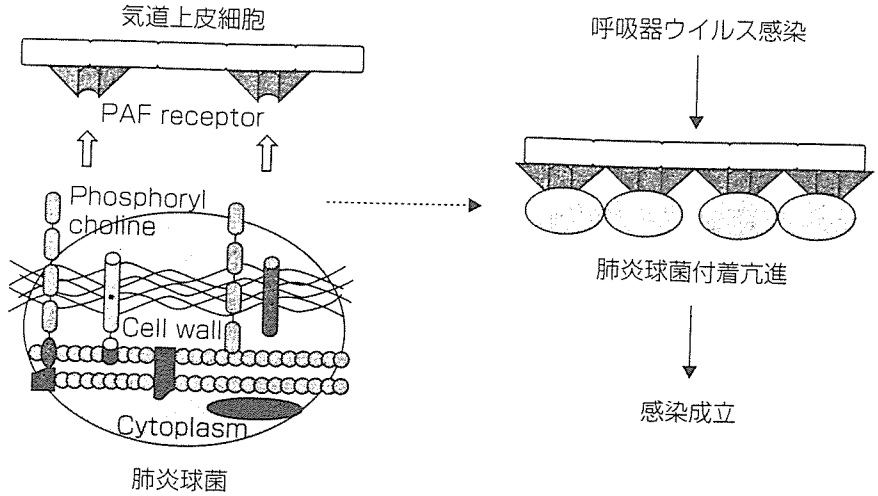

図1．肺炎球菌呼吸器感染症の成立機序
呼吸器ウイルス感染に伴う気道上皮細胞表面上のPAFレセプター発現が増加し，肺炎球菌の気道上皮付着が亢進する

イラミニダーゼは気道傷害を介して肺炎球菌付着に関与することが示されている．さらに，マウス肺炎実験モデルにおいてもインフルエンザウイルス先行感染において肺炎球菌性肺炎が重症化することも明らかになっている．

一方，Madhiらは南アフリカの乳幼児を対象とした研究において，ウイルス関連肺炎に肺炎球菌が重要な役割を果たすことを示唆している[2]．すなわち，乳幼児の肺炎球菌性肺炎の診断における血液培養の感度は低く，他に感度の高い診断法は無い．一方，HIV感染の無い乳幼児に対しては肺炎球菌コンジュゲートワクチン接種がその侵襲性肺炎球菌性感染症を85～97%抑制する事実から，このワクチン効果がウイルス肺炎における肺炎球菌の役割を明らかにするための感度の高いプローブとなると考えられる．この著者らは乳幼児のワクチン接種群（n＝18,245）におけるウイルス関連肺炎の発症頻度をプラセボ群（n＝18,268）と比較し，コンジュゲートワクチンはインフルエンザA，RSV，パラインフルエンザウイルスの検出された肺炎の発症を22～45%も抑制したとしている．この結果は，肺炎球菌がウイルス関連肺炎の発症に重要な役割を果たすこと，またこれらのウイルスが細菌性肺炎の発症に関与していることを示唆している．

同様にインフルエンザ菌と気道上皮の付着・侵入機構にもPAFレセプターが関与する事が報告されている．この事実から，肺炎球菌と同様に先行するウイルス感染がインフルエンザ菌感染の頻度を増加させることが推察される．

上述の報告を要約すると，呼吸器ウイルスは気道上皮細胞を傷害し，レセプター発現を亢進することで，肺炎球菌の付着を増加させ，感染増悪のリスクを高めていることが示唆される（図1）．

2．診断の進歩：肺炎球菌尿中抗原

最近，肺炎球菌尿中抗原検査（Binax NOW *Streptococcus pneumoniae*）が保険適応になり，その臨床応用が進んでいる[3]．この検査法は，肺炎球菌感染症患者の血中抗原が尿中に濃縮され，尿中抗原陽性になることを利用して開発された．尿検体は採取が容易であり，気道分泌物等のように口腔内細菌による汚染の可能性も無い．従って，肺炎球菌尿中抗原検査は肺炎球菌による肺炎，菌血症，髄膜炎等の簡便な細菌学的補助診断として意義がある．

1）肺炎球菌尿中抗原検査の原理

本キットはimmunochromatographic membrane assayの原理を利用した，肺炎患者の尿中および髄膜炎患者の髄液中の肺炎球菌莢膜共通多糖抗原（C-polysaccharide）の迅速検出法である．

2）臨床成績

51例の肺炎球菌性肺炎症例における肺炎球菌尿中抗原検出において，菌血症を伴う28例，菌血症を伴わない23例における尿中抗原陽性率はそれぞれ82.1%と78.3%といずれも高率で，両者間の差は認められなかった．全体の尿中抗原検査の感度は80.4%で特異度は97.2%であった．これらの結果は，菌血症の存在にかかわらず，肺炎球菌性肺炎における尿中抗原は，とりわけ菌血症を伴わない肺炎球菌性肺炎の診断に有用であることを示唆している．さらに英国における肺炎球菌性菌血症107例を対象とした検討においても，尿中抗原検査の感度は82%，特異度は97%であった．また，尿中抗原は治療7日後でも80～90%が陽性であった．

3）肺炎球菌抗原検査の問題点

小児における鼻咽頭への肺炎球菌の定着が尿中抗原結果に影響することが報告されている．鼻咽頭に肺炎球菌を保有している小児（66.6%）の尿中抗原陽性率は明らかに肺炎球菌を保有しない小児の陽性率（32.9%）より高かったと報告されている．また，肺炎球菌を保有していない小児において尿中抗原陽性率が高い理由として，低いレベルの肺炎球菌定着あるいはC-polysaccharideを保有する*Streptococcus mitis*の定着による可能性が考えられている．

さらに，肺炎発症後の患者において数週間も尿中抗原が陽性になることも報告されており，尿中抗原陽性の判定には肺炎球菌肺炎既往の有無に十分留意する必要がある．

3．本邦の肺炎球菌性肺炎の実態調査

1）肺炎球菌性市中肺炎の特徴（表1）

近年，本邦をはじめとする東アジア諸国において，肺炎球菌のβラクタム耐性やマクロライド耐性の頻度が高まり，その抗菌薬治療上の問題点が指摘されている．今回，我々は全国20施設で肺炎球菌性市中肺炎の臨床像，起炎菌の薬剤耐性，血清型の実態を調査した．研究実施期間は2001～2003年で，通常の方法で肺炎を診断し，細菌学的には喀痰および血液培養を実施して起炎菌を決定した．114例において，肺炎球菌は109例が喀痰，3例が血液，1例が胸水，1例が気管支肺胞洗浄液から分離され，菌血症を伴う肺炎球菌性肺炎の頻度は欧米に比較して低かった．また，114例中，89例（78.1%）は入院し，残りの25例は外来で治療された．患者の平均年齢は67.4歳（20～99歳）で，男性が59.6%を占めていた．全症例の71.9%に基礎疾患が認められ，その内訳は慢性呼吸器疾患（39.5%），糖尿病（12.3%），脳血管障害（8.8%）などであった．日本呼吸器学会のガイドラインに従った重症度分類では，重症33.3%，中等症42.1%，軽症24.6%であった．入院患者の平均在院日数は軽症でも15.9日，中等症24.1日，重症34.3日と，欧米に比較して明らかに在院日数は長かった（図2）．これらの114症例中，109例（95.6%）は治療により軽快したものの，5例（4.4%）が死亡した．この致命率4.4%は欧米の成績に比較すると低率であった．図3には入院症例のうち治癒例（n＝77）と死亡例（n＝5）の入院日数を示した．死亡例では入院日数が1週間未満であり，急速な経過を示した事が伺える．

2）肺炎球菌の薬剤感受性と耐性遺伝子

肺炎球菌114株のペニシリン感受性成績では，26株（22.8%）がペニシリン耐性であり（MIC 2μg/mlが25株，4μg/mlが1株），ペニシリン非感受性（MIC＞0.12μg/ml）以上は66株（57.9%）

表 1. 肺炎球菌性市中肺炎 114 症例の臨床像の特徴

平均年齢	67.4 歳（男性 59.6%）
菌血症陽性例	2.6%
基礎疾患合併	71.9%
慢性呼吸器疾患合併	39.5%
重症例の頻度	33.3%
致命率	4.4%

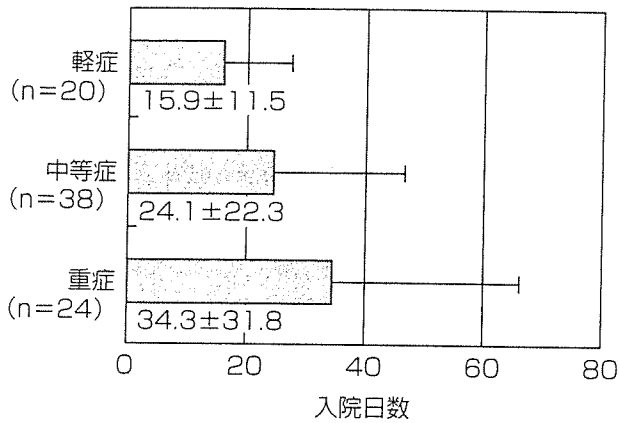

図 2. 肺炎球菌性肺炎患者の入院日数
軽症，中等症，重症例の順に入院日数は延長している．軽症でも入院日数は 2 週間を超えている．

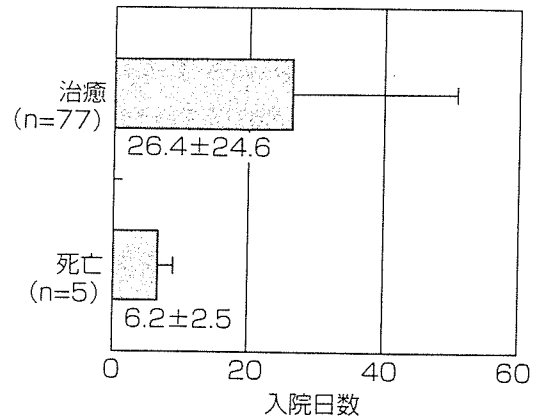

図 3. 肺炎球菌性肺炎患者の入院日数
死亡例の入院日数は 1 週間以内と短かく，死亡症例の経過が早い．

であった．肺炎球菌のβラクタム耐性を担っている*pbp*遺伝子変異の検討では，42 株（36.8%）が*pbp1a*＋*2x*＋*2b*遺伝子変異を有し（genotypic PRSP），28.1% が*pbp2x*遺伝子変異を有していた．*pbp1a*＋*2x*＋*2b*遺伝子変異株のMICは 0.25～4.0 μg/ml，MIC50 が 2μg/mlとペニシリン耐性を示すのに対し，*pbp2x*遺伝子変異株はMIC範囲 0.03～0.13μg/ml，MIC50 が 0.03μg/mlとペニシリン感受性であった．*pbp*遺伝子変異を認めなかったのは 13 株（11.4%）のみに過ぎなかった．

