬剤 | 型/亜型 | 平均IC50値(n)[a] | 範囲(カットオフ値)[b] | 平均$IC_{50}$値(n)[a] | 範囲(カットオフ値)[b] |
| ペラミビル三水和物 | A/H1N1pdm | 0.07(57) | 0.22-0.04(0.12) | 0.12(60) | 37.09-0.07(0.28) |
| | A/H3N2 | なし | なし | 0.17(59) | 0.33-0.10(0.37) |
| | B | なし | なし | 2.31(18) | 4.77-1.22(4.98) |
| オセルタミビルカルボン酸 | A/H1N1pdm | 1.88(57) | 10.91-1.48(3.43) | 1.37(60) | 443.95-0.72(2.61) |
| | A/H3N2 | なし | なし | 0.73(59) | 2.12-0.46(1.69) |
| | B | なし | なし | 27.09(18) | 59.12-16.97(70.08) |
| ザナミビル水和物 | A/H1N1pdm | 0.69(57) | 1.92-0.49(1.11) | 0.63(60) | 1.79-0.30(1.29) |
| | A/H3N2 | なし | なし | 0.63(59) | 2.66-0.41(1.50) |
| | B | なし | なし | 53.74(18) | 92.30-29.33(128.21) |
| ラニナミビル | A/H1N1pdm | 0.24(57) | 0.49-0.16(0.46) | 0.27(60) | 0.66-0.20(0.50) |
| | A/H3N2 | なし | なし | 0.65(59) | 1.09-0.30(1.20) |
| | B | なし | なし | 8.44(18) | 15.23-4.69(19.47) |

平均値は75%タイル+3IQR以上のはずれ値を除き算出。
aは$IC_{50}$試行株数。
bカットオフ値は75%タイル+3IQR以上のはずれ値を除き算出。

表 3　2009/2010 年シーズン,2010/2011 年シーズンの NA 阻害試験ではずれ値を示したウイルス分離株に対するペラミビル三水和物、オセルタミビルカルボン酸、ザナミビル水和物、ラニナミビルの $IC_{50}$ 値

| 型/亜型 | シーズン | 株名 | ペラミビル三水和物 | | オセルタミビルカルボン酸 | | ザナミビル水和物 | | ラニナミビル | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ (nM) | 増加率[a] | $IC_{50}$ (nM) | 増加率[a] | $IC_{50}$ (nM) | 増加率[a] | $IC_{50}$ (nM) | 増加率[a] |
| A/H1N1pdm | 2009/2010 | A/Fukushima/09FY007/2009 | 0.22※ | 3.15 | 10.91※ | 5.79 | 1.92※ | 2.8 | 0.49※ | 2.06 |
| | | A/Fukushima/09FY090/2010 | 0.09 | 1.21 | 3.78※ | 2.01 | 0.49 | 0.72 | 0.2 | 0.83 |
| A/H1N1pdm | 2010/2011 | A/Hyogo/10K291/2011 | 37.09※ | 307.8 | 443.95※ | 323.5 | 1.11 | 1.8 | 0.54※ | 2 |
| | | A/Kyoto/10K124/2011 | 24.85※ | 206.3 | 309.79※ | 225.8 | 0.85 | 1.4 | 0.4 | 1.5 |
| | | A/Kyoto/10K073/2011 | 0.34※ | 2.8 | 3.80※ | 2.8 | 1.51※ | 2.4 | 0.66※ | 2.4 |
| | | A/Nagasaki/10N073/2011 | 0.16 | 1.3 | 1.86 | 1.4 | 0.63 | 1 | 0.53※ | 1.9 |
| | | A/Kyoto/10K070/2011 | 0.22 | 1.8 | 2.57 | 1.9 | 1.79※ | 2.8 | 0.46 | 1.7 |
| A/H3N2 | 2010/2011 | A/Nagasaki/10N017/2011 | 0.14 | 0.9 | 2.12※ | 2.9 | 0.77 | 1.2 | 0.3 | 0.5 |
| | | A/Niigata/10F010/2011 | 0.2 | 1.2 | 0.68 | 0.9 | 2.66※ | 4.2 | 1.09 | 1.7 |
| B | 2010/2011 | はずれ値なし | | | | | | | | |

[a]増加率は各型/亜型における各薬剤の平均 $IC_{50}$ 値と各株の $IC_{50}$ 値により算出。
※は各型・亜型に対する 75%タイル＋3.0IQR 以上のはずれ値。

表 4　2009/2010 年シーズン,2010/2011 年シーズンの NA 阻害試験ではずれ値を示したウイルス分離株とオリジナル検体の NA のアミノ酸変異

| 型/亜型 | シーズン | 株名 | 臨床検体　NA 変異 | 分離株　NA 変異 |
|---|---|---|---|---|
| H1N1pdm | 2009/2010 | A/Fukushima/09FY007/2009 | I222T | I222T |
| | | A/Fukushima/09FY090/2010 | M241I | M241I |
| H1N1pdm | 2010/2011 | A/Hyogo/10K291/2011 | H274Y,E51G | H274Y,E51G |
| | | A/Kyoto/10K124/2011 | H274Y.T384A | H274Y,S62X,V176G,R419X |
| | | A/Kyoto/10K073/2011 | I240V,V263I,F322L,I362V, K372N,N401K,S443I | I240V,V263I,F322L,I362V, K372N,N401K,S443I |
| | | A/Nagasaki/10N073/2011 | なし | なし |
| | | A/Kyoto/10K070/2011 | P120L, N221D | P120L, N221D |
| H3N2 | 2010/2011 | A/Nagasaki/10N017/2011 | なし | D151G |
| | | A/Niigata/10F010/2011 | I30L | 148T/I |
| B | 2010/2011 | はずれ値なし | | |

N2 ナンバリングに基づいてアミノ酸配列を表記。

図 1　2009/2010 年シーズンに分離したインフルエンザ A/H1N1pdm に対するペナミビル三水塩、オセルタミビル

A. インフルエンザ A/H1N1 pdm
( 75%+3IQR)

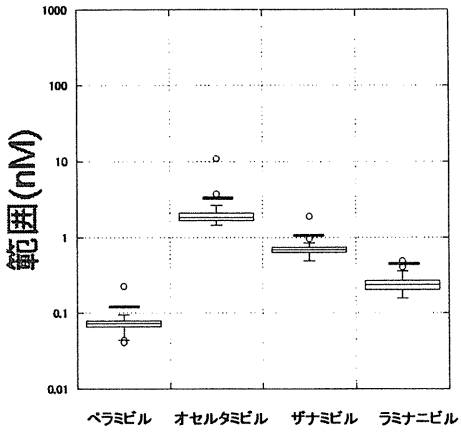
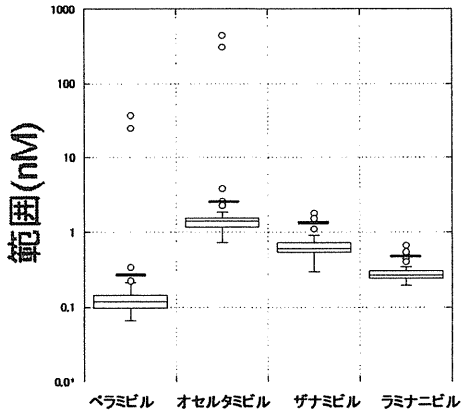
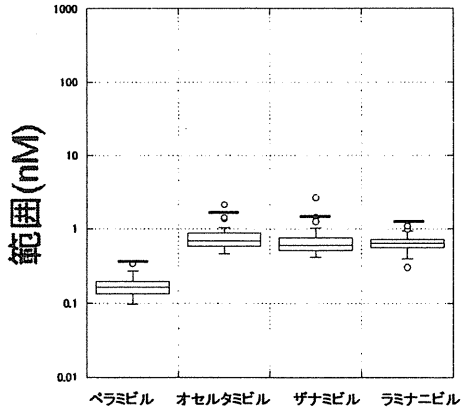



図 2　2010/2011 年シーズンに分離したインフルエンザ A/H1N1pdm,A/H3N2,B 型に対するペラミビル三水和物、オセルタミビルカルボン酸、ザナミビル水和物、ラニナミビル $IC_{50}$ 値の箱ひげ図

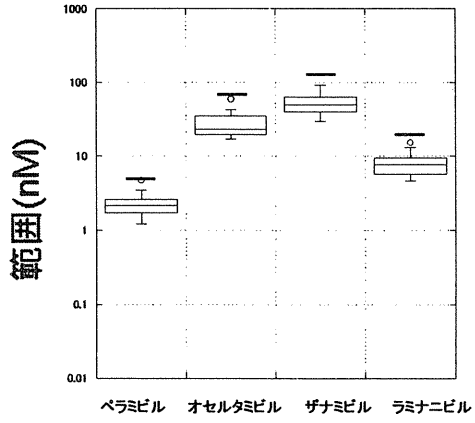
A. インフルエンザ A/H1N1 pdm
( 75%+3IQR)




B. インフルエンザ A/H3N2
( 75%+3IQR)




C. インフルエンザ B
(75%+3IQR)



研究成果の刊行に関する一覧表

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名 | 書　籍　名 | 出版社名 | 出版地 | 出版年 | ページ |
|---|---|---|---|---|---|---|---|
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雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
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# 研究成果の刊行物