一方，肺炎球菌 114 株のマクロライド耐性に関与する*erm* B遺伝子，*mef* A遺伝子の頻度についても検討した．*erm* B遺伝子は 23S rRNA methylaseのmetylationをコードし，*mef* A遺伝子はマクロライドのefflux に関与するとされている．結果として，114 株中*erm* B遺伝子保有株（50 株：56.1%）が最も多く，続いて*mef* A遺伝子保有株（26 株：22.8%），遺伝子非保有株（24 株：21.1%），*mef* A＋*erm* B遺伝子保有株（7 株：6.1%）の順であった．*erm* B遺伝子保有株は*mef* A遺伝子保有株に比較してエリスロマイシン耐性が顕著（MIC：0.5～128μg/ml，MIC50＝128μg/ml）であった．

3）肺炎球菌性肺炎の重症度および致命率と薬剤耐性

114 症例の肺炎球菌肺炎の重症度と起炎菌の薬剤耐性遺伝子保有との関連性について検討した．ペニシリン感受性株である*pbp2x*株は重症の傾向を示し，*pbp1a*＋*2x*＋*2b*株はより軽症の傾向が認められた．しかしながら，重症度および致命率とペニシリン耐性遺伝子の分布に明らかな有意差は認められなかった．同様に，重症度および致命率とマクロライド耐性遺伝子分布との関連性も認められなかった．

4. 治療の問題点と最近の動向

これまでに，Metlayらは成人の菌血症を伴う肺炎球菌性肺炎患者において，ペニシリン非感受性肺炎球菌がその院内死亡のリスク因子になると報告している[4]．しかしながら，我々の成績では菌血症を伴わない肺炎球菌性肺炎が大半で，致命率も 4.4% と低率であったこともあり，死亡と肺炎球菌のペニシリン耐性との間には明らか

な関係は認められていない．これまでの多くの研究においても，肺炎球菌性肺炎の死亡とペニシリン耐性の相関は明らかでない．さらには，最近の論文は不適切なβラクタム剤による治療であっても結果的に肺炎の致命率を上昇させていないとしている．一方，肺炎球菌性肺炎のマクロライド耐性と治療失敗が相関するとした報告がある．当然ながら，肺炎球菌のマクロライド耐性が高い地域では市中肺炎患者のマクロライド単剤治療には注意が喚起されている．

このように，現時点の肺炎球菌性肺炎の治療において，起炎菌の薬剤耐性に伴う治療失敗はある．しかしながら，適切な治療の有無にかかわらず，致命率の増加を招く事態は起こっていないと言えよう．

近年，海外では肺炎球菌肺炎に対するβラクタム剤とマクロライド剤の有用性が注目されている．Martinezらは，過去10年間の菌血症を伴う409症例の肺炎球菌性肺炎のβラクタム剤ベースのエンペリックな治療を評価し，マクロライド系薬の併用が肺炎死亡のリスクを低下させると報告している[5]．この結果は，起炎菌未定の，入院が必要な市中肺炎の治療にβラクタム剤とマクロライド系薬併用の推奨を支持している．さらに，この結果は他のレトロスペクティブ研究により確認されており，今後プロスペクティブ研究により重症肺炎に対するβラクタム剤とマクロライド系薬併用療法の評価が早急に求められている．

文　献

1) Ishizaka S, et al : Effects of rhinovirus infection on the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells. J Infect Dis 188 : 1928–1939, 2003.
2) Madhi SA, et al : A role for *Streptococcus pneumoniae* in virus-associated pneumonia. Nat Med 10 : 811–813, 2004.
3) 陳　　蒙，大石和徳：肺炎球菌尿中抗原検出法．日本臨床 63 : 159–162, 2005.
4) Metlay JP, et al : Impact of penicillin susceptibility on medical outcome for adult patients with bacteremic pneumococcal pneumonia. Clin Infect Dis 30 : 520–528, 2000.
5) Martinez JA, et al : Addition of a macrolide to β-lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia. Clin Infect Dis 36 : 389–395, 2003.

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Comparative Molecular Analysis of *Haemophilus influenzae* Isolates from Young Children with Acute Lower Respiratory Tract Infections and Meningitis in Hanoi, Vietnam

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Thirty-seven *Haemophilus influenzae* strains from nasopharyngeal swabs (NP) and 44 *H. influenzae* strains from cerebrospinal fluid (CSF) were investigated. Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas all of the 44 isolates from CSF were type b. The MICs of 16 antibiotics for the *H. influenzae* isolates from NP and CSF were similar, and no β-lactamase-negative ampicillin-resistant strain was found. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns, with none predominating, and the 44 *H. influenzae* strains from CSF had 9 PFGE patterns, with patterns α (22 isolates) and β (12 isolates) predominating. Our results indicate that two predominant types of *H. influenzae* type b strains have the potential to spread among children with meningitis in Hanoi, Vietnam.

Nontypeable *Haemophilus influenzae* (NTHi) can cause a variety of infections, including otitis media, bronchitis, and pneumonia (7), whereas *H. influenzae* type b (Hib) is a common cause of meningitis in children (11). Hib infection rates have been dramatically reduced in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunizations (10). It has also recently been reported that β-lactamase-negative ampicillin (AMP)-resistant (BLNAR) strains have increased in some countries (6, 12), although their global prevalence remains low (4, 5). The aim of our study was to investigate the characteristics of *H. influenzae* among children less than 5 years of age in Vietnam.

Thirty-seven *H. influenzae* strains were isolated from the nasopharyngeal swabs (NP) of 37 children aged 2 to 60 months (mean age, 11 months) who were diagnosed with acute lower respiratory tract infections between 2001 and 2002, and 44 *H. influenzae* strains were isolated from the cerebrospinal fluid (CSF) of 44 children aged 1 to 24 months (mean age, 9 months) who were diagnosed with meningitis between 2002 and 2003, in Hanoi, Vietnam. No patient with an acute lower respiratory tract infection overlapped a patient with meningitis. *H. influenzae* isolates were serotyped by slide agglutination with antisera purchased from Difco Laboratories (Detroit, Mich.), and β-lactamase production was detected by a disk impregnated with nitrocefin (Becton Dickinson, Sparks, Md.). PCR was carried out for *H. influenzae* isolates by using mixed primers (Wakunaga Pharmaceutical Co., Hiroshima, Japan), as described previously (3). MICs were determined by the agar dilution method according to the NCCLS guidelines (8). The susceptibilities of 81 *H. influenzae* isolates to the following 16 antibiotics were tested: penicillin G (Meiji Seika Kaisha, Tokyo, Japan), AMP (Meiji Seika Kaisha), amoxicillin-clavulanic acid (AMC) (GlaxoSmithKline K.K., Tokyo, Japan), cefatrizine (Taiyo Yakuhin Co., Nagoya, Japan), cefuroxime (Sankyo Co., Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan), cefotaxime (Aventis Pharma, Tokyo, Japan), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), minocycline [Lederle (Japan), Tokyo, Japan], chloramphenicol (Sankyo Co.), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), gentamicin (Schering-Plough K.K., Osaka, Japan), levofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), norfloxacin (Kyorin Pharmaceutical Co., Tokyo, Japan), and sulfamethoxazole-trimethoprim (Shionogi & Co., Osaka, Japan). After digestion with SmaI (Takara Shuzo Co., Shiga, Japan), pulsed-field gel electrophoresis (PFGE) was performed on the 37 *H. influenzae* isolates from the NP and the 44 *H. influenzae* isolates from the CSF, as described previously (16), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (13).

Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas the 44 isolates from CSF were all type b. Twenty-six strains (70.3%) from NP and 23 strains (52.3%) from CSF were β-lactamase producing, and the remaining strains were β-lactamase negative by the nitrocefin disk assay. PCR analysis to identify the resistance genes indicated that 25 strains from NP and 21 strains from CSF were β-lactamase-producing AMP-resistant isolates which had the TEM-1-type β-lactamase gene; 11 strains from NP and 22 strains from CSF were β-lactamase-negative AMP-susceptible isolates, all of which lacked all resistance genes; and 1 strain each from NP and CSF were β-lactamase-producing AMC-resistant isolates

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TABLE 1. Distribution of MICs against 16 antibiotics for *H. influenzae* strains isolated from nasopharyngeal swabs and cerebrospinal fluid from children in Vietnam

Antibiotic	MIC (μg/ml) for isolates from:					
	NP (n = 37)			CSF (n = 44)		
	Range	50%	90%	Range	50%	90%
Penicillin G	0.5–128	16	32	≤0.004–128	2	32
Ampicillin	0.25–64	8	32	0.125–32	1	8
Amoxicillin-clavulanic acid	0.25–2	0.5	0.5	0.25–1	0.25	0.25
Cefatrizine	2–32	4	8	2–16	4	16
Cefuroxime	0.5–4	1	4	0.016–4	1	2
Ceftriaxone	≤0.004–0.032	0.008	0.016	≤0.004–0.032	0.008	0.008
Cefotaxime	0.008–0.125	0.032	0.032	≤0.004–0.125	0.032	0.063
Imipenem	0.25–4	2	2	0.25–1	0.25	1
Minocycline	0.5–2	1	2	0.5–2	1	1
Chloramphenicol	0.5–16	4	8	0.5–16	8	16
Clarithromycin	0.25–16	8	16	4–16	8	8
Erythromycin	0.25–4	4	4	0.016–8	2	4
Gentamicin	1–2	1	2	0.016–2	0.5	2
Levofloxacin	0.016–0.063	0.032	0.032	≤0.004–0.032	0.032	0.032
Norfloxacin	0.063–0.125	0.125	0.125	0.063–0.125	0.063	0.125
Sulfamethoxazole-trimethoprim	1–≥128	≥128	≥128	0.032–≥128	128	≥128

which had the TEM-1-type β-lactamase gene and the *ftsI* gene with the same substitution as the low-BLNAR strains. Although all isolates from NP which had the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay, one isolate from CSF which had the TEM-1-type β-lactamase gene was β-lactamase negative and two isolates from CSF which did not have the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay. No BLNAR strain was found. Table 1 shows the MIC range, the MICs at which 50% of isolates were inhibited (MIC_{50}), and the MIC_{90} of 16 antibiotics for 37 *H. influenzae* isolates from NP and 44 *H. influenzae* isolates from CSF. Although the MICs of the *H. influenzae* isolates from NP against penicillin G and AMP appear to be higher than those from CSF, the antimicrobial susceptibilities of the *H. influenzae* isolates from NP and CSF were similar. Molecular typing by pulsed-field gel electrphoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns (A to V), without any predominant pattern (Fig. 1). The PFGE patterns of *H. influenzae* types a, b, and c were different from those of NTHi. Four isolates of type b had two PFGE patterns (I and K), and two isolates of type c had two PFGE patterns (H and Q). Forty-four *H. influenzae* strains from CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. The PFGE patterns of 4 *H. influenzae* type b strains from NP were quite different from those of the 44 *H. influenzae* type b strains from CSF (Fig. 2).