# インフルエンザ診療マニュアル 2011−2012年シーズン版

**Manual for Influenza in the 2011–2012 Influenza Season**

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## はじめに

2009年に初めて出現し同年秋に大流行した新型AH1N1（以下，H1N1 2009）は2010−2011年シーズンには季節性と同じ冬期に流行し，このシーズンはH3N2（香港型）やB型も流行する混合流行であった。また2010−2011年シーズンは従来のオセルタミビルリン酸塩（以下，オセルタミビル）とザナミビル水和物（以下，ザナミビル）に加えてペラミビル水和物（以下，ペラミビル）とラニナミビルオクタン酸エステル水和物（以下，ラニナミビル）の4種類のノイラミニダーゼ阻害薬（NAI）が初めてフルシーズン使用された。2011−2012年シーズンも引き続きH1N1 2009やH3N2等の流行が予想され，治療も上記のNAI4剤が中心になると思われる。日本臨床内科医会（日臨内）のインフルエンザ診療マニュアルもこれらNAIの比較を含めて2011−2012年版（第6版）が発行された[1]。ここではその要点を述べる。

## インフルエンザの流行状況

2009−2010年シーズンはH1N1 2009単独の流行であったが，2010−2011年シーズンはこれにH3N2，B型が加わった混合流行であり（**図1**），流行ピークは1〜3月であった。また20歳未満（特に小児）ではH3N2やBの流行が目立ったが，成人はH1N1 2009が流行の主体であった[2]。2011−2012年シーズンもA型ではH1N1 2009とH3N2の両亜型が引き続き流行する可能性が高い。

## 迅速診断

2010−2011年シーズンに日臨内解析（以下，本研究）により培養，PCRと比較して検討したキットの陽性試験予測率はA型89.7％，B型96.7％と高く，キットの診断能は良好であった。現在H1N1 2009に特異的な抗体を用いた診断キット，H1N1 2009とH3N2の鑑別キットなども利用可能である。

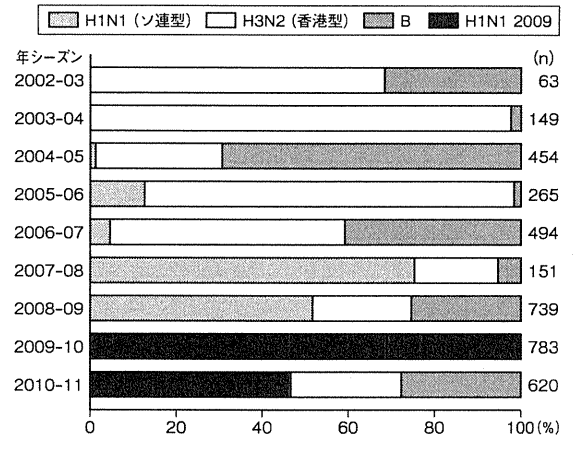


**図1　過去9シーズンのインフルエンザの型・亜型別頻度**[1]

## ワクチン

H1N1 2009一色の流行であった2009−2010年の本研究ではH1N1 2009単価ワクチンの有効率は70％前後と高かった。しかし2010−2011年シーズンはH3N2，Bのワクチン接種後の抗体価上昇はやや悪く（本研究でワクチン接種後の40倍以上のHI抗体価保有率はH1N1 2009が62.2％，H3N2が40.5％，Bが37.8％），H1N1 2009が流行の主体であった成人以外の有効性はやや低かったが，安全性は従来同様高かった。なお2011−2012年シーズンのワクチン株は前シーズンと同じだが小児の接種量が変更されており留意が必要である。

## 抗インフルエンザ薬

2008−2009年シーズンにH1N1（ソ連型）はH275Y変異のためほぼ100％オセルタミビル耐性型となり同薬の有効性が特に小児で低下したが[3]，H1N1 2009の出現とともにH1N1（ソ連型）は消えている。2010−2011年シーズンの本研究でA型の有効性はペラミビル以外の3剤はほぼ同等であった（**図2**）。またB型では各薬剤ともにA型よりも有効性が低いが，その中ではザナミビルがやや有効性が高い傾向がみられた（**図2**）。A亜型別の有効性は各NAIともにH3N2よりもH1N1 2009の方が高い傾向にあった[1]。NAI以外のアマンタジン塩酸塩はH3N2，H1N1 2009とも耐性型のため推奨されず，RNAポリメラーゼ阻害薬のファビピラビルも現時点で未承認である。以下，NAI4剤について**表1**で比較するとともに要点を記す。

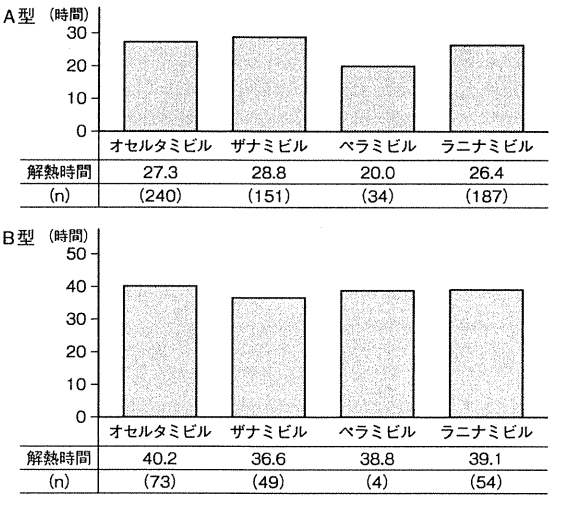


**図2　迅速診断のA型およびB型における抗インフルエンザ薬別の平均解熱時間**[1]

表1　ノイラミニダーゼ阻害薬の種類

| | オセルタミビル[1] | ザナミビル[2] | ペラミビル | ラニナミビル |
|---|---|---|---|---|
| 剤形（対象） | 内服薬<br>Cap：成人・小児≧37.5kg<br>DS：1歳以上 | 吸入薬<br>（成人，小児） | 点滴注射薬<br>（成人，小児） | 吸入薬<br>（成人，小児） |
| 用法・用量 | Cap：1回1Cap（75mg）<br>DS：1回2mg/kg<br>（1回最高用量は75mg）<br><br>1日2回<br>5日間内服 | 1回10mg<br>（5mgブリスターを2個）<br><br>1日2回<br>5日間吸入 | 成人：300mg（2バイアルまたは1袋）<br>小児：10mg/kg（1バイアルまたは1袋）<br><br>単回点滴または複数回点滴[3] | 10歳以上：40mg（20mgを2容器）<br>10歳未満：20mg（20mgを1容器）<br><br>単回吸入のみ |
| 予防投薬の適用 | 1CapまたはDS2mg/kg<br>（1回最高用量75mg） | 1日1回10mg<br>（5mgブリスターを2個） | 未承認 | 未承認 |
| 副作用 | 胃腸障害等 | まれ | 下痢 | まれ |
| 耐性ウイルス | H1N1ソ連型はほぼ100％耐性<br>H1N1 2009は低頻度 | まれ | H275Y変異株での感受性低下報告あり | まれ |

その他の注意事項　1）10歳以上の未成年においては，原則使用不可
2）4歳以下の安全性は確立していない
3）合併症等により重症化する恐れのある患者では600mg（4バイアルまたは2袋）まで可。なお本薬は腎排泄のため，腎機能障害患者では投与量の調整を要する

文献1）より一部改変

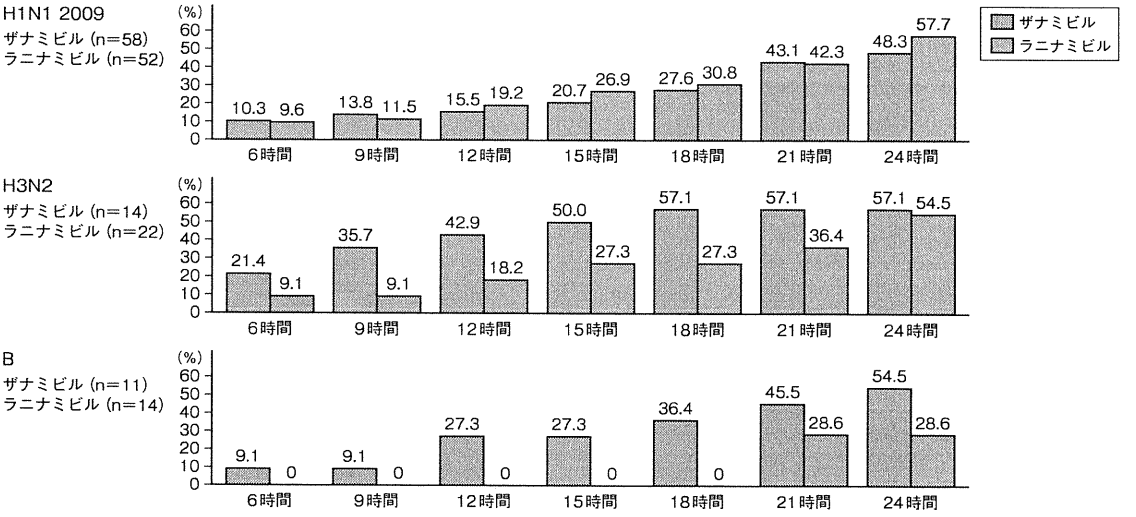


**図3　ザナミビルとラニナミビル投与開始3時間ごとの解熱症例率**[1]

### 1．オセルタミビル

内服薬で10歳以上の未成年ではハイリスク者を除いて原則使用禁止。H1N1 2009もH275Y変異が起きると本薬の有効性低下が懸念されるが，現状では耐性化率が1～2％と低く有効性は高い。2010－2011年も吸入困難な4歳以下の小児を中心に10歳未満で最もよく使用された[1,2]。

### 2．ザナミビル

吸入薬で全身への影響や耐性ウイルスの報告は少なく，H275Y変異を含めいずれの型・亜型にも有効で安全性も高い。患者調査では，吸入器の操作や吸入も，小児でも比較的容易であった。2010－2011年シーズンの本研究では10代で最も高頻度に使われた[2]。本薬剤はプロドラッグでなく，活性物質そのものであり，高濃度でウイルス増殖部位に直接作用するため即効性が期待される。ラニナミビルとの比較研究[4]で投与開始48時間の時点では有効性に大差なかったが24時間以内の早期ではH3N2やB型で臨床効果の出現が早い可能性が示唆された（**図3**）。

### 3．ペラミビル

米国より導入された長時間作用型の注射薬。薬効は1回点滴でオセルタミビル5日間投与に匹敵するとされ，A，B型の他に高病原性鳥インフルエンザウイルスH5N1にも強い抗ウイルス活性を示す。成人300mg，小児10mg/kgを15分以上かけて単回投与するが重症化の恐れがある場合はともに1日1回600mgまでの使用や連日の投与が可能。H275Y変異株で感受性低下が報告されているが，経口や吸入が困難な症例等で使用する意義が高い。

### 4．ラニナミビル

純国産で長時間作用型の吸入薬（プロドラッグ）。発症後1回投与で気道や肺に長時間貯留し，5日間投与のオセルタミビルと同等の薬効を示す。H275Y変異型にも有効だが，入院重症例では第一選択とはなりにくい。1回完結型のため，外来患者の利便性は高く症状改善による服薬中止の心配もない。H1N1 2009では有効性が高く（**図3**），2010－2011年シーズンの本研究でも多忙な成人でよく使用された。

## おわりに

本稿は『インフルエンザ診療マニュアル2011－2012年シーズン版』を抜粋したが詳細は同マニュアル（日本臨床内科医会事務局，電話：03-3259-6111で入手可能）を参照されたい。


**（文　献）**

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# Systemic Corticosteroids and Early Administration of Antiviral Agents for Pneumonia with Acute Wheezing due to Influenza A(H1N1)pdm09 in Japan

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## Abstract

***Background:*** Pneumonia patients with wheezing due to influenza A(H1N1)pdm09 were frequently treated with systemic corticosteroids in Japan although systemic corticosteroid for critically ill patients with pneumonia caused by influenza A(H1N1)pdm09 has been controversial. Applicability of systemic corticosteroid treatment needs to be evaluated.

***Methods/Principal Findings:*** We retrospectively reviewed 89 subjects who were diagnosed with influenza A(H1N1)pdm09 and admitted to a national hospital, Tokyo during the pandemic period. The median age of subjects (45 males) was 8 years (range, 0–71). All subjects were treated with antiviral agents and the median time from symptom onset to initiation of antiviral agents was 2 days (range, 0–7). Subjects were classified into four groups: upper respiratory tract infection, wheezing illness, pneumonia with wheezing, and pneumonia without wheezing. The characteristics of each group was evaluated. A history of asthma was found more frequently in the wheezing illness (55.6%) and pneumonia with wheezing (43.3%) groups than in the other two groups ($p = 0.017$). Corticosteroid treatment was assessed among subjects with pneumonia. Oxygen saturation was lower in subjects receiving corticosteroids (steroid group) than in subjects not receiving corticosteroids (no-steroid group) ($p<0.001$). The steroid group required greater oxygen supply than the no-steroid group ($p<0.001$). No significant difference was found by the Kaplan-Meier method between the steroid and the no-steroid groups in hours to fever alleviation from the initiation of antiviral agents and hospitalization days. In logistic regression analysis, wheezing, pneumonia and oxygen saturation were independent factors associated with using systemic corticosteroids.

***Conclusion:*** Patients with wheezing and a history of asthma were frequently found in the study subjects. Systemic corticosteroids together with early administration of antiviral agents to pneumonia with wheezing and possibly without wheezing did not result in negative clinical outcomes and may prevent progression to severe pneumonia in this study population.

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## Introduction

Although systemic corticosteroid treatment for severe pneumonia due to influenza A(H1N1)pdm09 has been controversial [1,2,3], systemic corticosteroid treatment in pneumonia patients especially presenting with acute wheezing induced by influenza A(H1N1)pdm09 was frequently administered at the early stage of their illness in hospitals in Japan during pandemic period. Wheezing is the end result of a narrowing of the intrathoracic airways and a limitation of expiratory air flow and is caused by many illnesses. Asthma and bronchiolitis were the main illnesses which caused wheezing in influenza A(H1N1)pdm09 virus infection [4,5,6,7,8]. Acute exacerbation of asthma is usually diagnosed in patients with wheezing and a history of asthma. It is treated with anti-asthma agents as well as systematic corticosteroids depending on the disease severity following the asthma treatment guidelines [9,10,11]. On the other hand, a previous study in preschool children with acute virus-induced wheezing indicated that systemic corticosteroid treatment was not superior to placebo [12]. Also, a study in infants with bronchiolitis concluded that treatment with systemic corticosteroid did not significantly affect hospitalization [13]. It has been physicians' questions whether pneumonia patients presenting with wheezing need to be treated with systemic corticosteroid during the pandemic period.

The aim of the present study was to evaluate if systemic corticosteroid treatment is suitable for hospitalized pneumonia patients with acute wheezing caused by influenza A(H1N1)pdm09.

## Materials and Methods

### Study design

We retrospectively reviewed the clinical data, chest radiologic and laboratory findings of all hospitalized patients diagnosed with

pandemic influenza A(H1N1)pdm09, admitted between August 2009 and March 2010 to the National Center for Global Health and Medicine (NCGM), which is a tertiary care hospital in Tokyo, Japan. Influenza A(H1N1)pdm09 infection was diagnosed according to case definitions developed by the World Health Organization [14]. Respiratory tract specimens of patients were either tested positive for the influenza A(H1N1)pdm09 virus by real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) or tested positive for influenza A virus by ImunoAce Flu® (TAUNS Laboratories, Inc.) or Espline® (Fujirebio Inc.), rapid diagnosis tests using an immunochromatography assay, which are approved by the Ministry of Health, Welfare, and Labour, Japan. Among all hospitalized patients, subjects who presented with respiratory disorders were eligible as study subjects and classified into four groups based on their respiratory disorders: upper respiratory tract infection, wheezing illness, pneumonia with wheezing, and pneumonia without wheezing (Figure 1). The four groups were compared and evaluated in terms of the relationships among clinical conditions, clinical time course, and treatments. The clinical effects for systemic corticosteroids treatment were evaluated among the subjects with pneumonia. Also, clinical factors which leaded to prescribe systemic corticosteroids were assessed among the study subjects. Systemic corticosteroid was administered based on the treatment for acute exacerbation of asthma in the asthma guidelines [9,10,11]. Wheezing was defined as a continuous high pitched sound emitting from the chest during expiration on auscultation. Pneumonia was diagnosed on the basis of infiltrative shadows on chest radiograph.

### Statistical analysis

Subjects' background data and clinical laboratory values were summarized and compared among groups of respiratory disorders as well as between those who did (steroid group) and did not (no-steroid group) receive systemic corticosteroid treatment. The Mann-Whitney test and Kruskal-Wallis test were used for continuous variables, and the Chi-square test and Fisher's exact test were used for categorical variables. Survival curves on the numbers of hours to fever alleviation from initiation of administration of antiviral agents and the duration of hospitalization in the steroid and the no-steroid groups were analyzed by the Kaplan-Meier method and comparisons were made using the log-rank test. For the evaluation of independent factors for using systemic corticosteroid treatment, a step-wise selection method was used to select significant factors if $p<0.1$ in the univariate analysis for a logistic regression analysis. Data analyses were conducted using SPSS statistics ver.19 (IBM, Armonk, NY, USA). For all analyses, significance levels were two tailed, and a p value of $<0.05$ was considered significant.

### Ethics statement

The study was approved by the Institutional Review Board of the NCGM. Informed consent was waived by the Board for this retrospective study, with the study notification to public being made by posters. Investigators kept the datasets in password-protected systems and presented data with the anonymity of study patients retained.

## Results

### Characteristics of the study subjects

During the study period, a total 104 patients were diagnosed with influenza A(H1N1)pdm09 and admitted to the NCGM. Among them, 89 (85.6%) patients who presented with respiratory disorders were eligible as study subjects (Figure 1). Some subjects were admitted with reasons other than respiratory disorders including encephalopathy, dehydration, and abdominal symptoms due to influenza infection.

The number of subjects in each category of respiratory disorders was as follows: upper respiratory tract infection (n = 22, 24.7%); wheezing illness (n = 9, 10.1%); pneumonia with wheezing (n = 30, 33.7%); and pneumonia without wheezing (n = 28, 31.5%). Of all 89 subjects, the number of subjects with pneumonia was 58 (65.2%).

The characteristics of subjects according to respiratory disorders are shown in Table 1. The median age of study subjects (45 male) was 8 years (range, 0–71), and 80 subjects (89.9%) were aged less than 15 years. More subjects with wheezing illness (55.6%) or pneumonia with wheezing (43.3%) had a history of asthma than did those with upper respiratory tract infection (13.6%) or pneumonia without wheezing (17.9%), and there were significant differences among the groups ($p = 0.017$). The median oxygen saturation ($SpO_2$) in room air on admission in subjects with wheezing illness (91.0%) or pneumonia with wheezing (90.0%) were lower than those in subjects with upper respiratory tract infection (96.5%) or pneumonia without wheezing (93.0%), and there were significant differences among the groups ($p<0.001$). Bacterial co-infection was detected in throat swabs and/or sputum in 44.9% of subjects, but there was no significant difference among the groups. In terms of laboratory findings, including total serum Immunoglobulin E level, there were no significant differences among the groups.