Infants and young children tend to acquire *H. influenzae* in the upper respiratory tract because of their low immunity (16), and subsequent colonization can become a risk factor for invasive diseases caused by *H. influenzae* (2, 11). Since it has recently been reported that BLNAR NTHi and Hib have increased in some countries (3, 6, 12), the primary objective of this study was to investigate such resistant strains among children in Vietnam. In fact, no BLNAR strains were found in either NP or CSF, although more than half the isolates were β-lactamase producing and had the TEM-1-type β-lactamase gene. Hib remains the major cause of meningitis after the introduction of Hib vaccine in many advanced nations, because that vaccine is not usually available in Vietnam (14). Therefore, a secondary objective of this study was to examine the transmission route of *H. influenzae*. It has recently been reported that children can acquire *H. influenzae* at day care centers (9, 16) or from their parents at home (15). Our PFGE studies showed that NTHi did not have dominant genetic patterns but that Hib had two dominant genetic patterns. The results provide evidence to show that at least two types of Hib strains are spreading horizontally among children with meningitis in Vietnam. The Hib conjugate vaccine appears to be effective, not only for the prevention of invasive diseases, but also for the reduction of nasopharyngeal carriage in young children (1, 10).

In conclusion, our results demonstrate that BLNAR strains are not prevalent and that two predominant types of Hib

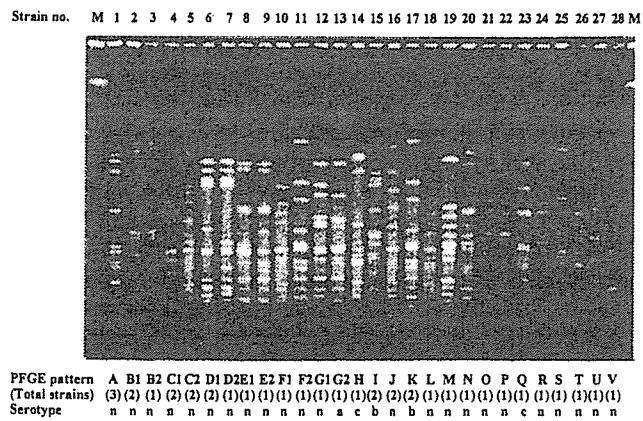

FIG. 1. PFGE patterns of SmaI-digested DNA from 37 *H. influenzae* isolates from NP of 37 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that 37 *H. influenzae* strains from the NP had 22 PFGE patterns (A to V), without any predominant pattern. The PFGE patterns of *H. influenzae* types a, b, and c were different from those of the nontypeable strains.

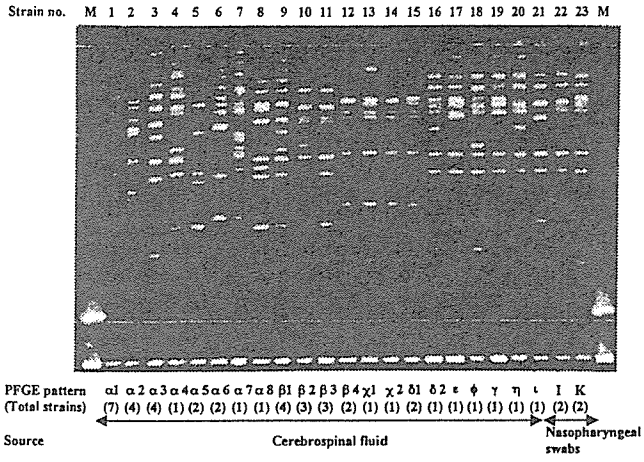

FIG. 2. PFGE patterns of SmaI-digested DNA from 48 Hib isolates from the CSF of 44 children with meningitis and the NP of 4 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that the 44 Hib strains from the CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. PFGE patterns of 4 Hib strains from the NP were quite different from those of 44 Hib strains from CSF.

strains have the potential for spreading among children with meningitis in Hanoi, Vietnam. Therefore, the introduction of the Hib conjugate vaccine for young children should be considered in order to prevent invasive diseases caused by Hib.

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REFERENCES

1. **Barbour, M. L., R. T. Mayon-White, C. Coles, D. W. Crook, and E. R. Moxon.** 1995. The impact of conjugate vaccine on carriage of *Haemophilus influenzae* type b. J. Infect. Dis. **171**:93–98.
2. **Faden, H., L. Duffy, R. Wasielewski, J. Wolf, D. Krystofik, and Y. Tung.** 1997. Relationship between nasopharyngeal colonization and the development of otitis media in children. J. Infect. Dis. **175**:1440–1445.
3. **Hasegawa, K., N. Chiba, R. Kobayashi, S. Y. Murayama, S. Iwata, K. Sunakawa, and K. Ubukata.** 2004. Rapidly increasing prevalence of β-lactamase-nonproducing, ampicillin-resistant *Haemophilus influenzae* type b in patients with meningitis. Antimicrob. Agents Chemother. **48**:1509–1514.
4. **Hoban, D., and D. Felmingham.** 2002. The PROTEKT surveillance study: antimicrobial susceptibility of *Haemophilus influenzae* and *Moraxella catarrhalis* from community-acquired respiratory tract infections. J. Antimicrob. Chemother. **50**:49–59.
5. **Karlowsky, J. A., I. A. Critchley, R. S. Blosser-Middleton, E. A. Karginova, M. E. Jones, C. Thornsberry, and D. F. Sahm.** 2002. Antimicrobial surveillance of *Haemophilus influenzae* in the United States during 2000–2001 leads to detection of clonal dissemination of a beta-lactamase-negative and ampicillin-resistant strain. J. Clin. Microbiol. **40**:1063–1066.
6. **Marco, F., J. Garcia-de-Lomas, C. Garcia-Rey, E. Bouza, L. Aguilar, C. Fernandez-Mazarrasa, and the Spanish Surveillance Group for Respiratory Pathogens.** 2001. Antimicrobial susceptibilities of 1,730 *Haemophilus influenzae* respiratory tract isolates in Spain in 1998–1999. Antimicrob. Agents Chemother. **45**:3226–3228.
7. **Murphy, T. F., and M. A. Apicella.** 1987. Nontypeable *Haemophilus influenzae*: a review of clinical aspects, surface antigens, and the human immune response to infection. Rev. Infect. Dis. **9**:1–15.
8. **National Committee for Clinical Laboratory Standards.** 1998. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
9. **Peerbooms, P. G., M. N. Engelen, D. A. Stokman, B. H. van Benthem, M. L. van Weert, S. M. Bruisten, A. van Belkum, and R. A. Coutinho.** 2002. Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. J. Clin. Microbiol. **40**:2832–2836.
10. **Peltola, H.** 2000. Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. Clin. Microbiol. Rev. **13**:302–317.
11. **Saito, M., K. Okada, K. Takemori, and S. Yoshida.** 2000. Clonal spread of an invasive strain of *Haemophilus influenzae* type b among nursery contacts accompanied by a high carriage rate of non-disease-associated strains. J. Med. Microbiol. **49**:845–847.
12. **Suzuki, K., T. Nishimura, and S. Baba.** 2003. Current status of bacterial resistance in the otolaryngology field: results from the Second Nationwide Survey in Japan. J. Infect. Chemother. **9**:46–52.
13. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
14. **Tran, T. T., Q. T. Le, T. N. Tran, N. T. Nguyen, F. K. Pedersen, and M. Schlumberger.** 1998. The etiology of bacterial pneumonia and meningitis in Vietnam. Pediatr. Infect. Dis. J. 17(Suppl. 9):S192–S194.
15. **Watanabe, H., K. Hoshino, R. Sugita, N. Asoh, K. Watanabe, K. Oishi, and T. Nagatake.** 2004. Possible high rate of transmission of nontypeable *Haemophilus influenzae* including β-lactamase-negative ampicillin-resistant strains between children and their parents. J. Clin. Microbiol. **42**:362–365.
16. **Yano, H., M. Suetake, A. Kuga, K. Irinoda, R. Okamoto, T. Kobayashi, and M. Inoue.** 2000. Pulsed-field gel electrophoresis analysis of nasopharyngeal flora in children attending a day care center. J. Clin. Microbiol. **38**:625–629.

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COMPARISON OF CLINICAL FEATURES AND HEMATOLOGIC ABNORMALITIES BETWEEN DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER AMONG CHILDREN IN THE PHILIPPINES

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Abstract. To demonstrate the differences of clinical features and hematologic abnormalities between dengue fever (DF) and dengue hemorrhagic fever (DHF), 359 pediatric patients admitted St. Luke's Medical Center in Quezon City, between 1999 and 2001 in Metro Manila, and adjoining provinces the Philippines, with a laboratory-confirmed dengue virus infection were evaluated. One third of the patients had DHF, and most of these patients were without shock. Restlessness, epistaxis, and abdominal pain were more associated with DHF. The platelet count was significantly lower in the DHF group than in the DF group before and after defervescence. In the DHF patients, the hematocrit was significantly increased before defervescence, and decreased the day after due to administration of intravenous fluid. Coagulation abnormalities associated with most DHF patients were thrombocytopenia and an increased fibrinolysis, but not disseminated intravascular coagulation. We present recent data on readily obtained clinical and laboratory data that can be used for early diagnosis and consequently earlier appropriate treatment of dengue virus infections.

INTRODUCTION

Dengue virus, a mosquito-borne human viral pathogen, has recently become a major public health concern particularly in tropical and subtropical countries, predominantly in urban and periurban areas. The geographic distribution of dengue viruses has greatly expanded and the number of cases has dramatically increased during the past three decades.[1] Two and a half billion people in more than one hundred countries are at risk of infection, with an estimated 50 million infections per year.[2] Since the first report of an outbreak of dengue hemorrhagic fever (DHF) in the Philippines in 1956,[3] dengue epidemics have occurred in the country at approximately five-year-intervals.[4,5] Previous reports have also characterized the view that dengue has been hyperendemic and a leading cause of childhood hospitalization during the 1980s in the Philippines.[6,7] Although dengue fever (DF) is a self-limited febrile illness, DHF is characterized by prominent hemorrhagic manifestations with thrombocytopenia, an increased vascular permeability, and is associated with a high mortality rate.[8]

An early clinical diagnosis of DHF is difficult because the World Health Organization (WHO) clinical and laboratory criteria for DHF may be manifested only in the late phase of acute illness.[9] Although previous reports have characterized the clinical features of DF and DHF,[9,10] differences in these features, including hematologic abnormalities between the two conditions, are poorly defined in hospitalized pediatric patients under appropriate treatment according to WHO guidelines. Therefore, this prospective study was undertaken to determine the differences in the clinical features and hematologic abnormalities between DF and DHF among hospitalized pediatric patients in Metro Manila, the Philippines from 1999 to 2001.

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PATIENTS AND METHODS

Patients and study design. All patients admitted at the St. Luke's Medical Center in Quezon City, the Philippines between January 1999 and December 2001 who satisfied the following criteria were enrolled in the study: 1) age between 2 and 17 years, 2) fever for ≤ 5 days, 3) temperature of at least 37.8°C, and 4) no apparent focus of infection. Informed consent was obtained from the patient's legal guardian.

Medical histories were obtained and physical examinations were conducted by one of the pediatrician investigators (CCC and MTDDC.) on recruitment and on a daily basis until discharge. The clinical symptoms and signs, including nutritional status, were recorded on case record forms. The day of defervescence was defined as day 0.[11] The days before and after defervescence were reported consecutively as follows: –2, –1, 0, +1, +2, etc.