### Treatment and clinical time course of study subjects

The treatments and clinical time courses of study subjects in each classified group of respiratory disorders during hospitalization are shown in Table 2.

All subjects were treated with antiviral agents, either oseltamivir or zanamivir. In some subjects antiviral medication was switched from oseltamivir to zanamivir or vice versa. The regular dose of oseltamivir was 150 mg/day for 5 days in adults, and 4 mg/kg/day for 5 days in pediatric patients. The regular dose of zanamivir was 20 mg/day for 5 days. The median number of days from symptom onset to initiation of administration of antiviral agents was 1.9 (range, 1–7), and the length to antiviral treatment from the symptom onset in the pneumonia with wheezing group tended to be longer (2.4 days; range, 1–5) ($p = 0.054$).

Systemic corticosteroid was used in 93.3% of pneumonia with wheezing subjects, 77.8% of wheezing illness subjects, and 64.3% of pneumonia without wheezing subjects ($p<0.001$). The dosage of corticosteroids was equivalent to methylprednisolone 1.0–1.5 mg/body weight (kg)/time, 2–4 times/day, in subjects under 15 years of age, and 40–80 mg/time, 2–4 times/day in those over 15 years of age. The median number of days from symptom onset to initiation of administration of systemic corticosteroids was 2.1 (range, 1–6), The median duration of systemic corticosteroid treatment was 5.2 days (range, 2–9).

Treatment with anti-asthmatic agents other than corticosteroids were included in drug regimens for asthmatic episodes, cough and sputum using at least one of the following: short-acting $\beta_2$-agonist, long-acting $\beta_2$-agonist, inhaled isoproterenol, inhaled disodium cromoglycate, aminophylline, and leukotriene receptor antagonist. All subjects in wheezing illness and pneumonia with wheezing groups received anti-asthma treatments; also, 27.3% of those with respiratory tract infections and 75.0% of those with pneumonia without wheezing had at least one administration with anti-asthma agent ($p<0.001$).

Oxygen was administered using a nasal cannula or face mask to 56.2% of subjects with respiratory disorders, but no subjects required mechanical ventilation.

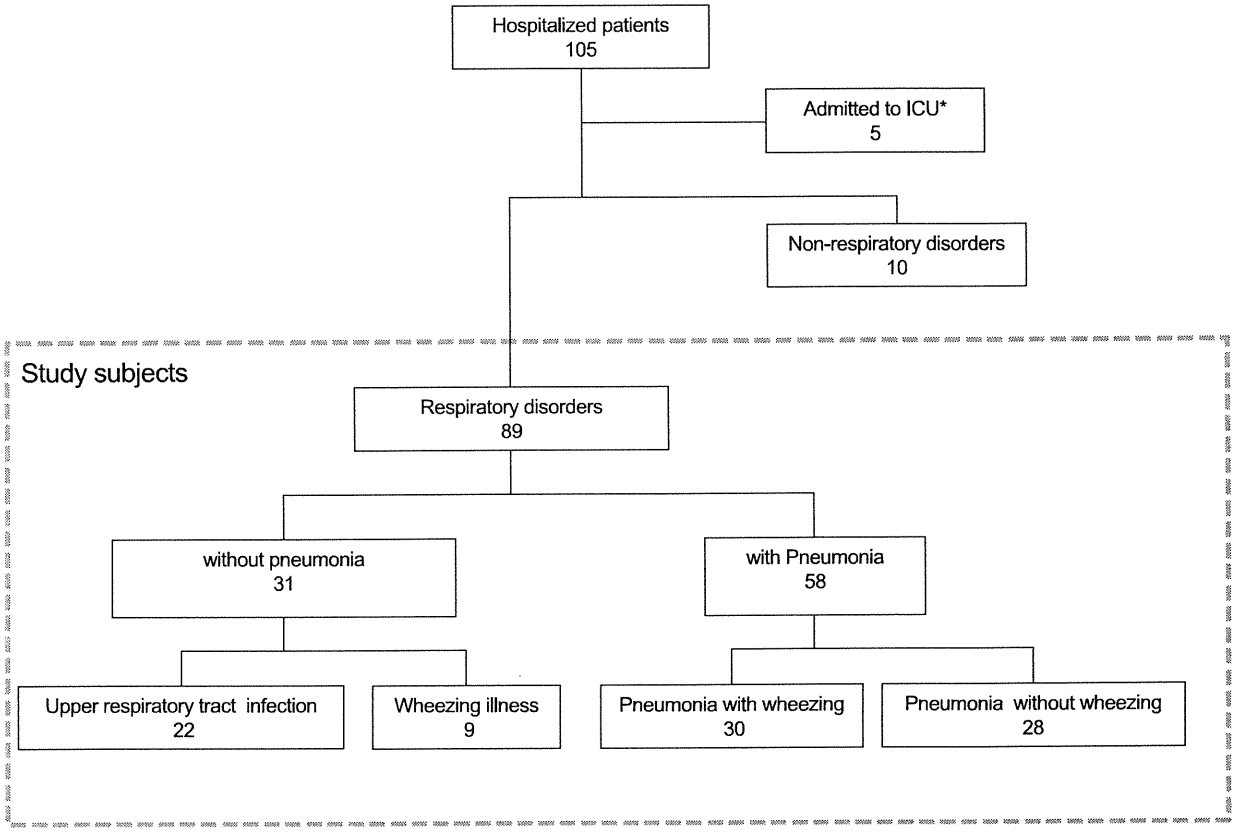


**Figure 1. Study population.** A total of 104 patients were diagnosed with pandemic influenza A(H1N1)pdm09. Five patients (one of whom died) were admitted to the ICU and were excluded from the study. The remaining 99 patients were the study subjects. Among them, 89 subjects presented with respiratory disorders and 10 presented with symptoms other than respiratory disorders, including encephalopathy. The subjects with respiratory disorders were classified into the following four groups: upper respiratory tract infection (n = 22), wheezing illness (n = 9), pneumonia with wheezing (n = 30), and pneumonia without wheezing (n = 28). The total number of subjects with pneumonia was 58.
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The time to fever alleviation from the initiation of administration of antiviral agents was not significantly different among the groups ($p = 0.967$). There was a longer duration of hospitalization in the pneumonia groups with and without wheezing compared with the other two groups, and there was significant difference among the groups ($p<0.001$).

## Evaluation of systemic corticosteroid treatment among subjects with pneumonia

Systemic corticosteroid treatment was evaluated in subjects with pneumonia (n = 58) and compared between subjects in the steroid group and in the no-steroid group (Table 3).

Wheezing was presented 58.7% in the steroid group and there was a significant difference between the groups ($p = 0.002$). $SpO_2$ in the steroid group was lower than that in the no-steroid group (SpO2, 90.0% vs. 95.6, respectively; $p<0.001$) and the steroid group required more oxygen supply than the no-steroid group (97.8% vs. 8.3%, respectively; $p<0.001$). Anti-asthma treatment was applied to 97.8% of the steroid group and 50.0% of the no-steroid group ($p<0.001$). Although bacterial co-infection was found in 52.2% of the steroid group and 25.0% of the no-steroid group at the time of admission ($p = 0.093$), antibiotics were administered to both the steroid and the no-steroid groups (89.1% vs. 50.0%, respectively; $p = 0.006$). There were no significant differences in terms of time to fever alleviation (<37°C) after administration of antiviral agents and in the duration of hospitalization between the groups ($p = 0.611$ and 0.599, respectively).

Clinical time course were assessed by the Kaplan-Meier method on time to fever alleviation from the initiation of administration of antiviral agents and duration of hospitalization in subjects with pneumonia (n = 58) and compared between the steroid and the no-steroid groups using the log-rank test (Figure 2). There were no significant differences between the groups in both time to fever alleviation ($p = 0.835$) and the duration of hospitalization ($p = 0.626$).

## Clinical factors for using systemic corticosteroids treatment among the study subjects

A multiple logistic regression analysis using baseline factors was conducted for subjects with respiratory disorders (n = 89). Wheezing, pneumonia and $SpO_2$ on admission were independent clinical factors associated with using systemic corticosteroids treatment (Table 4).