Blood was drawn on the first, third, fourth, and seventh days of the hospital stay. Serial complete blood counts were obtained until the day of discharge. Diagnostic tests for dengue included reverse transcriptase–polymerase chain reaction (RT-PCR) for flaviviruses and determination of IgM antibody to dengue viruses by an enzyme-linked immunosorbent assay (ELISA).[12,13] Because the diagnostic sensitivity was 90–93% for the IgM ELISA and 80–100% for the RT-PCR, the combined diagnostic sensitivity of the RT-PCR and the IgM ELISA will be greater than 90%.[14–16]

All cases with dengue virus infections confirmed by any of the diagnostic tests were categorized as either DF or DHF according to the criteria of the WHO.[17] The diagnostic criteria included a platelet count nadir of less than 100,000/μL, hemorrhagic manifestations, and an increase in hematocrit greater than 20% above the average or the presence of pleural effusion or ascites. Cases of DHF were further graded as I–IV. A chest radiograph (posterioanterior view) was routinely done to detect pleural effusion on the third day of hospitalization. Treatment, including intravenous fluids (IVF) and fresh frozen plasma (FFP) was given to each patient based on the WHO guidelines,[17] and the total

TABLE 1
Disseminated intravascular coagulation (DIC) score*

Items	Test results	Score
Underlying disease	Yes	1
Clinical symptoms		
Hemorrhagic manifestations	Yes	1
Visceral symptoms†	Yes	1
Test results		
Serum FDP level (μg/mL)	≥ 40	3
	≥ 20 to < 40	2
	≥ 10 to < 20	1
Platelet count (× 10^4/μL)	≤ 5	3
	> 5 to ≤ 8	2
	> 8 to ≤ 12	1
Plasma fibrinogen level (mg/dL)	≤ 100	2
	> 100 to ≤ 150	1
Prothrombin time ratio	≥ 1.67	2
(divided by the normal value)	≥ 1.25 to < 1.67	1

* FDP = fibrin degradation product.
† Signs of circulatory insufficiency due to microthrombus caused by DIC.

volume of IVF or FFP administered to each patient was recorded.

Disseminated intravascular coagulation (DIC) score. To assess the presence of DIC in the patients, all patients enrolled between September 2000 and December 2001 were examined for DIC scores.[18] The DIC scoring system used is shown in Table 1. The DIC score included an evaluation of the following parameters: the underlying disease and clinical symptom (hemorrhagic manifestations or visceral signs), and an assessment of platelet count, fibrinogen, prothrombin time (PT) ratio (divided by the normal value), and fibrin degradation product (FDP). Dengue virus infection was the underlying disease referred to the DIC scoring system for purposes of this study. The concentration of fibrinogen was measured with Dadethrombin reagent (Dade Behring, Inc., Newark, DE), and PT was determined with Thromborel S reagent (Dade Behring, Inc.). Citrated blood was used for the determination of the PT ratio and fibrinogen levels. The concentration of FDP was determined by means of a commercially available kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The study protocol was reviewed and approved by the Institutional Ethics Review Board of the St. Luke's Medical Center.

Statistical analysis. All data are expressed as the mean ± SD or as frequencies and proportions. Differences in laboratory data between patients with DF and DHF were analyzed using the Student's *t*-test for continuous variables. Differences in the demographic and clinical data and DIC scores between patients with DF and DHF were tested by the chi-square test or Fisher's exact test for nominal variables, whichever was appropriate. A *P* value less than 0.05 was considered significant. The statistical software SPSS version 12.0 (SPSS, Inc., Cary, NC) was used for data analysis.

RESULTS

Subject characteristics. Of the 503 subjects screened, 359 (71.4%) were confirmed as having a dengue virus infection: 322 (89.7%) by IgM-capture ELISA and 139 (38.7%) by RT-PCR. A total of 102 (28.4%) had positive results for both tests. Of the 359 laboratory-confirmed cases, 239 (66.6%) and 120 (33.4%) were diagnosed as DF and DHF, respectively (Table 2). Forty-two patients (23 with DF and 19 with DHF) were enrolled in 1999, 75 (37 with DF and 38 with DHF) in 2000, and 242 (179 with DF and 63 with DHF) in 2001. The proportion of DHF differed in each year (45.2% in 1999, 50.6% in 2000, and 26.0% in 2001). The distribution of dengue virus serotypes (DEN1, DEN2, DEN3, DEN4) determined by RT-PCR was (6, 1, 1, and 1) in 1999, (7, 5, 0, and 1) in 2000, and (24, 84, 4, and 0) in 2001, respectively. Double-positive reactions in the RT-PCR occurred in 10 cases for serotype DEN 1 + 2, one case each for serotype DEN 1 + 3 and DEN 1 + 4, and three cases for serotype DEN 2 + 3. An outbreak of dengue illness occurred between June and October 2001 (Figure 1). These cases were primarily associated with DEN 2 and DEN 1. The mean age of all subjects was 9.8 years. With respect to severity of disease, 120 patients diagnosed as having DHF were further classified as follows: DHF I (n = 7), DHF II (n = 110), DHF III (n = 2), and DHF IV (n = 1). Although a fatal case with DHF grade IV was observed, most DHF patients were without shock. Of these, 57 (47.5%) were associated with pleural effusion.

The duration of the hospital stay was significantly longer in those with DHF than in those with DF ($P < 0.001$; Table 2). A significant increase in the frequency of abdominal pain was

TABLE 2
Demographic and clinical profile of subjects*

Parameter	DF (n = 239)	DHF (n = 120)	Total (n = 359)	*P*
Mean age (years) (SD)	9.9 (4.2)	9.8 (3.8)	9.8 (4.0)	0.877
Male:female ratio	1.49	1.50	1.49	0.976
Days with fever before admission (SD)	3.4 (1.3)	3.5 (1.4)	3.5 (1.3)	0.670
Duration of hospital stay, days (SD)	4.4 (1.7)	5.6 (1.7)	4.8 (1.8)	< 0.001
Symptoms before admission, no./total no. (%)				
Abdominal pain	76/238 (31.9)	55/119 (46.2)	131/357 (36.7)	0.008
Epistaxis	46/233 (19.7)	23/119 (19.3)	69/352 (19.6)	0.926
Symptoms at time of admission, no./total no. (%)				
Restlessness	0/238 (0.0)	4/119 (3.4)	4/357 (1.1)	0.012†
Epistaxis	26/236 (11.0)	23/117 (19.7)	49/353 (13.9)	0.027
Abdominal pain	69/237 (29.1)	51/119 (42.9)	120/356 (33.7)	0.010
Petechiae	195/239 (81.6)	102/120 (85.0)	297/359 (82.7)	0.420
Gum bleeding	11/232 (4.7)	6/113 (5.3)	17/345 (4.9)	0.819
Hematemesis	1/222 (0.5)	2/108 (1.9)	3/330 (0.9)	0.251†
Breathlessness	0/238 (0.0)	2/119 (1.7)	2/357 (0.6)	0.110†

* DF = dengue fever; DHF = dengue hemorrhagic fever.
† Fisher's exact test.

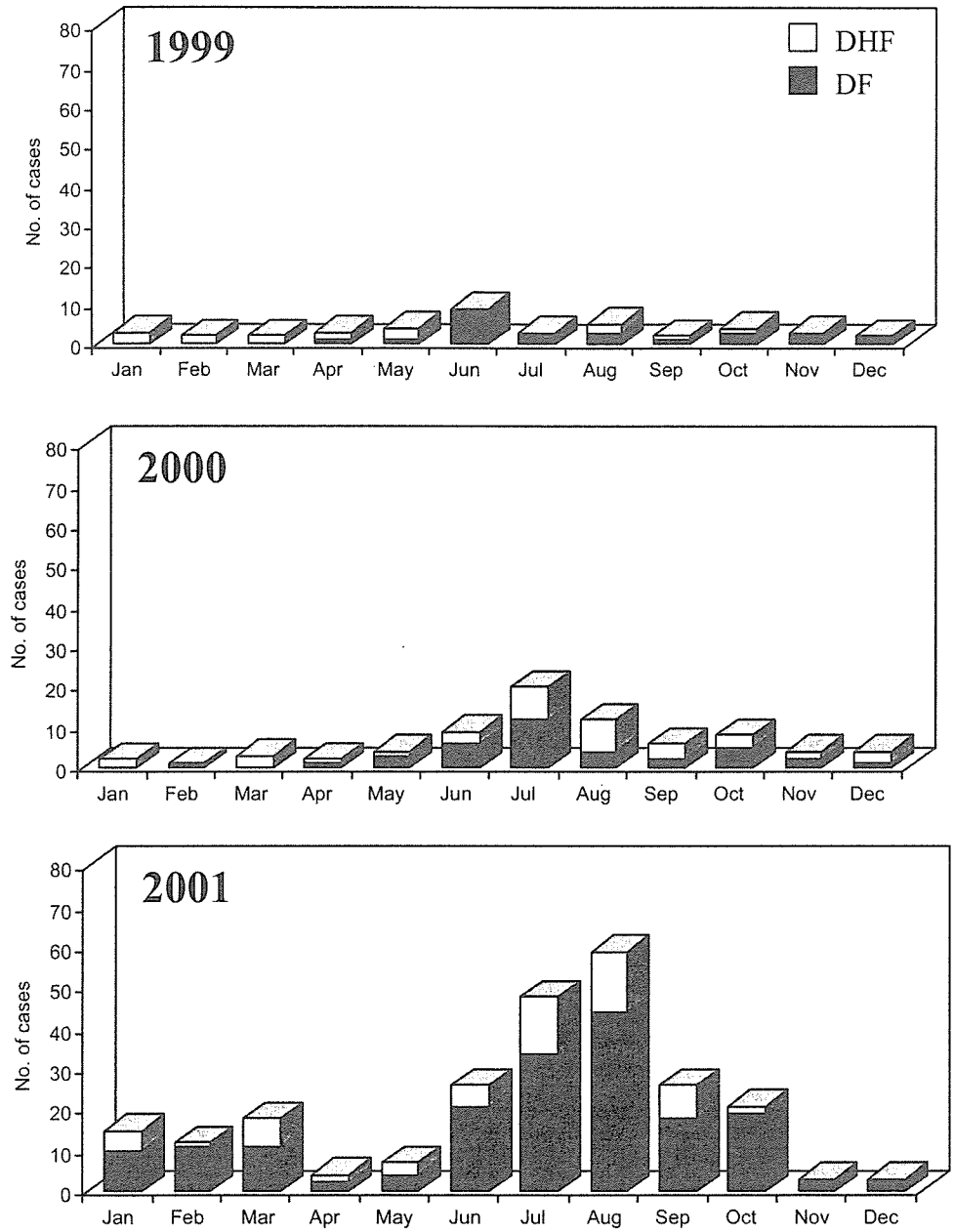

FIGURE 1. Distribution of dengue cases by month and year of enrollment. The number of laboratory confirmed dengue cases is plotted at monthly intervals from January 1999 to December 2001. DF = dengue; DHF = dengue hemorrhagic fever.

found in the DHF group before admission (P = 0.008) and at the time of admission (P = 0.010), compared with the DF group. The frequency of restlessness (P = 0.012) and epistaxis (P = 0.027) at the time of admission in the DHF group were also significantly higher than that in the DF group. No significant difference in nutritional status was found between the DF and DHF groups according to the Centers for Disease Control and Prevention classification.[19] The positive and negative predictive values of abdominal pain before admission for the development of DHF were 42.0% and 71.7%. These predictive values of symptoms upon admission were 42.5% and 71.2% for abdominal pain, 100% and 69.0% for restlessness, and 46.9% and 69.1% for epistaxis, respectively.