## Discussion

Our evaluation of hospitalized patients with pneumonia caused by influenza A(H1N1)pdm09, who were mostly young and

**Table 1.** Background and clinical characteristics of study subjects.

| | Upper respiratory tract infection | Wheezing* illness | Pneumonia† with wheezing | Pneumonia without wheezing | Total | P value |
|---|---|---|---|---|---|---|
| **Number of patients (%)** | 22 (24.7) | 9 (10.1) | 30 (33.7) | 28 (31.5) | 89 (100.0) | 0.007 |
| **Gender, male (%)** | 10 (45.5) | 9 (100.0) | 17 (56.7) | 9 (32.1) | 45 (50.6) | 0.004 |
| **Age-yr.** | | | | | | 0.143 |
| <15 | 18 (81.8) | 8 (88.9) | 30 (100.0) | 24 (85.7) | 80 (89.9) | |
| ≥15 | 4 (18.2) | 1 (11.1) | 0 (0.0) | 4 (14.3) | 9 (10.1) | |
| **Vaccination** | | | | | | |
| Seasonal influenza vaccine of 2009–2011 | 3 (13.6) | 2 (22.2) | 7 (23.3) | 5 (17.9) | 17 (19.1) | 0.956 |
| Influenza A(H1N1) pdm09 vaccine | 0 (0.0) | 5 (27.8) | 3 (16.7) | 2 (33.3) | 10 (18.5) | 0.145 |
| **Comorbidity** | | | | | | |
| Asthma‡ | 3 (13.6) | 5 (55.6) | 13 (43.3) | 5 (17.9) | 26 (29.2) | 0.017 |
| Others§ | 1 (4.5) | 1 (11.1) | 0 (0.0) | 1 (3.6) | 3 (3.4) | 0.236 |
| **Family asthma history** | 4 (18.2) | 3 (33.3) | 13 (43.3) | 5 (17.9) | 25 (28.1) | 0.107 |
| **Physical findings** | | | | | | |
| Body temperature °C, median (range) | 38.5 (35.9–40.4) | 38.6 (37.2–38.8) | 38.6 (36.5–40.3) | 38.6 (36.2–40.2) | 38.6 (35.9–40.4) | 0.729 |
| $SpO_2$¶ -%, median (tange) | 96.5 (87–98) | 91.0 (86–97) | 90.0 (82–97) | 93.0 (74–98) | 92.0 (74–98) | <0.001 |
| Co-infection‖ - No. (%) | 6 (27.3) | 7 (77.8) | 14 (46.7) | 13 (46.4) | 40 (44.9) | 0.081 |
| **Laboratory findings – median (range)** | | | | | | |
| WBC ($10^3/\mu L$) | 6730 (3260–13980) | 15810 (6100–13450) | 8000 (2790–16280) | 6820 (900–15580) | 7740 (900–16280) | 0.056 |
| Hemoglobin (g/dL) | 13.3 (10.2–16.8) | 13.2 (12.0–17.5) | 13.4 (4.9–14.9) | 13.4 (10.7–15.6) | 13.4 (4.9–17.5) | 0.911 |
| Platelet ($10^3/\mu L$) | 20.3 (8–39) | 26.6 (17–44) | 23.8 (14–193) | 23.2 (12–135) | 22.9 (8–193) | 0.103 |
| LDH (U/L) | 240.5 (168–407) | 287.0 (239–397) | 270.5 (218–418) | 264.5 (183–438) | 265.0 (168–438) | 0.057 |
| ALP (U/L) | 513.5 (7–1173) | 748 (240–1091) | 620 (449–1008) | 603 (123–756) | 614.0 (7–1173) | 0.224 |
| AST (U/L) | 28.0 (16–79) | 31.0 (25–50) | 29.0 (19–45) | 27.0 (21–100) | 29.0 (16–100) | 0.235 |
| ALT (U/L) | 15.0 (8–33) | 18.0 (14–33) | 13.5 (10–34) | 14.5 (8–70) | 15.0 (8–70) | 0.016 |
| Creatinine (mg/dL) | 0.44 (0.22–1.01) | 0.3 (0.21–1.03) | 0.35 (0.18–0.91) | 0.41 (0.26–2.69) | 0.40 (0.18–2.69) | 0.087 |
| Sodium (mEq/L) | 135.0 (129–141) | 136 (133–138) | 135 (130–141) | 135.5 (126–140) | 135.0 (126–141) | 0.364 |
| Potassium (mEq/L) | 3.9 (3.4–5.2) | 4.1 (3.5–5.0) | 4.0 (3.4–4.6) | 4.0 83.3–4.4) | 4.0 (3.3–5.2) | 0.698 |
| CRP (mg/dL) | 0.91 (0–11) | 0.83 (0.19–2.06) | 1.91 (0.05–9.23) | 1.0 (0.07–10.41) | 1.17 0.0–11.04) | 0.271 |
| Total serum IgE (U/mL) | 101.0 (21–6691) | 74.0 (3–382) | 473.5 (1–9179) | 283.0 (25–3440) | 243.0 (1–9179) | 0.164 |

*Wheezing was defined as a continuous high pitched sound emitting from the chest during expiration on auscultation.
†Pneumonia was diagnosed on the basis of infiltrative shadows on chest radiograph.
‡Asthma includes active asthma and inactive asthma.
§Other comorbidities include smoking , alcoholism, diabetis meritis, chronic heart diseases , obesity.
¶$SpO_2$: oxygen saturation measured by pulse oximetry in room air.
‖Pathogenic bacteria co-infection was detected by throat swabs and/or sputum.
Definition of abbreviations: WBC, white blood cell count; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; AST, aspartate amino transferase; ALT, alanine aminotransferase; CRP, C-reactive protein; IgE, Immunoglobulin E.
doi:10.1371/journal.pone.0032280.t001

presenting with wheezing, revealed that early systemic corticosteroid treatment did not result in negative clinical outcomes if patients were treated with antiviral agents during the early stage of illness.

Although asthma is not the only illness that causes wheezing, asthma is a risk comorbidity for influenza A(H1N1)pdm09 [8]. In the present study, wheezing was observed in 43.8% of all subjects, including in subjects who presented with pneumonia (Table 1). Systemic corticosteroid treatment is recommended in the asthma guidelines for treating acute exacerbation of asthma which requires hospitalization [9,10,11], but its use remains uncertain for asthma-exacerbated patients with pneumonia due to influenza A(H1N1)pdm09 [1,2,3].

The healthcare seeking behavior for people in Japan is customarily early especially for acute diseases including pandemic influenza and a median days to the initiation of treatment with antiviral agents from the symptom onset was 1.9 days (range, 1–7) in the study subjects (Table 2). The previous study in Mexico reported that the earlier administration of antiviral agent reduced severity of pneumonia, occurrence of pneumonia, and the duration of hospitalization [15]. In the present study, the study subjects were not admitted in the ICU, did not require mechanical ventilation support, and the median of duration of hospitalization was 7 days (range, 2–14) (Table 1). These results indicated that the study subjects, who were mostly young and were initiated the

**Table 2.** Treatment and clinical time course of study subjects.

| | Upper respiratory tract infection | Wheezing* illness | Pneumonia†¶ with wheezing | Pneumonia without wheezing | Total | P value |
|---|---|---|---|---|---|---|
| **Number of subjects (%)** | 22 (24.7) | 9 (10.1) | 30 (33.7) | 28 (31.5) | 89 (100.0) | 0.007 |
| **Treatments** | | | | | | |
| Time to initiation of antiviral agents from symptom onset - median days (range) | 1.8 (1–3) | 1.7 (1–3) | 2.4 (1–5) | 1.6 (1–7) | 1.9 (1–7) | 0.054 |
| **Antiviral agents** | | | | | | 0.006 |
| Oseltamivir | 12 (54.5) | 7 (77.8) | 22 (73.3) | 12 (42.9) | 53 (59.6) | |
| Zanamivir | 9 (40.9) | 2 (22.2) | 7 (23.3) | 13 (46.4) | 31 (34.8) | |
| Both oseltamivir and zanamivir‡ | 1 (4.5) | 0 (0.0) | 1 (3.3) | 3 (10.7) | 5 (5.6) | |
| **Systemic corticosteroid treatment§** | 4 (18.2) | 7 (77.8) | 28 (93.3) | 18 (64.3) | 57 (64.0) | <0.001 |
| Time to initiation of systemic corticosteroids from symptom onset - median days (range) | 2.0 (2–2) | 2.0 (1–5) | 2.4 (1–6) | 1.8 (1–5) | 2.1 (1–6) | 0.134 |
| Duration of systemic corticosteroid treatment-median days (range) | 5.0 (4–6) | 3.3 (2–6) | 5.8 (3–9) | 4.4 (2–8) | 5.2 (2–9) | <0.001 |
| Anti-asthma agents other than corticosteroid¶ | 6 (27.3) | 9 (100.0) | 30 (100.0) | 21 (75.0) | 66 (74.2) | <0.001 |
| Oxygen supply‖ | 7 (31.8) | 5 (55.6) | 19 (63.3) | 19 (67.9) | 50 (56.2) | 0.002 |
| Antibiotics** | 8 (36.4) | 8 (88.9) | 28 (93.3) | 19 (67.9) | 63 (70.8) | <0.001 |
| **Clinical time course** | | | | | | |
| Time to fever alleviation* - hours, median (range) | 35.4(11–120) | 44.0 (14–116) | 32.0 (12–150) | 37.3 (9–168) | 35.0 (9–168) | 0.967 |
| Length of Hospitalization, days, median (range) | 4.9 (2–9) | 6.8 (3–10) | 8.4 (6–14) | 7.6 (3–14) | 7.5 (2–14) | <0.001 |

*Wheezing was defined as a continuous high pitched sound emitting from the chest during expiration on auscultation.
†Pneumonia was diagnosed on the basis of infiltrative shadows on chest radiograph.
‡Antiviral medication was switched oseltamivir to zanamivire and vice versa.
§The dose of corticosteroid was equivalent to methylprednisolone 1.0–1.5 mg/body weight (kg)/time, 2–4 times/day, in subjects under 15 years of age, and 40–80 mg/time, 2–4 times/day in those over 15 years of age.
¶At least one medication of shortacting β2-agonist, longacting β2-agonist, inhaled isoproterenol, inhaled disodium cromoglycate, aminophylline, and leukotriene receptor antagonists.
‖Oxygen was administered using a nasal cannula or face mask.
**Antibiotics.
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treatment with antiviral agents earlier, did not progress to be critical.