Laboratory data. Although the peripheral white blood cell (WBC) count of all subjects was generally below normal values prior to defervescence, the peripheral WBC count was significantly higher in the DHF group than in the DF group on days -1, 0, +1, +2, and +3 of defervescence (Figure 2A). The lymphocyte fraction in the peripheral WBC count was also significantly higher in the DHF group than in the DF group from days -1 to +2 (Figure 2B). No difference was found in the absolute monocyte, eosinophil, and basophil counts between the two groups. The laboratory data also confirmed that the platelet count was significantly lower in the DHF group than in the DF group from days −3 to +5 (Figure 2C). The lowest peripheral platelet count was noted at day +1 for both groups. The nadir of platelet count ($\times 10^3/\mu L$) was 113.8 ± 58.3 in the DF group and 58.5 ± 84.1 in the DHF group, respectively. The hematocrit was significantly higher in the DHF group from days -2 to +0 (Figure 2D). The maxi-

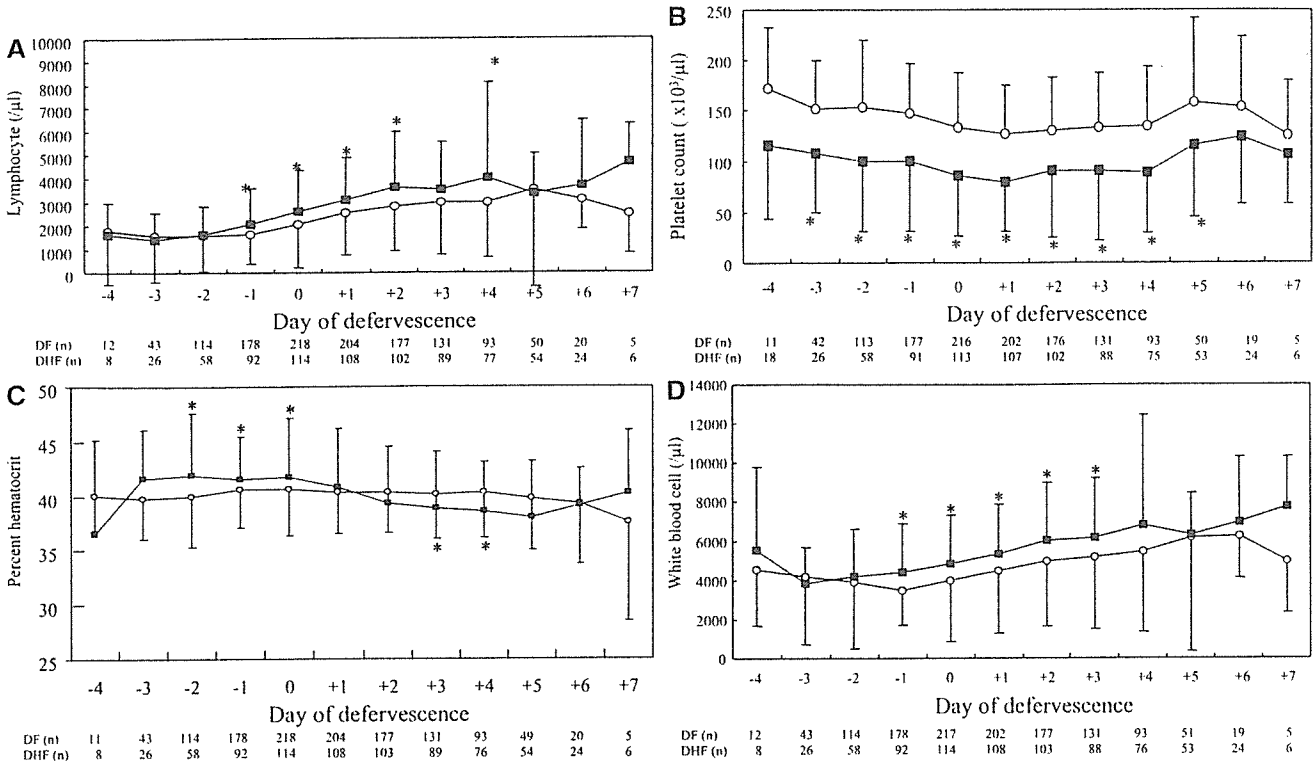

FIGURE 2. Comparison of the total white blood cell count (**A**), lymphocyte count (**B**), platelet count (**C**), and the hematocrit (**D**) in peripheral blood between pediatric patients with dengue fever (DF) and dengue hemorrhagic fever (DHF). The number of cases with DF and DHF are shown below each figure. Open circles show cases with DF and filled squares show cases with DHF. Data represent the mean ± SD $*P < 0.05$ versus patients with DF.

mum increase in the hematocrit was significantly higher in the DHF group (24.3 ± 13.8%) than that in the DF group (11.4 ± 7.7%) ($P < 0.001$). Interestingly, a significant decrease in the hematocrit was also found in the DHF group, compared with the DF group at day +4. This finding may be influenced by an intravenous fluid correction that was previously administered in addition to a shift of fluid to the intravascular space with recovery from the illness in patients with DHF. Therefore, we compared the total volume of IVF or FFP administered to each patient during admission between the DF and the DHF group. The total volume of IVF administered to each patient was significantly higher in the DHF group (n = 117, 3,265 ± 1,560 mL) than that in the DF group (n = 230, 2,687 ± 1,216 mL) during their hospital stay in this study ($P < 0.001$). No significant difference was found in the total volume of FFP used in between the DF group (n = 11, 307 ± 133 mL) and the DHF group (n = 38, 401 ± 471 mL).

DIC score. Among the parameters evaluated for the DIC scores, the frequency distribution of platelet and fibrinogen scores in the DHF group were significantly different than those in the DF group ($P < 0.05$), with higher scores observed for the DHF group (Table 3). Bleeding manifestations were frequently observed in both DF and the DHF patients, and no difference in clinical score was found between the two groups. A few cases of DF and DHF had an increased FDP and PT ratios with no significant differences in these scores between the two groups. Consequently, the frequency of cases with a DIC score ≥ 7 was significantly higher in the DHF group than in the DF group ($P < 0.001$). Of 17 cases with a DIC score of ≥ 7, 13 were DHF and 4 were DF. Of 13 DHF cases, 11 were DHF II and 1 case each of DHF I and IV, respectively. Only one death, a case of DHF grade IV, was associated with a marked increase in the PT ratio. Two cases of DHF were associated with a mild increase in the PT ratio. Only 7 of 17 cases were associated with a mild increase in FDP.

DISCUSSION

The findings herein serve to demonstrate the differences in clinical and laboratory features between DF and DHF during

TABLE 3
Comparison of DIC scores between those with DF and those with DHF*

Parameter	Score	DF (n = 163) No.	DF (n = 163) %	DHF (n = 94) No.	DHF (n = 94) %	Total (n = 257) No.	Total (n = 257) %	*P*
Platelet score	0	73	44.8	4	4.3	77	30.0	< 0.001
	1	39	23.9	15	16.0	54	21.0	
	2	30	18.4	18	19.1	48	18.7	
	3	21	12.9	57	60.6	78	30.4	
Clinical score	0	23	14.1	12	12.8	35	13.6	0.762
	1	140	85.9	82	87.2	222	86.4	
PT score	0	160	98.2	90	95.7	250	97.3	0.327
	1	3	1.8	3	3.2	6	2.3	
	2	0	0.0	1	1.1	1	0.4	
Fibrinogen score	0	111	68.1	53	56.4	164	63.8	0.027
	1	43	26.4	27	28.7	70	27.2	
	2	9	5.5	14	14.9	23	8.9	
FDP score	0	132	81.0	72	76.6	204	79.4	0.403
	1	31	19.0	22	23.4	53	20.6	
Total score	< 7	159	97.5	81	86.2	240	93.4	< 0.001
	≥ 7	4	2.5	13	13.8	17	6.6	

* DIC = disseminated intravascular coagulation; DF = dengue fever; DHF = dengue hemorrhagic fever; PT = prothrombin time; FDP = fibrin degradation product.

ission under appropriate treatment according to WHO elines. Abdominal pain and epistaxis were more com- ly associated with DHF patients during the acute phase ie illness in this study, although previous studies reported licting data on the frequency of abdominal pain and ding manifestations.[5,6] Since abdominal pain and epistax- ere also found in DF, a diagnostic value of these symp- s for the severity of disease is limited. Although DHF ired a longer hospital stay, DF also required a hospital longer than four days. This finding indicates that DF and F impose a considerable burden in the health care system e Metro Manila, the Philippines. Although the etiology of ominal pain in dengue illness remains obscure, Setia- and others reported that most pediatric patients with F and epigastric pain also had increased serum levels of lase or lipases and an enlarged pancreas.[20] Another study rted hemorrhagic gastritis as a most common finding of roendoscopy among patients with dengue fever in Tai- .[21] Since we could not specify any definite reasons for minal pain in this study, further studies will be necessary. ough abdominal pain or epistaxis yielded a low positive ictive value for the development of DHF, restlessness associated with a high positive predictive value. There- , this rare symptom could be used as a predictor of DHF. ur laboratory data confirmed the increasing mean total C and lymphocyte counts that approached normal levels nd the day of defervescence (Figure 2A and B). These ngs are consistent with previous reports, although an ex- ination for atypical lymphocytes was not done.[22–24] The imum increase in the hematocrit in the DHF group was er than 20%, and significantly higher than those in the group in this study, which supports the WHO definition of disease.[17] Increased vascular permeability would allow na to flow out of the intravascular compartments, leading ypovolemic shock. The present study also demonstrated the volume of IVF administered to prevent hypovolemic k in the DHF group was significantly higher than in the group. The increased administration of IVF for prevent- lengue shock syndrome subsequently could lead to a sig- ant decrease in the hematocrit in the DHF group, com- d with the DF group, after defervescence.

this study, we attempted to apply the diagnostic criteria IC to 257 patients with dengue virus infections.[18] Al- gh thrombocytopenia was more prominent in the DHF p than in the DF group, a few cases of DF and DHF had ncreased PT ratio. In addition, only a mild increase in ' was found in both the DF and DHF group. These data ot in agreement with previous reports,[25,26] and may be ained by the limited number of patients with dengue k syndrome in this study. The high frequency of low fi- ogen levels in the DHF group is indicative of increased nolysis, which is consistent with previous findings con- ing DHF.[25,27,28] Krishnamurti and others also reported ncreased activated partial thromboplastin time and de- ed fibrinogen levels in patients with DF and DHF.[29] e investigators suggested that platelet activation, rather consumptive coagulopathy, was likely to cause hemor- e in dengue without shock.

lthough an increased frequency of cases with a DIC score 7 was found in the DHF group compared with the DF p, most of these cases were free of consumptive coagu- thy. Serious bleeding manifestations such as melena caused by DIC were found in only one fatal case of DHF grade IV. Collectively, our data suggest that coagulation abnormalities involve a combination of thrombocytopenia and increased fibrinolysis, but not classic DIC in most patients in this study.

In summary, our present data demonstrated a low incidence of dengue shock syndrome among pediatric patients undergoing appropriate treatment in Metro Manila, the Philippines. Our data also show the differences in the frequency of clinical symptoms, such as restlessness, epistaxis, and abdominal pain, between patients with DF and DHF. Administration of increased volumes of IVF during the period of increased vascular permeability, a typical pathophysiologic feature of DHF 2–3 days after defervescence, can prevent dengue shock syndrome. Significantly low platelet counts and increased fibrinolysis in the peripheral blood were found in the DHF group, compared with the DF group. Coagulation abnormalities in most patients involve a combination of thrombocytopenia and increased fibrinolysis, but not classic DIC.

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REFERENCES

1. Igarashi A, 1997. Impact of dengue virus infection and its control. *FEMS Immunol Med Microbiol 18:* 291–300.
2. Guzman MG, Kouri G, 2002. Dengue: an update. *Lancet Infect Dis 2:* 33–42.
3. Hammon WM, Rudnick A, Sather GE, 1960. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. *Science 131:* 1102–1103.
4. Venzon EI, Rudnick A, Marchette NJ, Fabie AE, Dukellis E, 1972. The greater Manila dengue hemorrhagic fever epidemic of 1966. *J Philippine Med Assoc 48:* 297–313.

5. Dizon JJ, 1978. The occurrence of dengue haemorrhagic fever in the Philippines. *Asian J Infect Dis 2:* 15–17.
6. Hayes CG, Corazon RM, Gonzales A, Ranoa CP, 1988. Dengue infections in the Philippines: clinical and virological findings in 517 hospitalized patients. *Am J Trop Med Hyg. 39:* 110–116.
7. Songco RS, Hayes CG, Leus CD, Manaloto COR, 1987. Dengue fever/dengue haemorrhagic fever in Filipino children: clinical experience during the 1983–1984 epidemic. *Southeast Asian J Trop Med Public Health 18:* 284–290.
8. Nimmannitya S, 1987. Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J Trop Med Public Health 18:* 329–397.
9. Kalayanarooj S, Vaughn DW, Nimmannitya S, Gree S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpolpoj S, Innis BL, Rothman AL, Bisalak A, Ennis FA, 1997. Early clinical and laboratory indicator of acute dengue illness. *J Infect Dis 176:* 313–321.
10. Kabra SK, Jain Y, Pandey RM, Madhulika, Sinhai T, Tripathi P, Brooe S, Seth P, Seth V, 1999. Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. *Trans R Soc Trop Med Hyg. 93:* 294–298.
11. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A, 1997. Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis 176:* 322–330.
12. Bundo K, Igarashi A, 1985. Antibody-captured ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *J Virol Methods 11:* 15–22.
13. Morita K, Tanaka M, Igarashi A, 1991. Rapid identification of dengue virus serotypes by using polymerase chain reaction. *J Clin Microbiol 29:* 2107–2110.
14. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV, 1998. Dengue and dengue haemorrhagic fever. *Lancet. 352:* 971–977.
15. Baalmaseda A, Sandoval E, Perez L, Gutierrez CM, Harris E, 1999. Application of molecular typing techniques in the 1998 dengue epidemic in Nicaragua. *Am J Trop Med Hyg. 61:* 893–897.
16. Raengsakulrach B, Nisalak A, Maneekarn N, Yenchitsomanus P, Limsomwong C, Jairungsri A, Thirawuth V, Green S, Kalayanarooj S, Suntayakorn S, Sittisombut N, Malasit P, Vaughn DW, 2002. Comparisons of four reverse transcription-polymerase chain reaction procedures for the detection of dengue virus in clinical specimens. *J Virol Methods. 105:* 219–231.
17. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control.* Second edition Geneva: World Health Organization.
18. Nishiyama T, Matsukawa T, Hanaoka K, 2000. Is protease inhibitor a choice for the treatment of pre- or mild disseminated intravascular coagulation? *Crit Care Med 28:* 1419.
19. Ogden CL, Kaczmarski RJ, Flegal KM, Mei Z, Guo S, Wei R, Grummer-Strawn LM, Curtin LR, Roche AF, Jhonson CL, 2002. Centers for Disease Control and Prevention 2000 Growth Charts for the United States: improvements to the 1977 National Center for Health Statistics Version. *Pediatrics 109:* 45–60.
20. Setiawan MW, Samsi TK, Wulur H, Sugianto D, Pool TN, 1998. Epigastric pain and sonographic assessment of the pancreas in dengue hemorrhagic fever. *J Clin Ultrasound 26:* 257–259.
21. Wang J, Tseng C, Lee C, Cheng K, 1990. Clinical and upper gastroendoscopic features of patients with dengue virus infection. *J Gastroenterol Hepatol 5:* 664–668.
22. Boonpucknavig S, Lohachitranond C, Nimmanitya S, 1979. The pattern and nature of the lymphocyte population response in dengue hemorrhagic fever. *Am J Trop Med Hyg 28:* 885–889.
23. Suvatte V, Longsaman M, 1979. Diagnostic value of buffy coat preparation in dengue hemorrhagic fever. *Southeast Asian J Trop Med Public Health 10:* 7–12.
24. Thisyakorn U, Nimmannitya S, Ninganond V, Soogarun S, 1984. Atypical lymphocytes in dengue hemorrhagic fever: it's value in diagnosis. *Southeast Asian J Trop Med Public Health 15:* 32–36.
25. Mitrakul C, Poshyachinda M, Futralul P, Sangkawibha N, Ahandrik S, 1977. Hemostatic and platelet kinetic studies in dengue hemorrhagic fever. *Am J Trop Med Hyg. 26:* 975–984.
26. Srichaikul T, Nimmannitya S, Artchararit N, Sriasawakul T, Sungpeuk P, 1977. Fibrinogen metabolism and disseminated intravascular coagulation in dengue hemorrhagic fever. *Am J Trop Med Hyg 26:* 525–532.
27. Huang YH, Liu CC, Wang ST, Lei HY, Liu HS, Lin YS, Wu HL, Yeh TM, 2001. Activation of coagulation and fibrinolysis during dengue virus infection. *J Med Virol 63:* 247–251.
28. Wills B, Oraqui EE, Stephens AC, Daramola OA, Dung NM, Loan HT, Chau NV, Chambers M, Stepniewska K, Farrar JJ, Levin M, 2002. Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with dengue shock syndrome. *Clin Infect Dis 35:* 277–285.
29. Krishnamurti C, Kalayanarooj S, Cutting MA, Peat RA, Rothwell SW, Reid TJ, Green S, Nisalak A, Endy TP, Vaughn DW, Nimmannitya S, Innis BL, 2001. Mechanisms of hemorrhage in dengue without circulatory collapse. *Am J Trop Med Hyg 65:* 840–847.

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ORIGINAL ARTICLE

Usefulness of the Japanese Respiratory Society guidelines for community pneumonia: a retrospective analysis of community-acquired pneumonia between 2000 and 2002 in a general hospital

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Usefulness of the Japanese Respiratory Society guidelines for community pneumonia: a retrospective analysis of community-acquired pneumonia between 2000 and 2002 in a general hospital

MOTOMURA K, MASAKI H, TERADA M, ONIZUKA T, FURUMOTO A, ASOH N, OISHI K, NAGATAKE T. *Respirology* 2005; **10**: 208–214

Objective: The aim of this study was to investigate the causative organisms of community-acquired pneumonia (CAP) diagnosed between 2000 and 2002 and to evaluate the Japanese Respiratory Society (JRS) guidelines.

Methodology: A total of 124 cases of CAP diagnosed during the study period were analyzed, and the results were compared with those of a previous study by the authors' research group. Determination of the causative organisms of CAP was based on Gram stain, morphology of colonies, quantitative culture of sputum, identification of bacterial isolates, and serological tests.

Results: During the study period, the causative organisms were identified in 42 cases (33.8%). *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* were the major causative organisms. Patients were classified into three groups based on the severity of CAP according to the JRS guidelines. The survival rates of patients with moderate and severe CAP were significantly lower than those of the mild group as evaluated by the Kaplan–Meier method (moderate vs mild, 70% vs 100%; severe vs mild, 40% vs 100%; $P < 0.001$ for both). Seven patients died during the study, and the risk factors were old age, bedridden status with cerebral infarction, and microaspiration, which was associated with recurrent pneumonia within 17 days.

Conclusion: This study indicates that the JRS guidelines for CAP are useful for treating patients with CAP in Japan.

Key words: community-acquired pneumonia, Japanese Respiratory Society guidelines, prognosis factors.

INTRODUCTION

Acute respiratory infection (ARI) is one of the most common causes of death. In particular, community-acquired pneumonia (CAP) is associated with a high mortality rate in non-industrialized countries as well as in industrialized countries.[1,2] In Western countries, guidelines for the diagnosis of CAP were established in the 1990s and included classification of CAP patients by severity and treatment regimens for CAP, which take into account the underlying disease, laboratory tests, and the causative organisms.[3,4] In Japan, the Japanese Respiratory Society (JRS) guidelines were published in 2000.[5]

In the present study, we analyzed 124 patients with CAP, and determined the causative organisms and compared this data with that from our previous study.[6] We previously reported that strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were the major causative organisms of CAP.[6] In this

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study, we analyzed patients with CAP diagnosed at Tagami Hospital, Nagasaki, Japan, on the basis of the JRS guidelines.

SUBJECTS AND METHODS

Setting

Tagami Hospital is a 180-bed private and advanced emergency hospital. However, it is a non-teaching hospital and is located in an urban area. The hospital is of average size when compared with other hospitals in the area. Most of the in-patients are elderly. The hospital is affiliated with Nagasaki University, Japan, and CAP patients admitted from 1 January 2000 to 30 September 2002 were studied. The hospital's ethics committee approved the study.

Subjects

A total of 124 cases of CAP (76 men, aged 69.6 ± 12.7 years (mean ± SD) and 48 women, aged 69.8 ± 6.6 years) were enrolled. Of these, 122 (98.4%) were admitted to the wards and received antimicrobial therapy. The remaining two cases were treated in the outpatient department. Case analyses were based on the medical records of enrolled patients.

Diagnostic criteria for CAP

The diagnosis of CAP was based on the presence of the following two criteria:[7] (i) onset of pneumonia occurred outside the hospital—a new infiltrate shadow was detected on the chest X-ray in the outpatient department or within 24 h of hospitalization; and (ii) the presence of at least one symptom among fever, cough and sputum, or two symptoms among chest pain, dyspnoea and laboratory findings indicative of acute inflammation such as peripheral leukocytosis or elevated C reactive protein (CRP), or both.