Most subjects presented with respiratory disorders, including upper respiratory tract infection, wheezing illness, and pneumonia with or without wheezing. Wheezing is one of the manifestations of asthma and a previous report indicated that wheezing is associated with influenza mortality [16]. In the present study, total 43.8% of subjects presented wheezing and 33.7% presented both pneumonia and wheezing. Also, asthma history was more frequent among subjects with wheezing illness (55.6%) or pneumonia with wheezing (43.3%) than among the other subjects in upper respiratory infection (13.6%) and pneumonia without wheezing (17.9%) (Table 1). The results indicated that the occurrence of exacerbation of asthma might have been increased by influenza A(H1N1)pdm09 in this study population; however, exacerbation of asthma did not became critical. Systemic corticosteroid was mainly used for patients with wheezing either with pneumonia or without pneumonia. The presentation of wheezing can lead to a diagnosis of asthma if the patients have a history of asthma. Also, wheezing could also indicate a first episode of asthma attack or bronchiolitis, which are difficult to distinguish on the basis of wheezing alone. If a patient has asthma acute exacerbation or a first episode of asthma attack, not using systemic corticosteroids can increase disease severity and mortality [9,10,11]. Therefore, systemic corticosteroid treatment should be a consideration for clinical management of patients with wheezing despite the presence of viral pneumonia and/or bronchiolitis. Also, anti-asthmatic agents other than systemic corticosteroids were administered to all subjects with wheezing (Table 2). Treatments with anti-asthmatic agents together with corticosteroids need to be included as well as antibiotic agents in case of bacterial co-infection.

Respiratory condition, as reflected by $SpO_2$, was more severe in subjects of the steroid group than in the non-steroid group (Table 3). Also, oxygen was more supplied to the steroid group. Those results described that respiratory condition was more severe in the steroid group; however, systemic corticosteroid treatment has no influence to hours to fever alleviation after the initiation of treatment with antiviral agents and the duration of hospitalization (Table 3). In terms of assessment of clinical time course, the Kaplan-Meier curves for hours to fever alleviation from the initiation of antiviral agents and hospitalization days were not significantly different between the steroid and the non-steroid groups. (Figure 2). Systemic corticosteroids were administered for the most of subjects with wheezing (Table 2). These results suggests that systemic corticosteroid treatment for viral pneumonia with wheezing may not have negative effects to clinical time course.

Multiple logistic regression analysis among subjects with respiratory disorders evaluated that wheezing, pneumonia, and $SpO_2$ were independent factors associated with using systemic corticosteroid treatment (Table 4). The results indicated that these were factors that could motivate physicians to start systemic

**Table 3.** Clinical presentation of subjects with pneumonia according to systemic corticosteroid treatment.

| | Steroid group* | No-steroid group* | Total | P value |
|---|---|---|---|---|
| **Number of subjects No. (%)** | 46 (100) | 12 (100) | 58 (100) | |
| **Symptoms and signs on admission** | | | | |
| Wheezing† -No. (%) | 27 (58.7) | 1 (8.3) | 28 (48.3) | 0.002 |
| Co-infection‡-No. (%) | 24 (52.2) | 3 (25.0) | 27 (46.6) | 0.093 |
| Body temperature -°C, median (range) | 38.6 (36.5–40.3) | 38.2 (36.2–40.2) | 38.6 (36.2–40.3) | 0.261 |
| **Laboratory findings on admission, median (range)** | | | | |
| $SpO_2$§ (%) | 90.0 (74–97) | 95.6 (91–98) | 91.0 (74–98) | <0.001 |
| WBC ($10^3/\mu L$) | 8200 (2790–16280) | 6385.0 (900–13280) | 7715.0 (900–16280) | 0.024 |
| LDH (U/L) | 270 (201–418) | 255.5 (183–438) | 267.5 (183–438) | 0.687 |
| CRP (mg/dL) | 1.16 (0.05–9.23) | 2.69 (0.07–10.41) | 1.22 (0.05–10.41) | 0.154 |
| Sodium (mEq/L) | 135.1 (130–141) | 134.8 (126–139) | 135 (126–141) | 0.734 |
| Potassium (mEq/L) | 3.96 (3.3–4.6) | 3.39 (3.3–4.5) | 3.95 (3.3–4.6) | 0.438 |
| **Treatment -No. (%)** | | | | |
| Days to administration of antiviral agents¶ | 2.0 (1–5) | 1.8 (1–7) | 2.0 (1–7) | 0.589 |
| Anti-asthma treatments‖ | 45 (97.8) | 6 (50.0) | 51 (87.9) | <0.001 |
| Antibiotic agents | 41 (89.1) | 6 (50.0) | 47 (81.0) | 0.006 |
| Oxygen supply** | 45 (97.8) | 1 (8.3) | 46 (79.3) | <0.001 |
| **Clinical outcomes, median (range)** | | | | |
| Hours to alleviation of fever after admission†† | 36.0 (9–150) | 35.5 (9–168) | 35.5 (9–168) | 0.611 |
| Hospitalization days | 8.2 (5–14) | 7.7(3–14) | 8.1 (3–14) | 0.607 |

N = 58.
*No-steroid and steroid group denote group of subjects who were not treated and treated with systematic corticosteroids.
†Wheezing was defined as a continuous high pitched sound emitting from the chest during expiration on auscultation.
‡Pathogenic bacteria co-infection was detected by throat swabs and/or sputum.
§$SpO_2$: oxygen saturation measured by pulse oximetry in room air.
¶The number of days from symptom onset to the initiation of administration of antiviral agent either oseltamivir or zanamivir.
‖At least one medication of short-acting β2-agonist, long-acting β2-agonist, inhaled isoproterenol, inhaled disodium cromoglycate, aminophylline, and leukotriene receptor antagonists.
**Oxygen was administered using a nasal cannula or face mask.
††The time (hours) to alleviation of fever to less than 37°C after the administration of antiviral agents.
Definition of abbreviation: WBC, white blood cell count; LDH, lactate dehydrogenase; CRP, C-reactive protein.
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corticosteroid treatment at the study site. It also indicated that wheezing was not the only factor for using systemic corticosteroid treatment but also pneumonia and low level of respiratory condition which were reflected by $SpO_2$. In the present study, systemic corticosteroid treatment did not produce negative outcomes, even in patients with pneumonia and might be in patients with bronchiolitis. The results showed that the systemic corticosteroid treatment in the early stage of illness together with antiviral agents might work to reduce the time of critical conditions and to prevent disease progression to severe pneumonia among patients who were administered antiviral agents during the early stage of illness when their pneumonia were not so severe (Table 2).

Although secondary bacterial infection was reported as a negative outcome of systemic corticosteroid treatment in the severe ill patients with influenza H1N1pdm09 [2], in the present study, no significant effects of systemic corticosteroid treatment against bacterial co-infection were observed. It might be resulted from the antibiotics treatment from the hospital admission (Table 2, 3) and can be explained by the short duration of hospitalization of study subjects. (Table 2, 3).

Limitations of the present retrospective study are that the influenza HN1 2009 virus was confirmed in the limited number of subjects by RT-PCR and patients strongly considered to have 2009 influenza A/H1N1 virus infection were included. During the study period, influenza A(H1N1)pdm09 virus was the dominant influenza virus in Japan according to the Infectious Agent Surveillance Report in Japan [17]. Subjects who were identified as having influenza A virus infection were strongly considered to have influenza A(H1N1)pdm09 virus infection, so physicians diagnosed those patients as having influenza A(H1N1)pdm09 infection. Also, most of the study subjects were pediatric patients and the age distribution of the study subjects was representative of that for influenza A(H1N1)pdm09 in Japan [17,18]. The number of subjects in divided four groups according to the respiratory conditions were not equal as well as the small number of subjects without steroids treatment due to the retrospective study in a single hospital. Therefore, the further prospective study in patients with a variety of ages with large population is needed.

In conclusions, systemic corticosteroid treatment together with early administration of antiviral agents did not result in negative clinical outcomes in patients with influenza viral pneumonia with wheezing and without wheezing in the present study. The findings suggest that influenza pneumonia patients with wheezing and potentially without wheezing could be treated by systemic corticosteroids and early administration of antiviral agents if the severity of disease is before critical condition.