Patients with the following diseases were excluded from the study: (i) nosocomial pneumonia; (ii) pneumonia in institutions such as nursing homes for the elderly; (iii) recurrent pneumonia within 7 days of hospital discharge; and (iv) pneumonia due to non-infectious causes such as pulmonary infarction, pulmonary oedema or lung cancer.

Detection of causative organisms

A rabbit blood-agar culture medium (Difco, Detroit, MI, USA) was used for the quantitative culture of sputum, and aerobic and anaerobic blood culture bottles (SEPTI-CHEK; Becton Dickinson, San Jose, CA, USA) were used for the blood cultures. API 20E, API 20NE, API Strept, API Staph, API Coryne, and API Ne (BioMerieux Inc., Mary-l'Etoile, France) were used for bacterial identification. Specimens were obtained by expectoration or using sterilized suction tubes for sputum if respiratory infection was suspected. When it was difficult to obtain a sputum sample, or when recurrent pneumonia occurred, we aspirated bronchial secretion samples from the lower respiratory tract using a bronchoscope, before instituting antimicrobial chemotherapy.

The collected specimen was cultured using Tryptic Soy Agar II (Becton Dickinson) supplemented with 5% rabbit blood agar for 18 h at 35°C. The causative organisms were identified by Gram stain of the purulent portion of the sputum, colony morphology, and the detection of $>10^7$ CFU/mL by quantitative sputum culture.[8] When the presence of *Mycoplasma pneumoniae* was suspected, we measured cold agglutinins and *M. pneumonia* IgG antibodies (complement fixation test) on paired serum samples with at least a 4-week interval. When the presence of *Chlamydia pneumoniae* was suspected, *C. pneumonia* IgG and IgA antibodies were measured in paired serum samples by enzyme immunoassay (EIA). The antibody titres were evaluated according to the diagnostic criteria of Kishimoto *et al.*[9] The test was regarded as positive if the increase in IgG absorbance of the convalescent serum was more than 0.3 compared with the negative baseline in the acute phase, or if the increase in IgG absorbance of the convalescent serum was more than 0.2 compared with the positive baseline in the acute phase. When the presence of *Legionella pneumoniae* was suspected, an enzyme-linked immunosorbent assay (ELISA) for urinary Legionella antigen was performed. If viral pneumonia was suspected, the antibody titre for a presumptive virus was measured in paired serum samples.

The minimum inhibitory concentration (MIC) was determined by the microdilution technique using the antibiotic susceptibility test, according to the revised guidelines of the National Committee for Clinical and Laboratory Standards, USA.[10] Penicillin-intermediate *Streptococcus pneumoniae* (PISP) and penicillin-resistant *S. pneumoniae* (PRSP) were defined by MIC of 0.125–1.0 μg/mL and more than 2.0 μg/mL of penicillin for *S. pneumoniae*, respectively. In our analysis, PRSP included both PISP and PRSP.

Clinical analysis

In this study, we classified CAP cases into three groups: mild, moderate, and severe, according to the JRS guidelines. The patient's background, underlying disease, causative organisms, antimicrobial chemotherapy, and clinical outcomes were compared with our previous results.[6] The antimicrobial therapy was considered effective if the clinical symptoms and acute inflammatory responses improved (decreased cough, reduced sputum volume, and decrease in sputum purulence) or if the causative organisms decreased in number or disappeared from the sputum. For the management of CAP, the severity of pneumonia was classified using the JRS guidelines,[5] based on the physical examination, CXR, WCC, serum CRP value and the arterial PaO_2. Patients were classi-

fied into three groups: mild, moderate, and severe. These groups were compared in terms of duration of admission, duration of treatment, and mortality rate using the Kruskal–Wallis test for multiple group comparisons. *P*-values <0.05 were considered statistically significant. The Kaplan–Meier survival curves were analyzed using the Mini-stat computer software program (ATMS, Tokyo, Japan).

RESULTS

Patient background

The age distribution at onset of CAP was 16–94 years (16–19 years, six patients (4.8%); 20–64 years, 27 patients (21.8%); >65 years, 91 patients (73.4%)). Table 1 shows the analysis of CAP cases using the JRS severity of pneumonia criteria. The mean age was 66.6 ± 20.8 years for the mild group, 75.3 ± 17.5 years for the moderate group, and 79.9 ± 9.2 years for the severe group. Multiple group comparisons showed significant differences (mild vs moderate, $P < 0.001$; mild vs severe, $P < 0.001$). The duration of admission was significantly shorter in the mild group (mild, 30.8 days vs moderate, 53.9 days, $P < 0.001$; mild, 30.8 days vs severe, 58.9 days, $P < 0.001$). The duration of treatment was 18 days in the severe group, 7 days in the mild group and 6 days in the moderate group (mild vs severe, $P < 0.001$; moderate vs severe, $P < 0.001$). The number of deaths during the hospitalization period was zero, three and four in the mild, moderate and severe groups, respectively (mild vs severe, $P < 0.001$; mild vs moderate, $P < 0.001$; moderate vs severe, $P = 0.281$). The subsequent observation period was up to 534 days. Figure 1 shows the Kaplan–Meier survival curves calculated for the three severity groups. The cumulative survival rate decreased to 70% at 70 days in the moderate group, and 40% at 105 days in the severe group (mild vs moderate, $P < 0.01$; mild vs severe, $P < 0.001$).

Laboratory data were similar to those reported in our previous study.[6] The mean level of CRP in the mild group was 5.97 ± 4.36 mg/dL; in the moderate group, 7.25 ± 4.78; and in the severe group, 10.26 ± 6.78. The severity of CAP was significantly associated with the CRP levels in the three severity groups (mild vs moderate and mild vs severe, $P < 0.01$). The mean level of serum albumin in the mild group was 4.43 ± 2.12 g/dL; in the moderate group, 4.17 ± 1.98; and in the severe group, 3.76 ± 1.85. The mean level of serum albumin was significantly lower in the severe group than in the mild or moderate groups ($P < 0.01$ for both).

Underlying diseases

The most common underlying diseases were respiratory, which were present in 53 (43%) of the 124 cases. The breakdown of these included bronchial asthma in 19 (36.4%), emphysema in 14 (26.4%), and old tuberculosis in 12 (22.6%). The second common underlying disease group was cerebrovascular accident, including cerebral infarction, which accounted for 20 patients (16%). Of the 124 patients studied, 11 (9%) had diabetes mellitus.

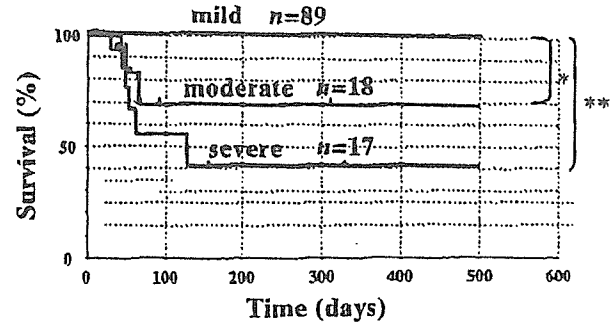

Figure 1 Kaplan–Meier analysis of 124 cases of community-acquired pneumonia in each of the three severity groups. Kaplan–Meier survival plots were computed using Mini-stat statistical software. The *P*-values for the prediction for each severity group were computed using a log-rank test. *$P < 0.01$, **$P < 0.001$.

Table 1 Analysis of the study population in each severity group according to the JRS Guideline for CAP

	Severity by JRS		
	mild	moderate	severe
No. cases	89 (71.8%)	18 (14.5%)	17 (13.7%)
Age (years, mean ± SD)	66.6 ± 20.8	75.3 ± 17.5	79.9 ± 9.2
Duration of admission (days)	30.8	53.9*	58.9*
Duration of treatment (days)	9	9.3	11.9**
Deaths	0	3	4
Serum C-reactive protein (mg/dL, mean ± SD)	5.97 ± 4.36	7.25 ± 4.78*	10.26 ± 6.78*
Serum albumin (g/dL, mean ± SD)	4.43 ± 2.12	4.17 ± 1.98*	3.76 ± 1.85*

*$P < 0.01$ compared to mild group; **$P < 0.01$ compared to moderate group.

Causative organisms of CAP

Causative organisms of CAP were identified in 42 (33.8%) patients. Among these, the diagnosis was based on sputum culture in 38, while in the other four patients it was based on serological tests. Among the 42 cases, 35 (83.3%) were monomicrobial infection. Polymicrobial infection with *C. pneumoniae*, *S. pneumoniae* and *H. influenzae* was detected in one patient (2.4%).

The strains of *S. pneumoniae* were determined in 15 cases (12.1%), and seven (46.7%) of 15 isolates were PRSP. *S. pneumoniae* was the most frequently isolated organism. Figure 2 shows the causative organisms of CAP in this study and in our previous study.[6] The rate of isolated PRSP was not determined in our previous study, as the oxacillin sensitivity test was not performed.[6]

H. influenzae strains were determined in 10 patients (8.1%) and all were non-β-lactamase-producing strains. Only one strain of *H. influenzae* did not disappear after antimicrobial therapy. This strain was shown to be a low β-lactamase negative ampicillin resistant *Haemophilus influenzae* (BLNAR) strain, based on the pattern of penicillin-binding proteins determined by polymerase chain reaction.[11] The infection rates for *H. influenzae* as a causative organism in our previous and present studies were 27.2% and 23.8%, respectively. *Moraxella catarrhalis* was isolated in four patients (Fig. 2). *Pseudomonas aeruginosa* was isolated in three patients, in whom the underlying disease was bronchiectasis. Other organisms isolated included *Klebsiella pneumoniae*, *Corynebacterium pseudodiphtheriticum* and *Corynebacterium propinquum* (Fig. 2).

In this study, serological tests were performed for *M. pneumoniae* and *C. pneumoniae*, using paired serum samples obtained from 68 (54.8%) patients. *Mycoplasma pneumoniae* serology was positive in three (2.4%) patients. Their age range was 18–50 years. Only one patient was positive for *C. pneumoniae* as per the diagnostic criteria of Kishimoto *et al.*[9]

Causative organisms were not determined in 82 (66.1%) patients. There were a number of reasons for this. A total of 26 patients (21%) had received antibiotics before attending the hospital. They had received antibiotics for a mean period of 3.9 days, but prior to any CXR being taken. Cerebrovascular disease was identified as the underlying disease in 10 (8.1%) patients. In such patients, microaspiration-induced pneumonia caused by anaerobic bacteria was suspected. No factor that may have influenced the detection of the causative organisms could be identified in the remaining 46 cases. Unfortunately, blood cultures were performed in only seven (5.6%) of the 124 cases of CAP.