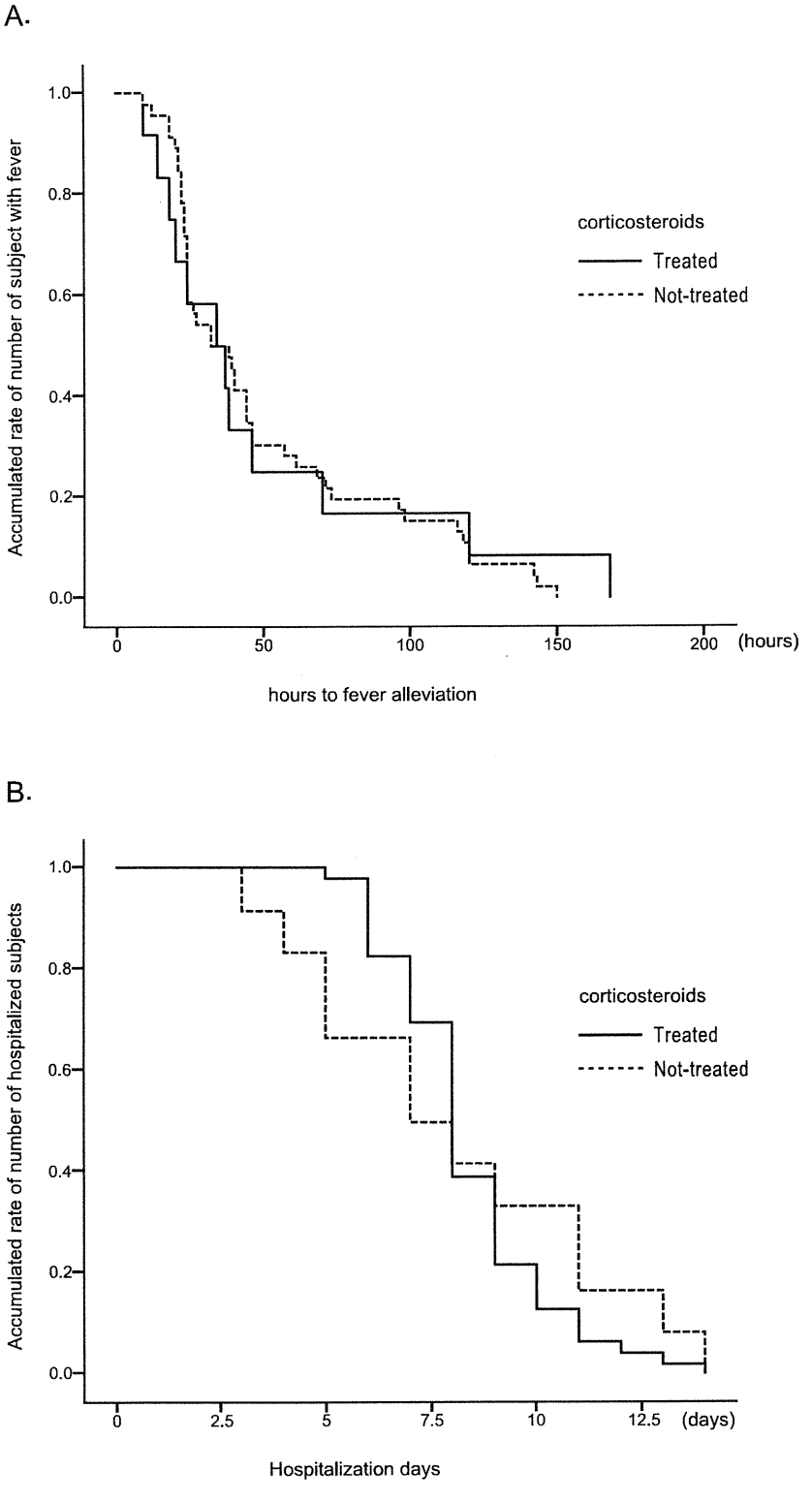


**Figure 2. Systemic corticosteroids treatment in the relation0 to clinical time course assessed by Kaplan-Meier methods.** Kaplan-Meier curves of the number of hours to fever alleviation (A) and hospitalization days (B) according to systemic corticosteroid treatment among subjects with viral pneumonia in steroid (n = 46) and non-steroid (n = 12) groups There were no significant differences between the groups in terms of either hours to fever alleviation (log rank test, p = 0.835) or hospitalization days (log rank test, p = 0.626).
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**Table 4.** Clinical factors for using systemic corticosteroids treatment among the study subjects by multiple logistic regression analysis.

| Parameter | Regression coefficient | Standard error | P value | Odds ratio | 95% confidence interval |
|---|---|---|---|---|---|
| Intercept | 20.444 | 8.927 | | | |
| Wheezing* | 2.401 | 0.841 | 0.004 | 11.03 | 2.12–57.33 |
| Pneumonia† | 1.298 | 0.618 | 0.036 | 3.66 | 1.09–12.30 |
| SpO2‡ | −0.229 | 0.094 | 0.015 | 0.80 | 0.66–0.96 |

n = 89.

*Wheezing was defined as a continuous high pitched sound emitting from the chest during expiration on auscultation.

†Pneumonia was diagnosed on the basis of infiltrative shadows on chest radiograph.

‡$SpO_2$: oxygen saturation measured by pulse oximetry in room air on admission.

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## Author Contributions

Conceived and designed the experiments: KK. Performed the experiments: KK JT HU RY TM EK NK TJM. Analyzed the data: TM KK. Wrote the paper: KK TM.

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# Reducing Occurrence and Severity of Pneumonia Due to Pandemic H1N1 2009 by Early Oseltamivir Administration: A Retrospective Study in Mexico

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## Abstract

***Background:*** Anti-viral treatment has been used to treat severe or progressive illness due to pandemic H1N1 2009. A main cause of severe illness in pandemic H1N1 2009 is viral pneumonia; however, it is unclear how effective antiviral treatment is against pneumonia when administered >48 hours after symptom onset. Therefore, we aimed to determine how time from symptom onset to antiviral administration affected the effectiveness of antiviral treatment against pneumonia due to pandemic (H1N1) 2009.

***Methods/Principal Findings:*** A retrospective medical chart review of 442 patients was conducted in a hospital in Mexico. Subjects had tested positive for pandemic H1N1 2009 virus by real-time reverse-transcriptase-polymerase-chain-reaction and were administered oseltamivir. Median time from symptom onset to oseltamivir administration was 5.0 days (range, 0–43). 442 subjects, 71 (16.1%) had severe pneumonia which required mechanical ventilation, 191 (43.2%) had mild to moderate pneumonia, and 180 (40%) did not have pneumonia. Subjects were divided into four groups based on time to oseltamivir administration: ≤2, 3–7, 8–14, and >14 days. Severity of respiratory features was associated with time to treatment, and multivariate analysis indicated that time to oseltamivir administration was associated with severity of respiratory features. A proportional odds model indicated that 50% probability for occurrence of pneumonia of any severity and that of severe pneumonia in patients who would develop pneumonia reached at approximately 3.4 and 21 days, respectively, after symptom onset. Patients with a shorter time to oseltamivir administration were discharged earlier from the hospital.

***Conclusions:*** Earlier initiation of oseltamivir administration after symptom onset significantly reduced occurrence and severity of pneumonia and shortened hospitalization due to pandemic H1N1 2009. Even when administered >48 hours after symptom onset, oseltamivir showed considerable potential for reducing pneumonia. Application of these results would benefit patients affected by future influenza pandemics.

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## Introduction

Pandemic H1N1 2009 emerged in Mexico in March 2009 [1] and rapidly spread throughout the world. The fatality rate and the frequency of severe cases varied among countries and regions even among different regions within the same country [2,3]. Mexico reported a number of cases with severe clinical presentations and deaths, especially in the early period of the outbreak. This occurred at least in part because the novelty of the influenza strain was not recognized until April 23, 2009 [1,4]. The World Health Organization [5] and the Center for Disease Control and Prevention [6] recommended early initiation of treatment with antiviral drugs in patients with pandemic H1N1 2009 virus infection with severe or progressive illness as well as in high-risk populations. The main cause of severe illness in pandemic H1N1 2009 was viral pneumonia [1,3,7,8,9] which is relatively rare in seasonal influenza. Oseltamivir has been used to treat influenza virus infection. The efficacy of oseltamivir treatment commencing >48 h after symptom onset in seasonal influenza has not been established [10]. There has been concern whether the time-interval from symptom onset to administration of oseltamivir affects clinical features on patients with pandemic H1N1 2009 virus infection. This study investigated how the post-onset window to oseltamivir administration affected the occurrence and severity of pneumonia and the duration of hospitalization in patients treated at the National Institute of Respiratory Diseases (INER) in Mexico City, Mexico during the pandemic period.