Antimicrobial therapy of CAP

Antimicrobial therapy for CAP was terminated following improvement of clinical symptoms and normalization of the CRP level, even if the pulmonary shadows on the CXR were still present. However, if there was a worsening of clinical symptoms and worsening pneumonia on CXR on the third day after antibiotics were commenced, the antibiotics were immediately changed. Of the 124 patients, 123 were treated by i.v. infusion, and only one patient with suspected atypical pneumonia was treated orally with a quinolone. The first choice antimicrobial agents for CAP were cephalosporins and cephamycin which were used in 36 (29.0%) patients (second generation cephalosporin, five cases; third generation cephalosporin, 31 cases). Penicillins were used in 32 (25.8%) patients, carbapenems in 23 (18.5%), tetracyclines in four (3.2%), macrolides in two (1.6%), and a new quinolone in one (0.8%). Antimicrobial agents were changed in 26 (20.9%) patients with carbapenem being used in 10 patients, third generation cephalosporins in seven, tetracycline in seven, and penicillin, clarithromycin clindamycin and levofloxacin each in one patient. All patients were switched to a single antibiotic. The reasons for changing antibiotics were adverse effects in seven cases, atypical pneumo-

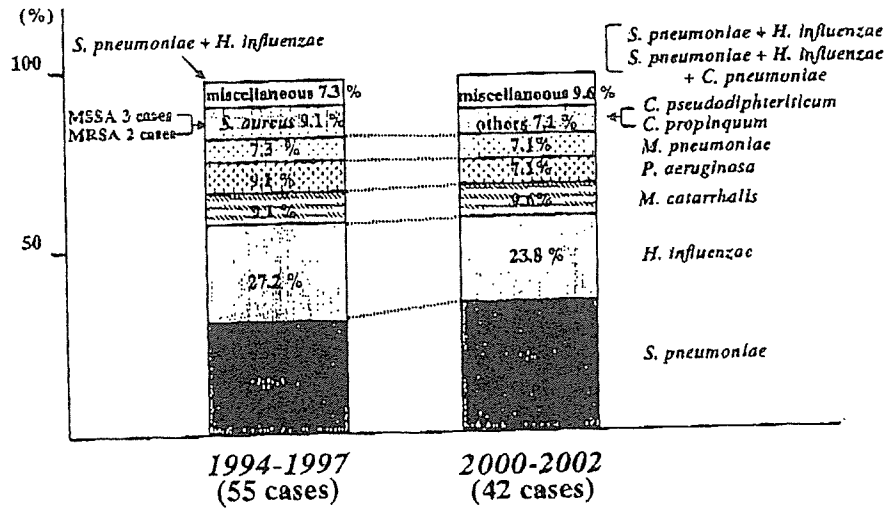

Figure 2 Causative organisms of community-acquired pneumonia during each study period. The 1994–1997 study has been published previously.[6]

nia in three, aspiration pneumonia in four, and others (insufficient therapeutic effect) in 12. A second antimicrobial agent was added in one patient with PRSP-induced pneumonia and in four patients with *H. influenzae* pneumonia.

Analysis of patients who died during treatment of community-acquired pneumonia

Table 2 shows the clinical details of those patients who died during the treatment of CAP. Seven (5.6%) died during the treatment and all were males. The mean age was 81.1 ± 8.6 years. Of these, *H. influenzae* was isolated in one patient but no causative organisms were isolated in the remaining six (85.7%). Pleural effusion was found in five cases (71.4%), of whom three had congestive heart failure. Four had respiratory failure, which was treated either with ventilatory support or non-invasive positive pressure ventilation in addition to corticosteroid therapy. The risk factors for death included male gender, performance status of 4,[12] cerebral infarction, and aspiration pneumonia relapsing within 17 days.

DISCUSSION

The present study was designed to identify the causative organisms of CAP and analyze cases with CAP based on severity as classified by the JRS guidelines. The major causative organisms of CAP were *S. pneumoniae* and *H. influenzae*,[13–15] which accounted for 69% of the cases in our study. PRSP accounted for 46.7%, which is similar to the results from another survey of adults.[16] No relationship was observed between PRSP pneumonia and the severity of CAP in our study. Watanabe *et al.*[17] reported that PISP pneumonia did not differ significantly from PRSP pneumonia in clinical symptoms and severity.

Interestingly, *C. propinquum* in the Corynebacterium absolute non-fermenter (ANF) group has been identified as a new causative organism of CAP.[16] Corynebacterium species are usually known as organisms that constitute the normal bacterial flora. Therefore, it is important to examine the Gram-stained sputum specimen for such microorganisms. The rate of identification of causative organisms in our previous study[6] was 45.8%, however, the rate decreased to 33.8% in the present study. The reasons for this decrease are likely to be the increase in the number of patients receiving antibiotics before presentation and the increase in the number of patients with cerebral infarction and possible anaerobic infection in such patients.[19] A low frequency of *M. pneumoniae* infection was observed in the present study, which was similar to our previous study.[6] The reason for this might be related to the age distribution of our patients, which was biased towards the older age group (the proportion of patients aged >65 years was 73.4%).

Table 2 Analysis of dead cases during the treatment course of community-acquired pneumonia

No.	Age	Gender	PS	Underlying diseases	Consciousness disturbance	Pleural effusion*	Aetiology	Infiltration on chest X-ray	Severity	Days before recurrence
1	78	Male	4	Cerebral infarction	No	No	ND	Three lobes	Moderate	6
2	92	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	14
3	87	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	8
4	79	Male	4	Cerebral infarction	Yes	Yes	ND	One lobe	Moderate	9
5	85	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	17
6	84	Male	4	Cerebral infarction	No	No	ND	One lobe	Moderate	10
7	63	Male	4	Cerebral infarction	Yes	Yes	*H. influenzae*	All	Severe	3

Pleural effusion yes means unilateral or both; ND, not determined; PS, performance status.
* Yes indicates one or both lungs.

Bed-ridden elderly patients with multiple cerebral infarctions and recurrent aspiration pneumonia had a poorer prognosis. Arancibia *et al.*[20] reported that infections caused by Gram-negative bacteria such as *P. aeruginosa*, microaspiration and the presence of underlying diseases indicated a poor prognosis in patients with pneumonia.[20]

In our previous report,[6] we classified the patients' severity according to the Japanese Society of Chemotherapy guidelines.[21] We reported that the severe cases had high CRP and low serum albumin levels.[6] Similar results were obtained in the present study in which the JRS guidelines were applied. Moreover, the duration of admission, duration of treatment, and the cumulative survival rates were significantly different among the three severity groups. The duration of admission was long even in the mild group. The main reason for the long hospitalizations was weakness of lower limb muscles and a further general weakness after CAP treatment, which was seen in most of the elderly patients, and rehabilitation was provided until they were discharged. In some patients, a pulmonary rehabilitation program was added, as COPD was incidentally diagnosed during their admission.

We classified and analyzed those who died from CAP into five stages, based on the classification of Fine *et al.*[22] Our results were different from their prediction (data not shown) as most of their cases were labelled severe, if their underlying disease was malignancy,[22] while cases with multiple cerebral infarctions were not graded as being severe. Thus, their classification was not suitable for our patients, as multiple cerebral infarction was a major underlying disease.

Our results suggest that the JRS guidelines for CAP are a suitable and useful tool for analysis of the treatment of patients with CAP in Japan.

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REFERENCES

1 Garibaldi RA. Epidemiology of community-acquired respiratory tract infections in adults. Incidence, etiology, and impact. *Am. J. Med.* 1985; 78: 32–7.

2 Yoshimine H, Oishi K, Mubiru F *et al.* Community-acquired pneumonia in Ugandan adults: short-term parenteral ampicillin therapy for bacterial pneumonia. *Am. J. Trop. Med. Hyg.* 2001; 64: 172–7.

3 Niederman MS, Mandell LA, Anzueto A *et al.* Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. *Am. J. Respir. Crit. Care Med.* 2001; 163: 1730–54.

4 British Thoracic Society Standards of Care Committee. BTS guidelines for the management of community acquired pneumonia in adults. *Thorax* 2001; 56 (Suppl. 4): IV1–64.

5 The Japanese Respiratory Society. *The Japanese Respiratory Society Guidelines for the Management of Community-Acquired Pneumonia in Adults*, Japan Respiratory Society, Tokyo, 2000; 20–1.

6 Motomura K, Takasugi M, Furumoto A *et al.* Clinical features and etiology of community-acquired pneumonia at a general hospital between 1994 and 1997. *Nihon Kokyuki Gakkai Zasshi* 2000; 38: 267–72 (in Japanese).

7 Mundy LM, Auwaerter PG, Oldach D *et al.* Community-acquired pneumonia: impact of immune status. *Am. J. Respir. Crit. Care Med.* 1995; 152: 1309–15.

8 Matsumoto K, Uzuka Y, Taguchi M *et al.* Sputum bacteria quantification culture. *Media Circle* 1985; 19: 181–99.

9 Kishimoto T, Kubota Y, Matsushima T *et al.* Assay of specific anti-Chlamydia pneumoniae antibodies by ELISA method. 2. studies on clinical usefulness and serological diagnostic standards. *Kansenshogaku Zasshi* 1996; 70: 830–9 (in Japanese).

10 National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests Fro Bacteria That Grow Aerobically*, 5th edn. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, PA, 2000.

11 Ubukata K, Shibasaki Y, Yamamoto K *et al.* Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* 2001; 45: 1693–9.

12 Oken MM, Creech RH, Tormey DC *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am. J. Clin. Oncol.* 1982; 5: 649–55.

13 Bartlett JG, Mundy LM. Community-acquired pneumonia. *N. Engl. J. Med.* 1995; 333: 1618–24.

14 Ishida T, Hashimoto T, Arita M, Ito I, Osawa M. Etiology of community-acquired pneumonia in hospitalized patients: a 3-year prospective study in Japan. *Chest* 1998; 114: 1588–93.

15 Nishiyama O, Taniguchi H, Kondoh Y, Kimura M, Shimizu J. The utility of the Pneumonia PORT prediction rule for evaluating the severity of community-acquired pneumonia in Japan. The relationship between disease severity and drug-resistant Streptococcus pneumoniae. *Kansenshogaku Zasshi* 2002; 76: 1003–9 (in Japanese).

16 Shimada K, Terai T, Igari J *et al.* Susceptibilities of bacteria isolated from patients with lower respiratory infectious diseases to antibiotics (2000). *Jpn. J. Antibiot.* 2002; 55: 537–67 (in Japanese).

17 Watanabe H, Sato S, Kawakami K *et al.* A comparative clinical study of pneumonia by penicillin-resistant and -sensitive Streptococcus pneumoniae in a community hospital. *Respirology* 2000; 5: 59–64.

18 Furumoto A, Masaki H, Onizuka T *et al.* A case of community-acquired pneumonia caused by Corynebacterium propinquum. *Kansenshogaku Zasshi* 2003; 77: 456–60 (in Japanese).

19 Marik PE. Aspiration pneumonitis and aspiration pneumonia. *N. Engl. J. Med.* 2001; 344: 665–71.
20 Arancibia F, Bauer TT, Ewig S *et al.* Community-acquired pneumonia due to gram-negative bacteria and pseudomonas aeruginosa: incidence, risk, and prognosis. *Arch. Intern. Med.* 2002; 162: 1849–58.
21 The Japanese Chemotherapy Society. The clinical assessment of new antimicrobial agents in respiratory infection. *Jpn. J. Chemother.* 1997; 45: 762–78.
22 Fine MJ, Auble TE, Yealy DM *et al.* A prediction rule to identify low-risk patients with community-acquired pneumonia. *N. Engl. J. Med.* 1997; 336: 243–50.