**Table 1.** Characteristics of study patients.

| Variable | Oseltamivir administration | | | | Total | P value |
|---|---|---|---|---|---|---|
| **Days from symptom onset until oseltamivir administration** | **Group 1 ≤2** | **Group 2 3–7** | **Group 3 8–14** | **Group 4 >14** | **Median 5.0* (range, 0–43)** | |
| **No. of patients (% of all study patients)** | **n = 92 (20.8%)** | **n = 213 (48.2%)** | **n = 101 (22.9%)** | **n = 36 (8.1%)** | **n = 422 (100%)** | |
| **Hospitalized/ambulatory, No. (% in each group)** | | | | | | 0.000[2] |
| Hospitalized | 21 (22.8) | 107 (50.2) | 79 (78.2) | 34 (94.4) | 241 (54.5) | |
| Ambulatory | 71 (77.2) | 106 (49.8) | 22 (21.8) | 2 (5.6) | 201 (45.5) | |
| **Deaths, No. (% in each group)** | | | | | | 0.004[4] |
| | 1 (1.1) | 10 (4.7) | 9 (8.9) | 4 (11.1) | 24 (5.7) | |
| **Age (y), mean ± SD** | 31.4±16 | 32.8±16.4 | 37.8±16.2 | 38.5±17.4 | 34±16.5 | 0.010[1] |
| Range | 1.3–73.5 | 0.4–74.6 | 0.7–81.4 | 0.7–81.4 | 0–85 | |
| **Age (y), No. (% in each group)** | | | | | | |
| <1 | 0 (0.0) | 5 (2.3) | 2 (2.0) | 1 (2.8) | 8 (1.8) | |
| 1 – <5 | 5 (5.4) | 14 (6.6) | 4 (4.0) | 2 (5.6) | 25 (5.7) | |
| 5 – <10 | 4 (4.3) | 5 (2.3) | 2 (2.0) | 0 (0.0) | 11 (2.5) | |
| 10 – <18 | 10 (10.9) | 16 (7.5) | 4 (4.0) | 1 (2.8) | 31 (7.0) | |
| 18 – <50 | 60 (65.2) | 141 (66.2) | 67 (66.3) | 24 (66.7) | 292 (66.1) | |
| 50 – <65 | 12 (13.0) | 29 (13.6) | 20 (19.8) | 6 (16.7) | 67 (15.2) | |
| ≥65 | 1 (1.1) | 3 (1.4) | 2 (2.0) | 2 (5.6) | 8 (1.8) | |
| **Male sex, No. (% in each group)** | 55 (59.8) | 114 (53.5) | 56 (55.4) | 24 (66.7) | 249 (56.3) | 0.436[2] |
| **Socioeconomic background†, No. (% in each group)** | | | | | | 0.001[3] |
| 0 | 5 (5.4) | 12 (5.6) | 19 (18.8) | 6 (16.7) | 42 (9.5) | |
| 1 | 7 (7.6) | 29 (13.6) | 14 (13.9) | 11 (30.6) | 61 (13.8) | |
| 2 | 75 (81.5) | 146 (68.5) | 61 (60.4) | 14 (38.9) | 296 (67.0) | |
| 3 | 4 (4.3) | 18 (8.5) | 4 (4.0) | 4 (11.1) | 30 (6.8) | |
| 4 | 0 (0.0) | 4 (1.9) | 3 (3.0) | 1 (2.8) | 8 (1.8) | |
| 5 | 1 (1.1) | 4 (1.9) | 0 (0.0) | 0 (0.0) | 5 (1.1) | |
| **Influenza vaccination in 2008 and/or 2009, No. (% in each group)** | 3 (3.3) | 4 (1.9) | 4 (4.0) | 0 (0.0) | 11 (2.5) | 0.499[2] |
| **Comorbidities and others, No. (% in each group)** | | | | | | |
| Obesity | 7 (7.6) | 28 (13.1) | 15 (14.9) | 4 (11.1) | 54 (12.2) | 0.442[2] |
| Diabetes | 2 (2.2) | 13 (6.1) | 8 (7.9) | 2 (5.6) | 25 (5.7) | 0.370[2] |
| Hypertension | 5 (5.4) | 17 (8.0) | 11 (10.9) | 5 (13.9) | 38 (8.6) | 0.357[2] |
| Chronic heart failure | 0 (0.0) | 2 (0.9) | 1 (1.0) | 0 (0.0) | 3 (0.7) | 0.745[2] |
| Asthma | 10 (10.9) | 22 (10.3) | 5 (5.0) | 5 (3.9) | 42 (9.5) | 0.307[2] |
| COPD‡ | 1 (1.1) | 0 (0.0) | 1 (1.0) | 1 (2.8) | 3 (0.7) | 0.243[2] |
| Immunocompromised | 0 (0.0) | 2 (0.9) | 3 (3.0) | 1 (2.8) | 6 (1.4) | 0.256[2] |
| Steroid treatment | 0 (0.0) | 1 (0.5) | 2 (2.0) | 2 (5.6) | 5 (1.1) | 0.032[2] |
| Smoking | 19 (20.7) | 66 (31.0) | 34 (33.7) | 16 (44.4) | 135 (30.5) | 0.046[2] |
| Alcohol dependence | 7 (7.6) | 18 (8.5) | 18 (17.8) | 6 (16.7) | 49 (11.1) | 0.038[2] |
| Drug dependence | 0 (0.0) | 1 (0.5) | 3 (3.0) | 3 (8.3) | 7 (1.6) | 0.002[2] |

[1]One-way ANOVA,
[2]Chi-square test,
[3]Kruskal-Wallis test,
[4]Cochran-Armitage test.
Grouping of patients was based on the number of days from symptom onset to oseltamivir administration: Group 1, ≤2 days; Group 2, 3–7 days; Group 3, 8–14 days; Group 4, >14 days.
*Median number of days from symptom onset to oseltamivir administration among all study patients.
†Socioeconomic background, based on patient's approximate daily income: 0 = <$5 US, 1 = $6–$10, 2 = $11–$15, 3 = $16–$25, 4 = $26–$40, 5 = >$40.
‡COPD: chronic obstructive pulmonary disease.
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## Materials and Methods

### Study design

INER is a national tertiary care organization that includes a research center devoted to respiratory diseases. INER provides medical services primarily for economically deprived and uninsured populations, many of whom are from the Mexico City Metropolitan area. Medical records of patients with RT-PCR confirmed pandemic H1N1 2009 infections who were treated at the INER between April 1, 2009 and January 31, 2010 and were administered oseltamivir, the only available antiviral drug in the INER during the pandemic period, were retrospectively reviewed. All patients who were administered oseltamivir during the study period were included in this study. The regular dosage and duration of oseltamivir treatment was 150 mg/day for 5 days. However, the duration was extended when deemed necessary because of the patient's clinical conditions.

Clinical data, chest radiologic findings and laboratory findings were reviewed in terms of time from symptom onset to oseltamivir administration. Socioeconomic background of patients was classified into 6 levels based on their daily income. The study patients were divided into the following 4 groups based on the number of days from symptom onset to oseltamivir administration: Group 1 (≤2 days), Group 2 (3–7 days), Group 3 (8–14 days), and Group 4 (>14 days). The groups were compared in terms of clinical symptoms and findings, severity of the respiratory features and the duration of hospitalization. Severity of respiratory features was classified into three categories: severe pneumonia, mild to moderate pneumonia, or upper respiratory tract involvement without pneumonia. Pneumonia was confirmed on the basis of abnormal shadows on chest radiographs and was considered severe if it required mechanical ventilation, and mild to moderate if it did not. The third category consisted of upper respiratory tract involvement without pneumonia. How these aspects were influenced by the time to oseltamivir administration from symptom onset was subsequently examined.

The study was approved by the Institutional Review Boards of the INER, Mexico and National Center for Global Health and Medicine, Japan. Written informed consent was obtained from all hospitalized study patients or their relatives and verbal consent from all study outpatients was obtained in accordance with the Review Boards in the INER. Investigators kept the datasets in password-protected systems and presented data with the anonymity of study patients.

### Statistical analysis

Statistical analyses were performed by comparing Groups 1 through 4, and testing the linearity of incidence. Patient background data (qualitative and quantitative values) and clinical laboratory

**Table 2.** Clinical features of the study patients on admission.

| Variable | Oseltamivir administration | | | | Total | P value* |
|---|---|---|---|---|---|---|
| Days from symptom onset until oseltamivir administration | Group 1 ≤2 | Group 2 3–7 | Group 3 8–14 | Group 4 >14 | | |
| No. of patients (% of all study patients) | n = 92 (20.8%) | n = 213 (48.2%) | n = 101 (22.9%) | n = 36 (8.1%) | n = 422 (100%) | |
| Abnormal respiratory sounds | 17 (18.5) | 78 (36.6) | 48 (47.5) | 13 (36.1) | 156 (35.3) | <0.001 |
| Hemoptysis | 0 (0.0) | 10 (4.7) | 14 (13.9) | 4 (11.1) | 23 (6.3) | <0.001 |
| Abnormal pulmonary shadows† | 23 (25.0) | 121 (56.8) | 84 (83.2) | 34 (94.4) | 262 (59.3) | <0.001 |
| Pneumothorax† | 0 (0.0) | 2 (0.9) | 5 (5.0) | 2 (5.6) | 9 (2.0) | 0.004 |
| Pleurisy† | 0 (0.0) | 1 (0.5) | 3 (3.0) | 2 (5.6) | 6 (1.4) | 0.005 |
| Chest pain | 18 (19.6) | 61 (28.6) | 31 (30.7) | 12 (33.3) | 122 (27.6) | 0.068 |
| Dyspnea | 27 (29.3) | 100 (46.9) | 70 (69.3) | 27 (75.0) | 224 (50.7) | <0.001 |
| Cyanosis | 2 (2.2) | 33 (15.5) | 29 (28.7) | 11 (30.6) | 75 (17.0) | <0.001 |
| Intubation | 2 (2.2) | 31 (14.6) | 26 (25.7) | 12 (33.3) | 71 (16.1) | <0.001 |
| Vomiting | 4 (4.3) | 10 (4.7) | 4 (4.0) | 3 (8.3) | 21 (4.8) | 0.573 |
| Diarrhea | 3 (3.3) | 11 (5.2) | 6 (5.9) | 4 (11.1) | 24 (5.4) | 0.105 |
| Myalgia | 45 (48.9) | 112 (52.6) | 48 (47.5) | 16 (44.4) | 221 (50.0) | 0.540 |
| Asthenia | 7 (7.6) | 41 (19.2) | 20 (19.8) | 10 (27.8) | 78 (17.6) | 0.006 |
| Cough | 62 (67.4) | 168 (78.9) | 79 (78.2) | 31 (86.1) | 340 (76.9) | 0.027 |
| Purulent sputum | 13 (14.1) | 36 (16.9) | 25 (24.8) | 10 (27.8) | 84 (19.0) | 0.018 |
| Arthralgia | 40 (43.5) | 119 (55.9) | 52 (51.5) | 19 (52.8) | 230 (52.0) | 0.381 |
| Chills | 2 (2.2) | 25 (11.7) | 5 (5.0) | 2 (5.6) | 34 (7.7) | 0.872 |
| Nasal obstruction | 16 (17.4) | 21 (9.9) | 4 (4.0) | 1 (2.8) | 42 (9.5) | 0.001 |
| Sore throat | 21 (22.8) | 34 (16.0) | 11 (10.9) | 2 (5.6) | 68 (15.4) | 0.004 |
| Abdominal pain | 0 (0.0) | 3 (1.4) | 2 (2.0) | 0 (0.0) | 5 (1.1) | 0.568 |
| Conjunctivitis | 20 (21.7) | 17 (8.0) | 5 (5.0) | 3 (8.3) | 45 (10.2) | 0.002 |

Grouping of patients was based on the number of days from symptom onset to oseltamivir administration: Group 1, ≤2 days; Group 2, 3–7 days; Group 3, 8–14 days; Group 4, >14 days.
*Cochran-Armitage test.
†Chest radiological findings.
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